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Biomedical Applications

CHROMBIO. 410

Note

Determination of 1-diethylcarbamoyl-4-methylpiperazine (diethylcarbamazine) in human plasma and urine

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Diethylcarbamazine (Banocide, Hetrazan) (DEC) has been used for many years for the treatment of human and animal filariases.

Spectrophotometric methods for measuring DEC through formation of ion-pairs have been utilized by Lubrum [1] and Ramachandran [2]. Procedures based on this principle, however, lack the specificity and sensitivity of gas chromatographic methods.

Bogan [3] has developed a gas chromatographic method for measuring diethylcarbamazine in animal plasma and tissues, and while the procedure is an improvement over the above methods, the sensitivity is somewhat lacking as the minimum detectable concentration is 400 ng/ml and there are operational difficulties with column priming and poor chromatography.

The present method can detect concentrations of 10 ng/ml from 0.5 ml human plasma, and therefore can be applied to the clinical situation where levels below 500 ng/ml are obtained later than 12 h after oral dosing of 200 mg of DEC citrate.

EXPERIMENTAL

Reagents and materials

Diethylcarbamazine (I) is a basic compound, soluble in most organic solvents and in aqueous solutions of organic acids, and stable in the presence of 2 *M* aqueous sodium hydroxide.

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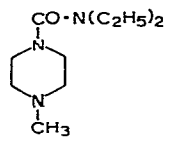
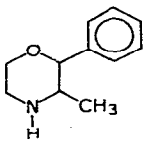


I



II

Phenmetrazine (3-methyl-2-phenylmorpholine hydrochloride) (II) was chosen as internal standard because of its similarity to DEC in extraction properties, its appropriate retention time and its ready availability; analogues of DEC are not commercially available. Other chemicals used were ethyl acetate (redistilled), *n*-hexane and sodium hydroxide (all AnalaR grade; BDH, Poole, Great Britain). The sodium hydroxide was employed as a 2 *M* solution in water.

Standard plasma solutions of DEC citrate were prepared from an aqueous solution of concentration 2 mg/100 ml, serially diluted using plasma as appropriate. Urine standards were prepared from a solution in human urine of concentration 10 mg/100 ml, serially diluted using urine as appropriate. The internal standard was prepared by dissolving phenmetrazine hydrochloride in distilled water to a concentration of 20 μg/ml.

Glassware

Screw-capped 10-ml Sovirel tubes (V.A. Howe, London, Great Britain) were used in the extraction and BC24/C14T tapered test-tubes (Quickfit and Quartz, J.A. Jobling, Staffordshire, Great Britain) were used for solvent evaporation.

Gas—liquid chromatographs

A Perkin-Elmer F30 instrument, modified to allow sample injection from a Hewlett-Packard 7670A autosampler, was used with a nitrogen flame ionization detector. This was operated under standard conditions with hydrogen and air flow-rates of 3 and 50 ml/min, respectively, and the standing current adjusted to 10 pA.

A Hewlett-Packard 5735A gas chromatograph, equipped with 7671A autosampler was also used with a nitrogen flame ionization detector. Again this was operated under standard conditions with hydrogen and air flow-rates of 3 and 50 ml/min, respectively, and the standing current adjusted to 16 pA.

Operation of the autosamplers and processing of data were carried out using a Hewlett-Packard 3352B minicomputer-based laboratory automation system.

