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GAS–LIQUID CHROMATOGRAPHIC METHOD FOR THE ROUTINE ESTIMATION OF DISOPYRAMIDE IN PLASMA OR SERUM

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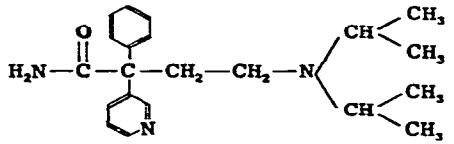
SUMMARY

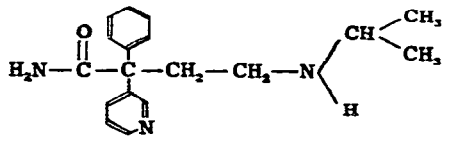
A rapid, sensitive and specific gas–liquid chromatographic method is presented for the routine monitoring of plasma concentrations of the anti-arrhythmic compound, disopyramide. The procedure involves extraction of the drug from alkaline plasma into ether, purification of the extract and gas chromatographic analysis using OV-101 liquid phase and flame ionization detection. The results demonstrate the accuracy and reproducibility of the method. Contrary to a previous report, it has been shown that delay in separating plasma from erythrocytes does not affect the disopyramide level in plasma.

INTRODUCTION

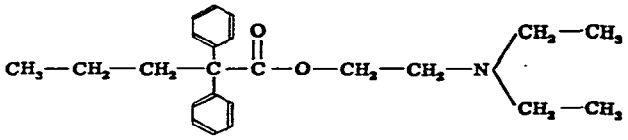
Disopyramide [4-diisopropylamine-2-phenyl-2-(2-pyridyl)butyramide] is an anti-arrhythmic drug effective in the treatment of a broad spectrum of atrial and ventricular arrhythmias[1]. Its action is similar to that of quinidine, but disopyramide is usually better tolerated and its use has not been associated with toxic blood reactions or sudden death[2,3].



disopyramide



N-mono-dealkylated disopyramide



SKF 525A

In healthy adult males disopyramide has a plasma half-life of *ca.* 6 h, and a large proportion (56%) of the dose is excreted unchanged in the urine within 48 h. A small proportion is eliminated as the secondary amine metabolite, N-monodealkylated disopyramide[4,5]. However, in patients with cardiac disease renal function is often impaired, resulting in a prolonged half-life[6].

The suggested therapeutic plasma concentration of the drug is in the range 1.5–6 μg/ml, and high levels are often associated with side-effects, such as a dry mouth, blurred vision, urinary hesitancy and impotence[6–8].

Naylor[9] has shown that disopyramide has a dose-dependent negative inotropic effect in cardiac muscle of several species. To ensure effective therapy and minimize side-effects there is a need to monitor plasma levels. This particularly applies to three groups of patients: those with myocardial infarction where ventricular function is depressed, those receiving disopyramide for the long-term prevention of arrhythmias and those with impaired renal function. This paper describes a method suitable for the routine estimation of disopyramide plasma levels.

EXPERIMENTAL

Materials

Disopyramide and SKF 525A were supplied by Roussel Labs. (Wembley Park, Great Britain) and Smith, Kline & French Labs. (Welwyn Garden City, Great Britain), respectively. All reagents were of 'Analar' grade, purchased from May & Baker (Dagenham, Great Britain). The diethyl ether used in the final extraction was distilled prior to use.

Extraction procedure

A 1-ml sample of plasma or serum is placed into a 10-ml glass tube and 100 μl of aqueous internal standard solution (SKF 525A, 20 μg/ml), 50 μl of 0.88 ammonia and 9 ml of ether are added. The tube is capped, and shaken for 5 min on a wrist action shaker. After centrifugation, the organic layer is removed to a fresh tube containing 1 ml of 0.1 *M* hydrochloric acid, and shaken for 5 min. After further centrifugation, the organic layer is discarded. To the remaining aqueous layer 100 μl of 0.88 ammonia and 5 ml of distilled ether are added, and shaken for 5 min. After centrifugation, the ether is removed to a pointed tube and evaporated to dryness in a water bath at 55°. The residue is dissolved in 50 μl of chloroform, and a sample of 1–5 μl is injected into the gas chromatograph.

