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Note

Rapid gas chromatographic determination of disopyramide in serum using a nitrogen detector

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Disopyramide is a new antiarrhythmic agent that seems to be a drug with a greater therapeutic index than quinidine. Pharmacokinetic studies in man have indicated that disopyramide phosphate taken orally is absorbed rapidly and almost completely from the gut. The plasma half-life of disopyramide is about 7 h and 60% of the disopyramide is excreted unchanged in the urine within 48 h (ref. 1).

Quantitation of disopyramide has been performed by using a spectrofluorimetric method based on the fluorescence of disopyramide in 50% sulphuric acid[1]. However, this method is non-specific because the N-monodealkylated metabolite has the same fluorescence characteristics as the parent compound. Studies on the biotransformation and pharmacokinetics of disopyramide require a more sophisticated method, in which it is possible to quantitate disopyramide without interference by the metabolite. A gas chromatographic separation and quantitation of the drug is the most suitable method. Because of the interference by impurities from extraction solvents and serum, the use of a flame ionization detector necessitates a time-consuming clean-up procedure. The use of a nitrogen detector, a sensitive and selective detection system, combined with a simple extraction procedure, is described here.

EXPERIMENTAL

Apparatus

The gas chromatograph used was a Pye Unicam G.C.V. (Philips Nederland) equipped with a nitrogen detector. The column was a Pyrex glass coil of length 3 ft and I.D. 2 mm.

Operating conditions

Gas chromatography was carried out under the following conditions: stationary phase, 3% OV-17 on Gas-Chrom Q, 100–120 mesh; carrier gas, nitrogen at 30 ml/min; hydrogen flow-rate, 30 ml/min; air flow-rate, 300 ml/min; oven temperature, 255°; injector temperature, 275°; detector temperature, 275°.

Extraction procedure

Pipette into a glass-stoppered separating funnel 1.0 ml of serum, 2.0 ml of 0.1 *N* sodium hydroxide solution, 100 μl of internal standard solution (*p*-chlorodiso-

pyramide, 100 μg per solution in ethanol) and 10 ml of chloroform. Shake well for 30 sec, dry the chloroform layer with 1 g of anhydrous sodium sulphate and filter. Extract the aqueous layer with 10 ml of chloroform for 15 sec, dry and filter off the chloroform phase. Evaporate the pooled chloroform extracts to dryness on a water-bath at 50° under a stream of nitrogen. Transfer the dried residue with small portions of chloroform into a 3-ml glass tube, evaporate the chloroform under a stream of nitrogen and dissolve the residue in 25 μl of ethanol. Inject 1 μl of the final solution into the gas chromatograph.

RESULTS AND DISCUSSION

A calibration graph was prepared by adding known amounts of disopyramide phosphate to pooled blank serum, resulting in the following standards: 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0 and 10.0 μg disopyramide per ml. The standards were analyzed in triplicate by the extraction procedure described above. The ratio of the peak area of disopyramide to that of internal standard was plotted against the concentration of the standards and Fig. 1 shows a typical standard graph.

The extraction efficiency was measured by extracting the 10.0 μg/ml standard. After extraction, *p*-chlorodisopyramide was added, and the relative peak area was calculated and compared with the peak area of the same amounts of disopyramide

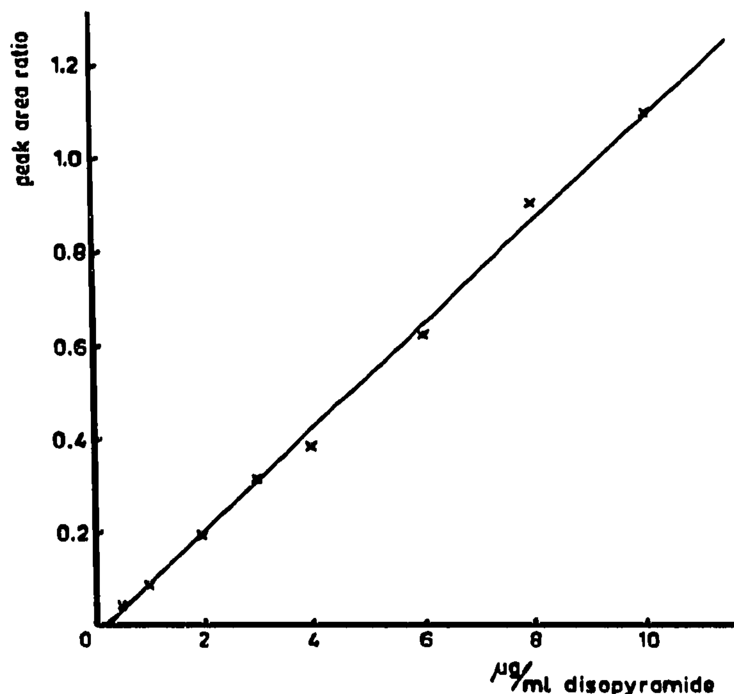

Fig. 1. Typical standard graph in which the peak area ratio of disopyramide to *p*-chlorodisopyramide is plotted against the concentration of disopyramide in serum.