Extraction procedure for plasma and urine

Preliminary experiments having shown that extraction of DEC and phenmetrazine was quantitative into ethyl acetate using aqueous sodium hydroxide, and that this solvent produced a clean gas chromatogram in the region of the measured compounds when blank fluids were extracted, the following procedure was devised. To each plasma (0.5 ml) or urine (1 ml) sample (standard as prepared above, or unknown) in a Sovirel tube were added 100 μl of phen-

metrazine standard solution (2 μg as hydrochloride). The mixture was made alkaline with 500 μl of 2 *M* sodium hydroxide solution for plasma, or 1 ml for urine, and ethyl acetate (5 ml) was added. The phases were mixed by mechanical tumbling end-over-end at 15/min for 10 min, separated by centrifugation at 3000 *g* for 5 min and the organic layer transferred as completely as possible to a tapered test-tube. Five ml more ethyl acetate were added to the aqueous

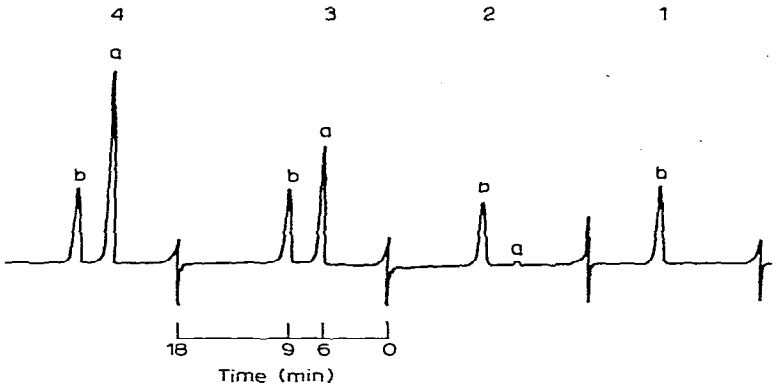


Fig. 1. Gas—liquid chromatography traces of plasma samples extracted and analysed for DEC citrate. Peaks: a = DEC; b = phenmetrazine. 1, Pre-dose patient plasma sample; 2 and 3, patient plasma samples containing 0.27 and 5.16 μg/ml, respectively; 4, plasma standard of concentration 8.26 μg/ml.

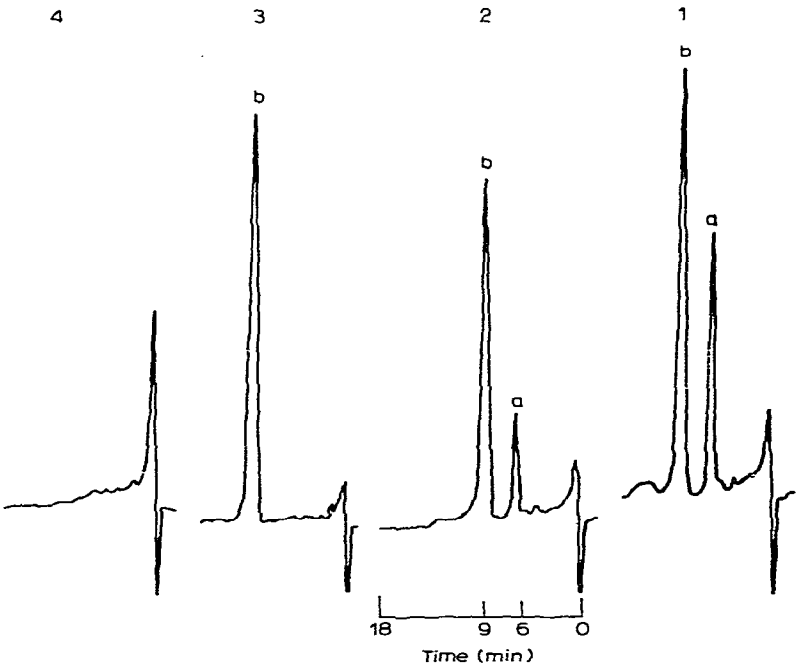


Fig. 2. Gas—liquid chromatography traces of urine samples extracted and analysed for DEC citrate. Peaks: a = DEC; b = phenmetrazine. 1, Urine standard of concentration 13.00 μg/ml; 2, urine sample containing 5.01 μg/ml; 3, pre-dose urine sample containing phenmetrazine; 4, pre-dose patient urine sample.

phase, the extraction repeated, and the organic phase added to the first extract. The ethyl acetate was removed using dry nitrogen at room temperature and the residue redissolved in 200 μl of hexane using a Vortex mixer to wash the sides of the tube. Samples were transferred to Hewlett-Packard microvials for analysis by gas chromatography, sample injection being 5 μl.