Chromatography

A Varian Model 2440 gas chromatograph equipped with a flame ionization detector and a Pyrex glass column (1.8 × 0.006 m I.D.) packed with OV-101 (3% w/w) on Gas-Chrom Q support (80–100 mesh) are used. The operating conditions

are as follows: oven temperature, 230°; injector temperature, 250°; detector temperature, 270°; nitrogen flow-rate, 60 ml/min.

Calibration

A calibration graph is obtained by adding known amounts of disopyramide to blank human plasma and extracting as described above. Fig. 1 shows a typical graph from a set of such standards. A plot of the peak height ratio of disopyramide to the internal standard against plasma concentrations in the range 0.5–20 μg/ml is a straight line passing through the origin.

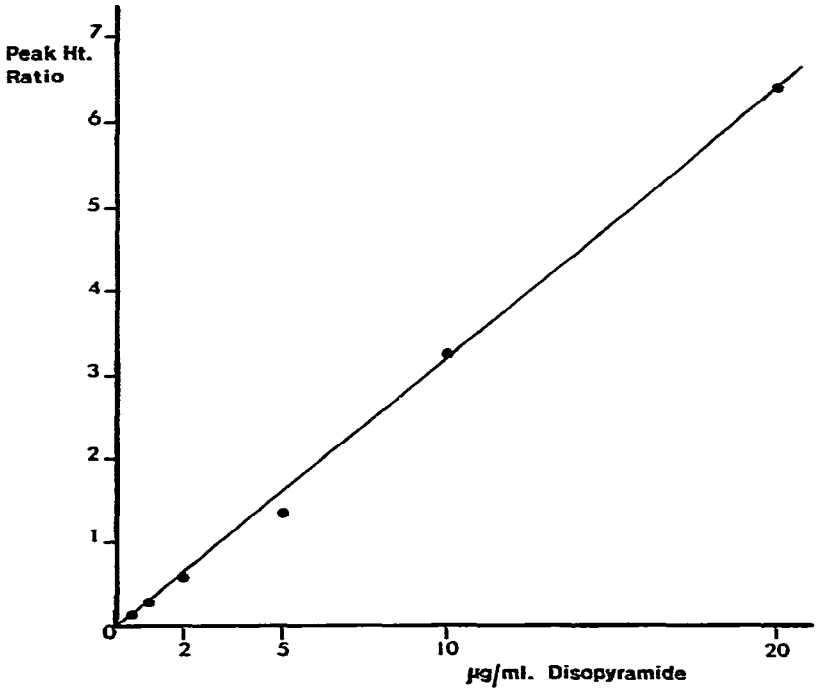


Fig. 1. A typical calibration graph obtained under routine analytical conditions. The peak height ratio of disopyramide to SKF 525A is plotted against the concentration of disopyramide in plasma. Linear regression of this line using the method of least squares yields a correlation coefficient of 0.996.

RESULTS

Under the conditions described, SKF 525A and disopyramide gave symmetrical peaks with retention times of 3.6 and 5.8 min, respectively. Fig. 2 is a representative chromatogram (N-mono-dealkylated disopyramide has a retention time of 2.4 min). There were no interfering peaks from plasma.

Analysis of replica samples spiked with disopyramide in the range 0.5–20 μg/ml produced results with coefficients of variation of less than 4% (Table I).

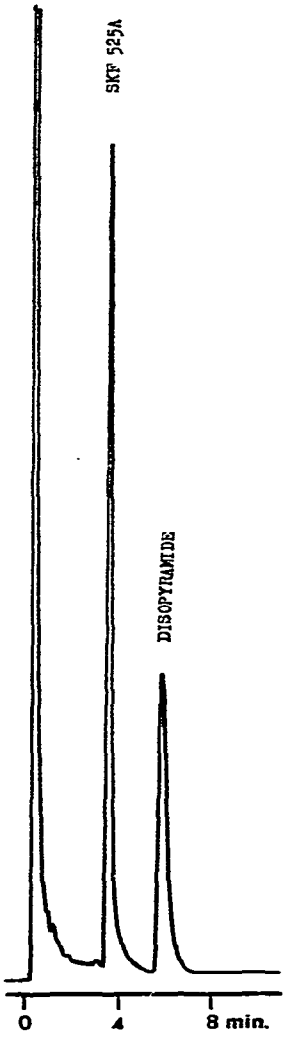


Fig. 2. A gas–liquid chromatogram obtained from a plasma sample taken from a patient receiving 100 mg Rythmodan (disopyramide) every 6 h. The peak represents a disopyramide plasma level of 1.1 μg/ml immediately prior to a dose.

