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Note

Determination of nanogram amounts of dicyclomine with gas chromatography and nitrogen-selective detection

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Dicyclomine · HCl [β-(diethylamino)ethyl 1-cyclohexylcyclohexanecarboxylate hydrochloride] is a parasympatholytic agent, employed for many years in the symptomatic treatment of gastrointestinal disorders [1, 2]. In a comparative study of the bioavailability of three marketed dicyclomine preparations the pioneering gas chromatographic (GC) method of Meffin et al. [3], albeit very sensitive, appeared unsuitable for routine use because of the too laborious preparation of the sample extract to be injected. Therefore an assay procedure was developed which involved extraction and purification steps followed by GC measurement with a nitrogen-selective detector.

EXPERIMENTAL

Materials

Dicyclomine · HCl (Fig. 1, I) was supplied by Merrell (Cincinnati, OH, U.S.A.). Chlorcyclizine · HCl (Fig. 1, II), the internal standard, was purchased from Pfalz & Bauer (Flushing, NY, U.S.A.). All the other chemicals were obtained from Merck (Darmstadt, F.R.G.). The organic solvents were distilled

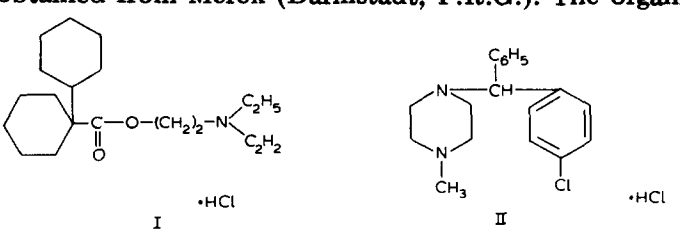

Fig. 1. Chemical structures of dicyclomine · HCl (I) and chlorcyclizine · HCl (KI), internal standard.

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before use; in particular, diethyl ether shortly before. All the glassware (tubes, pipettes) was previously washed twice with ethyl acetate containing 5% methanol and heated at 500°C for 4 h. Dichlordimethylsilane (Merck), 5% in toluene (Merck), was employed as silanizing agent for the conical evaporation tubes. The standard solutions contained 5 mg of I and/or II in 10 ml of methanol. From these suitably diluted working solutions were prepared.

Extraction procedure

A 1-ml volume of plasma and 6 μl of internal standard solution (30 ng) were transferred to a 100 × 16 mm screw-capped centrifuge glass tube. After the addition of 0.5 ml of 1 *M* sodium hydroxide the sample was extracted with 5 ml of diethyl ether for 5 min in a horizontal shaker and then centrifuged. The extract was transferred to another glass tube and shaken with 1 ml of 1 *M* hydrochloric acid for 3 min and centrifuged. The upper phase was discarded by suction, whereas the aqueous phase was washed by shaking with 3 ml of diethyl ether for 3 min. The upper layer was aspirated and discarded, then the aqueous phase was alkalinized with 1.2 ml of 1 *M* sodium hydroxide, extracted with 5 ml of diethyl ether for 5 min and centrifuged. The upper phase was transferred to a 120 × 24 mm silanized glass conical tube and evaporated to dryness under a nitrogen stream. The residue was taken up with 10 μl of ethyl acetate containing 5% (v/v) methanol for injection into the chromatograph.

Chromatographic system

A coiled glass column (1.8 m × 2 mm I.D.) packed with 5% OV-225 on 80—100 mesh Chromosorb HP Hewlett-Packard (Cernusco sul Naviglio, Milan, Italy) was mounted in a Hewlett-Packard 5830 A computer-controlled gas chromatograph equipped with a nitrogen-selective detector and connected with a 18850 A terminal. Working conditions: temperatures, 250°C (injector), 245°C (oven) and 300°C (detector); gas flow-rates, 3 ml/min (hydrogen), 49 ml/min (air) and 30 ml/min (nitrogen, carrier gas). The operating conditions were programmed into the system through the terminal which also controlled them and produced a report containing the chromatogram and the determination results (peak areas and retention times of I and II).