Gas—liquid chromatography

Several stationary phases were investigated, and it was concluded that 2% Carbowax 20M, 5% KOH, on Chromosorb G AW DMCS (100—120 mesh) was the optimum in terms of separating the drug and internal standard from endogenous biological material, and of eliminating adsorption and tailing seen on silicone phases [3]. The column was maintained at 160°, the injection port at 180° and the detector at 240°. Nitrogen was used as carrier gas at a flow-rate of 40 ml/min. Under these conditions the retention time of DEC was 6 min and that of phenmetrazine 9 min.

The peaks from DEC and phenmetrazine were identified by retention times, and the 3352B data system was programmed to calculate the concentrations of DEC by peak area ratios referred to standards extracted and run with the samples.

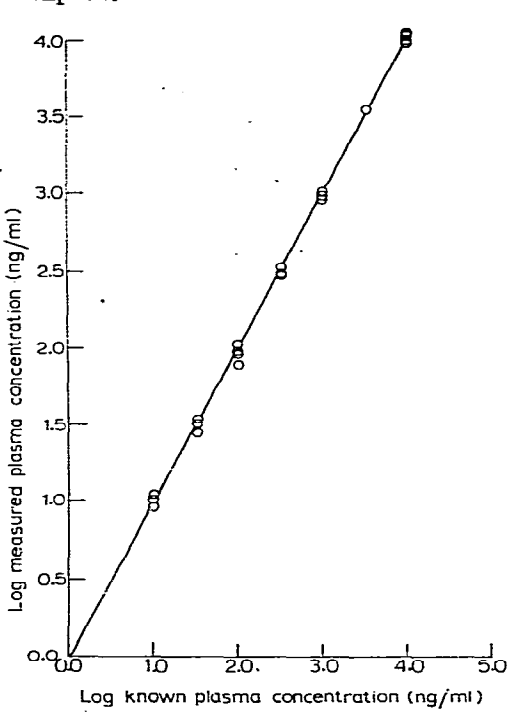


Fig. 3. Validation graph for analysis of DEC citrate in human plasma. Each concentration was measured in quadruplicate; the line is a linear regression of the logarithms of the measured concentrations on the spiked concentrations with a correlation coefficient of 0.9996.

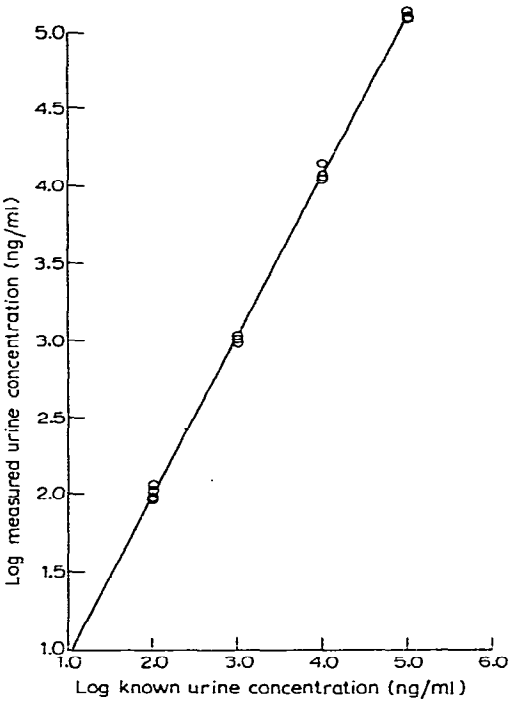
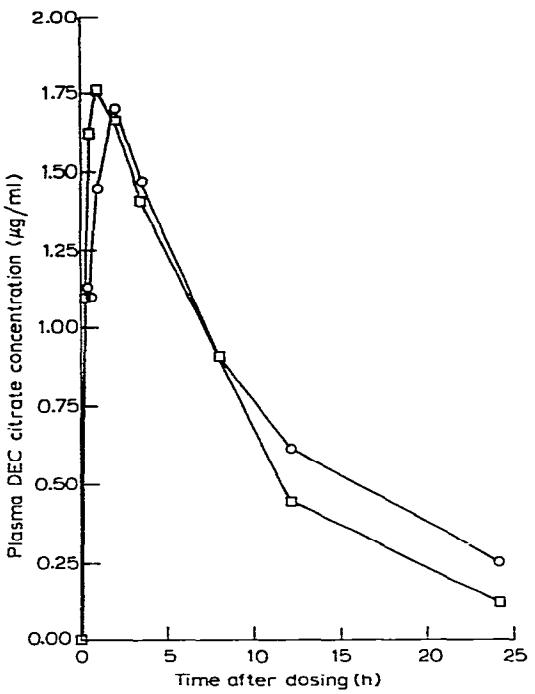