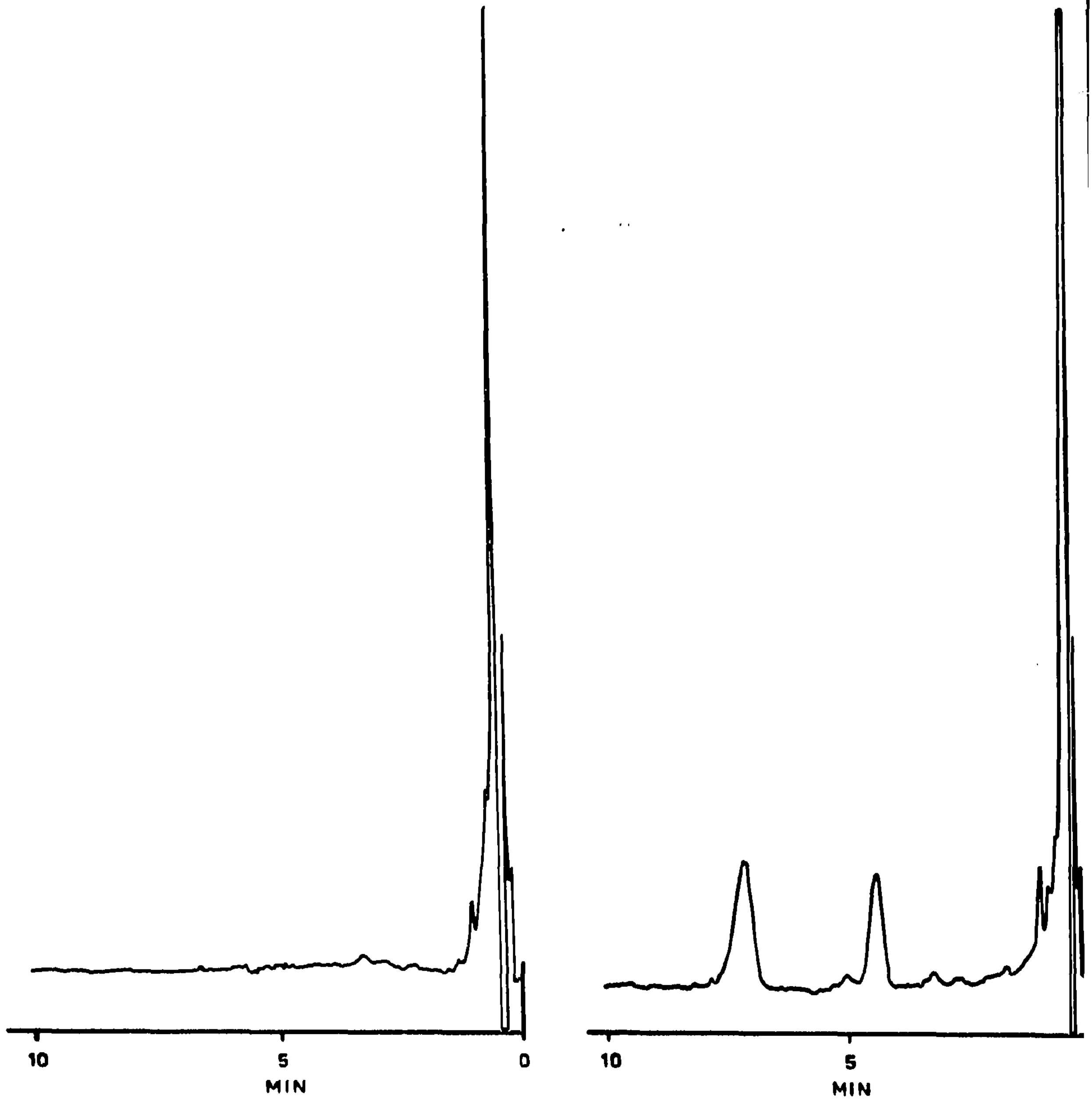

Fig. 2. Chromatogram of the blank serum extract. Attenuation: 512 × 1.

Fig. 3. Chromatogram of the standard containing 10.0 μg disopyramide per ml. Attenuation: 512 × 1.

and *p*-chlorodisopyramide. The extraction efficiency of disopyramide was 88.6% with a standard deviation of 2.7% ($n = 5$).

After the extraction of disopyramide and *p*-chlorodisopyramide together, comparison of the peak area ratio with the ratio of the known amounts of disopyramide and *p*-chlorodisopyramide showed (after extraction) a calculated amount of disopyramide of 102.5% with a standard deviation of 2.0% ($n = 5$).

These results indicate that in this assay *p*-chlorodisopyramide is a good internal standard to compensate for the extraction efficiency of disopyramide. Using a flame ionization detector there is a serum peak in the chromatogram that interferes with the peak of *p*-chlorodisopyramide. When using the nitrogen detector, this serum peak is not detected. The high sensitivity and selectivity of the nitrogen detector results in a very small solvent peak and a nearly horizontal base line when a blank serum extract is injected (see Fig. 2). A time-consuming clean-up procedure is not necessary when using the nitrogen detector (see Fig. 3).

ACKNOWLEDGEMENT

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REFERENCE

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