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Note

Simplified method for the measurement of disopyramide in plasma

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Although several assay procedures are reported[1–8] for measurement of the antiarrhythmic agent, disopyramide, most present methodologic drawbacks such as long analytical time, special detection system, column instability or interference from other therapeutic agents in plasma samples. We wish to present a gas chromatographic (GC) assay which is relatively rapid (approximately 3 h), sensitive (<0.1 μg/ml), does not present interference from commonly used therapeutic agents and employs a flame-ionization detector (FID).

EXPERIMENTAL

Materials

Disopyramide phosphate (Norpace) and SC13068 (*p*-chlorodisopyramide) were supplied by G. D. Searle & Co. (Chicago, Ill., U.S.A.).

Extraction

All test tubes were rinsed with diethyl ether prior to use. Plasma standards of disopyramide phosphate were made in concentrations of 0.5, 1, 2, 5 μg base/ml. To 2 ml of plasma sample or standard in a 10-ml screw-top tube was added 100 μl internal standard (SC 13068, 125 μg/ml in 0.01 *N* hydrochloric acid), 500 μl 2 *N* sodium hydroxide solution and 5 ml diethyl ether. The tube was capped and shaken for 10 min on a wrist action shaker and centrifuged for 5 min at 700 *g*. The organic layer was transferred to a fresh tube and another 5 ml ether was added to the aqueous phase, the extraction was repeated and the two organic extracts pooled. To this was added 2 ml 0.1 *N* sulfuric acid, the tubes were capped, shaken for 10 min and centrifuged for 5 min. The organic phase was aspirated and the aqueous phase transferred to a conical tube with ground glass stopper. To the aqueous phase was added 1 ml 2 *N* sodium hydroxide solution and 1 ml chloroform. The tube was vortexed for 25 sec and centrifuged for 5 min. The top aqueous layer was then aspirated and the organic layer evaporated to dryness under air. The residue was redissolved in 15 μl methylene chloride and 3–5 μl were injected on to the column.

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Chromatography

A Shimadzu Model GC-3B gas chromatograph equipped with an FID was used. A glass column (1.7 m × 2.6 mm I.D.) was packed with 3% UCW 98 (Applied Science Labs., State College, Pa., U.S.A.) on Gas-Chrom Q (100–120 mesh). The operating conditions were: oven temperature 210°; injector temperature approximately 230°; nitrogen flow-rate, 100 ml/min; air, 80 ml/min; hydrogen, 50 ml/min; sensitivity 10^3; range 32.

RESULTS

Under the assay conditions described above, the retention times of disopyramide and the internal standard were 4.5 and 7.4 min, respectively. A calibration curve was obtained by plotting peak height ratio (disopyramide:internal standard) against plasma concentration (expressed as the base). Fig. 1 shows representative chromatograms. Six calibration curves were run with quadruplicate points. The regression coefficient was 0.996 ± 0.003 (S.D.) and the slope was 0.308 ± 0.11 with an x intercept of 0.075 ± 0.056.

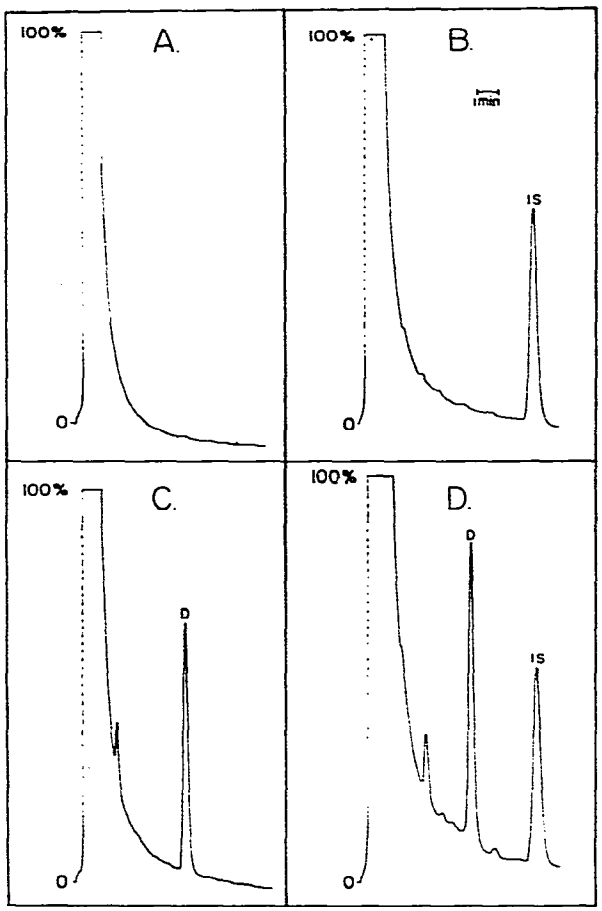

Fig. 1. Chromatograms of A, plasma blank; B, internal standard (IS); C, disopyramide (D); and D internal standard and disopyramide.