TABLE I

ANALYSIS OF SPIKED REPLICA SAMPLES OF DISOPYRAMIDE IN HUMAN PLASMA

$n = 10$ for each concentration.

Expected value (μg/ml)	*Assayed value (μg/ml)*	*Standard deviation*	*Coefficient of variation*
0.5	0.51	±0.02	±3.9%
1	1.00	±0.03	±3.0%
2	2.01	±0.07	±3.5%
5	4.97	±0.18	±3.6%
10	10.03	±0.28	±2.8%
20	20.13	±0.43	±2.1%

It has been suggested by Daniel and Subramanian[10] that after blood has been taken, disopyramide is progressively bound to red blood cells thus giving artificially low plasma levels in samples where plasma and erythrocytes are not separated within a short period. On two separate occasions we examined this hypothesis by drawing 100 ml of blood from a volunteer who had taken 400 mg of disopyramide (four Rythmodan® capsules). The blood was immediately decanted into nine identical lithium heparin tubes. At various times between 5 min and 48 h, tubes were centrifuged and plasma removed for analysis. The results showed that there was no decrease in plasma disopyramide with time (Table II).

TABLE II

EFFECT OF STORAGE ON THE RECOVERY OF DISOPYRAMIDE FROM HUMAN BLOOD

The results are from two separate 100-ml blood samples (I and II). Aliquots were separated at the given time and assayed for disopyramide.

Time	*Recovery (%)*	
	I	*II*
5 min	100*	100**
10 min	95	97
15 min	93	102
30 min	97	95
1 h	105	98
2 h	106	102
4 h	101	102
24 h	103	97
48 h	100	106

* Initial level 3.4 μg/ml.
** Initial level 2.5 μg/ml.

DISCUSSION

Although several methods already exist for measuring disopyramide in plasma none has proved suitable for routine therapeutic monitoring.

Ranney *et al.*[5] first quantitated disopyramide using a fluorescence technique, but this is subject to interferences and fails to differentiate between disopyramide and N-mono-dealkylated disopyramide. Hutsell and Stachelski[11] described a method that permits the estimation of both disopyramide and its major metabolite, but its complexity precludes routine use. The procedure outlined by Duchateau *et al.*[12], although rapid, achieves specificity by relying on the availability of a selective nitrogen detector. Because of the absence of either an internal or external standard, the technique of Daniel and Subramanian[10] gives rise to inconsistent results in our laboratory. The high-performance liquid chromatographic determination described by Meffin *et al.*[13] is rapid, selective and accurate, but the equipment used is not widely enough available for routine application.

In our experience no drug which has been administered concurrently with disopyramide has been found to interfere with the assay, including: clofibrate, diazepam, digoxin, frusemide, salbutamol and warfarin. Other compounds which

have been tested for interference are: mexiletine, lignocaine, procainamide, N-acetyl procainamide, quinidine and verapamil. As it is impossible to exclude all possible therapeutic agents, however, a plasma sample should be obtained from the patient for analysis prior to the first dose of disopyramide.

In over a year of regular use only one compound has been encountered which interferes with the assay, *viz.* di-2-ethylhexyl phthalate. This plasticizer is present in small amounts in the diethyl ether used and in relatively large quantities in human blood stored in PVC transfusion bags. Distillation of the ether and the use of "blank" plasma stored in glass or rigid plastic containers eliminated the problem. This compound is also found in the flexible plastic tubing used in cardiac by-pass procedures, and Jaeger and Rubin[14] have suggested that there is a risk that it may contaminate the blood of patients undergoing open-heart surgery. We would therefore recommend caution in interpreting disopyramide levels obtained in these patients and in those who have recently received blood transfusion.

The routine monitoring of plasma drug levels requires methods that are specific, sensitive, rapid, reliable and inexpensive. The method outlined for disopyramide satisfies these criteria and has proved its worth in clinical use.

ACKNOWLEDGEMENTS

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