Fig. 4. Validation graph for analysis of DEC citrate in human urine. Each concentration was measured in quadruplicate; the line is a linear regression of the logarithms of the measured concentrations on the spiked concentrations with a correlation coefficient of 0.9995.



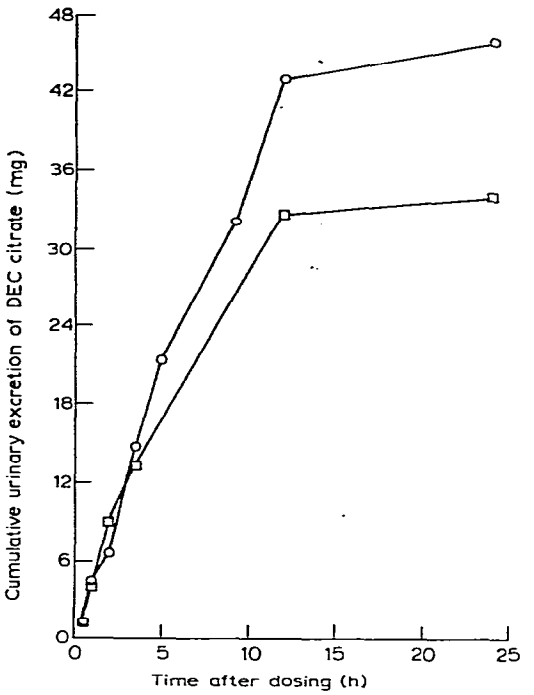


Fig. 5. Plasma concentrations of DEC citrate in two volunteers dosed orally with 200 mg Banocide.

Fig. 6. Accumulated urinary excretion of unchanged DEC citrate in the same volunteers as in Fig. 5.

RESULTS

Typical chromatograms of plasma (Fig. 1) and urine (Fig. 2) standards and samples from patients are shown, together with validation graphs showing the linearity and range (Figs. 3 and 4). Standard samples were re-analysed after storage at −20°, including freshly prepared standards; plasma samples are stable for at least six weeks, and urine samples stable for at least three months at this temperature.

Recoveries of DEC were measured by comparing gas chromatogram peak areas from DEC citrate standards, carried through the procedure, with peak areas from DEC base in methanolic solution. Each determination was carried out in triplicate, and the mean recoveries (± standard deviation) from plasma and urine were 95 ± 8% and 33 ± 7%, respectively.

Clinical studies

This analytical procedure was applied to samples from informed and consenting volunteers and from patients dosed with Banocide (DEC citrate); preliminary results have been reported by Rée et al. [4], and data on the concentration—time course are shown in the following graphs (Figs. 5 and 6). Urine

samples which were beyond the calibration range of 100 μg/ml were diluted with control urine and re-analysed.

Results of a field study in patients receiving a topical application of DEC citrate, and detailed pharmacokinetic calculations on the oral data presented here will be published at a later date.

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