Accuracy and precision

Quadruplicate plasma samples, spiked with disopyramide at concentrations unknown to the investigators were analysed and the results are presented in Table I.

TABLE I

SPIKED REPLICATES OF DISOPYRAMIDE BASE IN HUMAN PLASMA

$n = 4$ for each concentration.

Expected value (μg/ml)	*Assayed value (μg/ml)*	*Standard deviation*	*Coefficient of variancy (%)*
0.1	0.13	0.013	10
0.2	0.20	0.019	9.7
0.25	0.24	0.030	13
0.3	0.30	0.048	16
0.88	1.04	0.074	7.1
0.88	1.01	0.08	7.9
1.40	1.44	0.10	6.9
1.45	1.43	0.13	9.0
1.50	1.50	0.072	4.8
2.00	2.11	0.18	8.8
2.75	2.88	0.27	9.3
3.25	3.07	0.20	6.6
4.00	4.14	0.59	14
4.25	4.19	0.42	9.9
7.50	8.03	0.21	2.6

Specificity

The commonly used antiarrhythmics were tested for interference with our disopyramide assay. Lidocaine, quinidine, procainamide and N-actyl procainamide (all 10 μg/ml) and propranolol (250 ng/ml) were examined by spiking human plasma with these agents. None was found to produce interference. Valium, a drug reported to interfere with at least one other assay[7], did not interfere. In addition, plasma samples from patients on drugs such as nitroglycerin, thorazine, nafcillin, aminoglycosides (tobramycin and gentamycin), and bretylium have been tried without interference.

Sensitivity

Quadruplicate plasma samples spiked with 0.1, 0.2 and 0.3 μg/ml were assayed and results were analysed using the paired-t test. 0.1 μg/ml was found to be significantly different from 0.2 μg/ml ($p < 0.0005$) and 0.2 μg/ml was found to be significantly different from 0.3 μg/ml ($p < 0.01$). The accuracy and precision at these lower limits may be found in Table I.

DISCUSSION

There are currently a number of assays published with various drawbacks. The spectrophotofluorometric assay[1] for disopyramide does not distinguish between

the drug and its mono-N-dealkylated metabolite. High-pressure liquid chromatography[2] and GC assays using a nitrogen detector[3] have the disadvantage of requiring equipment not yet routinely found in laboratories. Of the GC–FID assays, that of Hutsell and Stachelski[4] requires a long extraction with a long retention time. That of Johnston and McHaffie[5] requires ether distillation while the assay of Hayler and Flanagan[6] causes rapid column decomposition which can be retarded only by frequent injections with a column silylating agent. Doedens and Forney[7], in publishing a GC–FID assay, found that diazepam interferes and had a reported accuracy below that of other assays. That of Daniel and Subramanian[8] requires large solvent volumes and the time consuming process of drying down a large solvent volume.

In developing this assay we initially found an interfering peak directly under the disopyramide peak and further investigation showed the origin of the contaminant to be from the glass tubes being used. Our usual procedure was to wash tubes in Harleco dichromate cleaning solution followed by distilled water rinses but a final ether rinse eliminated the interfering peak.

It has been reported[5] that a plasticizer, di-2-ethylhexyl phthalate, found in polylvinyl chloride transfusion bags and in ether, interferes with some disopyramide assays. Using blood bank plasma from transfusion bags and using non-distilled ether we have found no such interfering peaks in our assay.

The 3% UCW 98 packing appears not be to subject to the rapid column deterioration reported by another assay[6]. Resolution on it appears good enough so that, if desired, one could consider cutting the retention time in half by increased oven temperature and/or carrier gas flow-rate.

In routine use of the present disopyramide assay we have found it to be simple and reliable while avoiding many of the shortcomings of other assays.

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