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Note

Gas chromatographic analysis of disopyramide

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Several methods for the determination of disopyramide have appeared in the literature. Fluorimetric analysis[1], gas–liquid chromatography with flame ionization[2,3] or nitrogen-specific detector[4], and high-pressure liquid chromatography[5] have been used.

We required a rapid, reliable method which could be accomplished using a gas chromatograph with a flame ionization detector. Previously reported methods using this system required a lengthy extraction scheme[3] or lacked an internal standard[2].

EXPERIMENTAL

Apparatus

The gas chromatograph used was a Varian Model 1200 equipped with a flame ionization detector (Varian, Palo Alto, Calif., U.S.A.). The column was glass, 60 cm × 2 mm I.D., treated with dimethyldichlorosilane and packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q (Ohio Valley Specialty Chemical, Marietta, Ohio, U.S.A.). Column temperature was 260°. Injector and detector temperatures were 300°. Carrier gas was nitrogen at a flow-rate of 30 ml/min. Oxygen at 200 ml/min and hydrogen at 15 ml/min were supplied to the detector. Extractions were carried out with the aid of a Vortex-genie mixer (Scientific Industries, Springfield, Mass., U.S.A.).

Extraction procedure

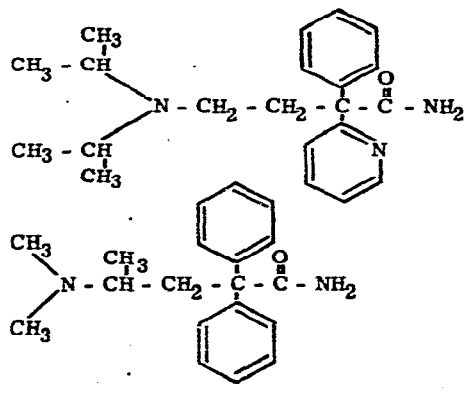
An extraction scheme similar to that investigated by Foerster and Mason[6] was used. Two ml of blood and 0.5 ml of a solution of 25 μg/ml aminopentamide (Bristol Laboratories, Syracuse, N.Y., U.S.A.) were pipetted into a 14 × 125 mm glass tube equipped with a PTFE-lined screw cap. After the addition of 0.2 ml of concentrated NH_4OH and 5 ml of *n*-butyl chloride (Fisher reagent grade; Fisher Scientific, Fair Lawn, N.J., U.S.A.), the contents of the tube were gently shaken by hand for 2 min. Following centrifugation, the upper *n*-butyl chloride phase was transferred with a disposable pipet to a second 14 × 125 mm tube containing 3 ml of 0.2 *N* HCl. The tube was agitated for 1 min with a vortex mixer and centrifuged. The upper organic layer was completely removed by aspiration and discarded. The

HCl solution was transferred to a 5-ml conical glass-stoppered tube and made alkaline with 0.6 ml of concentrated NH_4OH. Reagent grade chloroform was added (50 μl) and the tube was agitated with a vortex mixer for 1 min. The tube was centrifuged and 3 μl of the lower chloroform phase were injected into the chromatograph.

Therapeutic blood concentrations of disopyramide vary between 2 and 8 μg/ml[7]. Standards were prepared in blood at 1, 3, 5, 8 and 10 μg/ml and extracted as described above. Peaks were quantified on the basis of peak height. A linear relationship between the concentration of disopyramide and the peak height ratio disopyramide:internal standard was observed.

RESULTS AND DISCUSSION

Previous chromatographic methods for disopyramide used *p*-chlorodisopyramide as the internal standard[3–5]. We were unable to obtain this compound and, therefore, used aminopentamide, a drug no longer used therapeutically in the United States. The structures of aminopentamide and disopyramide are similar, as shown in Fig. 1. Aminopentamide eluted from the column immediately prior to disopyramide but was separated completely from it (Fig. 2).



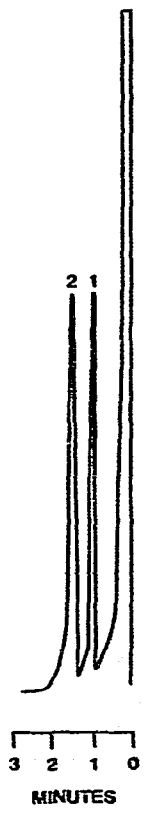


Fig. 1. Structural formulas for disopyramide (upper) and aminopentamide (lower).

Fig. 2. Gas–liquid chromatogram of an extract from blood of aminopentamide (1) and disopyramide (2). Attenuation: 256 × 1.

In our initial experiments, blood was extracted with *n*-butyl chloride, the solvent was concentrated and an aliquot was injected into the chromatograph. This simplified extraction did not result in any extraneous peaks at the retention times of interest. It was necessary, however, to wait for the elution of a large peak with a retention time of 8.4 min. Using the described method with extraction into HCl completely eliminated this late peak, allowing injections to be made at 3-min intervals.

Accuracy and precision were estimated at disopyramide concentrations of 5 and 10 μg/ml. Ten analyses carried out on the same day on a sample containing 10 μg/ml of disopyramide resulted in an average concentration found of 10.3 μg/ml with a standard deviation of 0.2 and a coefficient of variation of 2. Extraction at a concentrations of 5 μg/ml yielded an average concentration found of 5.6 μg/ml, a standard deviation of 0.3 and a coefficient of variation of 5.

Potential for interference from 16 drugs was tested by extracting aqueous solutions of 10 μg/ml of the substances. This concentration exceeds the reported therapeutic concentrations of these drugs in blood[8]. Drugs found not to interfere were quinidine, procainamide, lidocaine, propranolol, α-methyldopa, clonidine, guanethidine, acetaminophen, chlordiazepoxide, codeine, meperidine, pentazocine, propoxyphene, coumadin, and dicoumarol. Possible interference from the metabolites of these drugs was not investigated. Diazepam was found to have a retention time identical to disopyramide. Chronic therapy with diazepam usually does not result in a blood level greater than 1 μg/ml[8]. At this concentration, diazepam was found to give a false concentration of disopyramide of 0.4 μg/ml. Thus, interference from diazepam would be minimal under normal circumstances.

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