Calculations

The detector response factors to be used in the calculations came from spiked control human plasma samples (30 ng of both I and II in 1 ml), processed as above along with the samples to be assayed (one for every five unknown samples). Aliquots of an ethyl acetate—methanol (95:5) solution containing 30 ng of both I and II were injected before and after a series of unknown samples (at least twenty) to obtain the reference values for the response factor and the retention times.

RESULTS AND DISCUSSION

The clean-up steps on the plasma sample provided an extract with minute interfering peaks at the retention times of I and II (5.2 and 8.9 min, respectively). The time of the analysis was prolonged until 30 min to permit the exit of

TABLE I

RECOVERY OF DICYCLOMINE ADDED TO CONTROL PLASMA IN REPLICATE (n = 3) ANALYSIS

Dicyclomine added (ng/ml)	Dicyclomine found (ng/ml, ± S.D.)	Relative S.D. (%)	Recovery (%)
5	6.4 ± 0.6	9.4	128
10	11.2 ± 0.6	5.4	112
20	20.7 ± 1.8	8.7	104
40	39.0 ± 1.4	3.6	98
80	76.3 ± 6.4	8.4	95

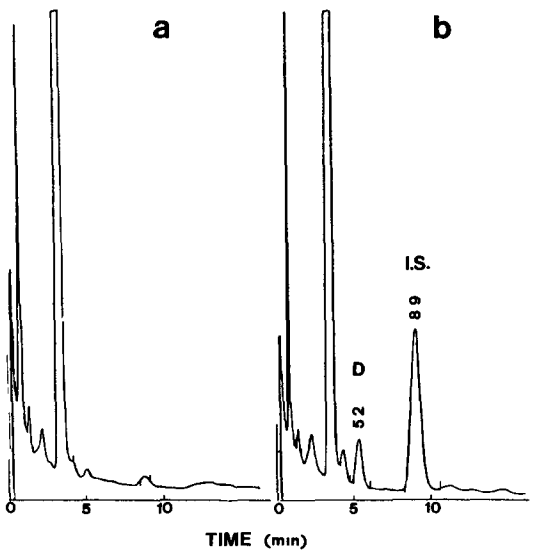

Fig. 2. Chromatograms of human plasma extracts. (a) Control plasma; (b) 3-h plasma from a subject receiving 20 mg of I, containing 18 ng/ml dicyclomine (D) and 30 ng/ml chlorcyclizine (I.S., internal standard).

a peak with a retention time of 24 min which proved to give trouble in the subsequent tracing when not previously removed. For the construction of a calibration curve control human plasma was spiked with I over a concentration range from 5 to 80 ng/ml of dicyclomine base and II (30 ng/ml of chlorcyclizine base). Triplicates for each concentration were submitted to the assay (Table I).

The results showed that the response was linear, the determination was satisfactorily reproducible (relative standard deviation from 3.5 to 9.2%) and that the recovery ranged from 95 to 128% for the concentrations tested. The recovery exceeding 100% for the low concentrations might be attributed to a small amount of interfering material that was found at the retention time of I (Fig. 2a).

The regression equation for the straight line calculated from the values in Table I was $Y = 1.8472X + 0.9307$ with $r = 0.9953$. The limit of quantitation of the method was 5 ng/ml; nevertheless, it was possible to detect dicyclomine

in samples at concentrations less than 5 ng/ml. The reproducibility of the method in the chromatographic system was tested on ten diverse samples containing 30 ng of both I and II, separately processed on two consecutive days (5 + 5) and showed a relative standard deviation of 3.4%.

In order to minimize the loss of I and/or II for adsorption on the column, two or three control human plasma extracts were injected just before the unknown sample series was assayed.

Although the supplier of the nitrogen-selective detector recommended avoiding the use of OV-225 (nitrogen-containing phase), the overall system proved to give reliable results with noticeable savings in time when the work was restarted by maintaining the collector bead overnight at a temperature high enough to prevent contamination from the column bleed and under the same flow-rates of air and hydrogen as during working operations.

Under the conditions described the method was successfully employed for dicyclomine determinations on 200 plasma samples from six healthy volunteers who received 20 mg of drug orally in a cross-over study of its bioavailability from drops, tablets and gel preparations.

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