CASE REPORT

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Disopyramide (Norpace®) Distribution at Autopsy of an Overdose Case

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ABSTRACT: A 44-year-old female died as the result of an overdose of disopyramide. An analytical method was developed and the distribution of the drug in various tissues was determined. Analysis of the blood sample indicated a drug concentration far exceeding that of the therapeutic concentration.

KEY WORDS: toxicology, disopyramide, death

Norpace® is the Searle Laboratories product name for disopyramide phosphate, a relatively new drug used in the treatment of cardiac arrhythmias, with pharmacological action similar to quinidine. A search of the literature revealed only one fatal case with disopyramide, and it involved a child [*1*].

Case History

A 44-year-old female called several friends and informed them of her intent to commit suicide by the ingestion of diazepam and disopyramide. A rescue squad was dispatched to her residence from which she was transported to the nearest medical facility. Upon arrival, emergency treatment with syrup of ipecac and activated charcoal and close monitoring of the patient were initiated. The patient suddenly went into cardiopulmonary arrest. All attempts at resuscitation failed, and the patient was pronounced dead.

Pathology

Autopsy revealed an obese female with generalized vascular disease, visceral congestion, pulmonary edema, gastric and duodenal mucosal petechiae, hepatomegaly, and a distended gall bladder containing 60 cm^3 of bile.

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Methods and Materials

Disopyramide, mono-*N*-dealkylated metabolite, and a chlorinated derivative of disopyramide that served as the internal standard for quantitative analysis (Fig. 1) were supplied by Searle Laboratories, Chicago.

The methylene chloride, methanol, ethyl acetate, and chloroform were "distilled in glass"-grade reagents from Burdick and Jackson, Muskegan, Mich. All other chemicals were standard reagent-grade materials.

Gas chromatography for both flame ionization detectors and gas chromatography/mass spectrometry (GC/MS) was performed on a Hewlett-Packard 5711-A gas chromatograph using a 1.5-m (15-ft) glass column, 6.35-mm (¼-in.) outside diameter by 2-mm inside diameter, packed with 3% OV-101 on Supelcoport, 100-120 mesh. The GC/MS was performed with a Hewlett-Packard 5930-A mass spectrometer. Ultraviolet spectroscopy was performed with a Varian 635 spectrophotometer. Liquid chromatography was performed with a system composed of an Altex Model 110 pump and an Altex Model 153 fixed wavelength detector. The column was 30-cm by 4.6-mm inside diameter stainless steel packed in-house by the authors with Vydac TP 101 absorbent. Thin-layer chromatographic (TLC) data were obtained on 20- by 20-cm plates of silica gel 60.

The initial drug screen on the blood specimen was that of Foerster and Mason [*2*], and it produced the ultraviolet spectrum of Fig. 2. Gas chromatographic analysis of the extract produced a peak with a retention time of 1.38 relative to codeine at 240°C. The electron impact and chemical ionization mass spectra of the compound are shown in Figs. 3 and 4, respectively. All of these data are identical to those of standard disopyramide.

The urine drug screen was performed in two TLC systems. System 1 consisted of ethyl acetate/methanol/ammonium hydroxide (85:10:5) and System 2, methanol/ammonium hydroxide (100:1.5). In each system two iodoplatinate-positive spots were observed; the R_f values of System 1 were 0.61 and 0.31 and of System 2, 0.53 and 0.44. The spot with the high R_f value in each system corresponds to that of disopyramide and the lower spot to that of mono-*N*-dealkylated disopyramide. The presence of disopyramide in the urine was confirmed by GC retention time and GC/MS. Attempts to gas chromatograph pure standard of the dealkylated metabolite were unsuccessful because of thermal decomposition.

Published methods for disopyramide quantitation include a fluorescence method [*3*], gas chromatographic methods [*4,5*], and a liquid chromatographic method [*6*]. The fluorescence method is not specific for disopyramide and was eliminated from consideration. The gas chromatographic methods were not used because they failed to measure the metabolite [*4*] or required excessive manipulations [*5*]. The liquid chromatographic method [*6*] is an acceptable and reliable method but it uses reversed phase ion-paired chromatography, which makes collection and identification by mass spectroscopy difficult; therefore, a normal phase chromatographic method was developed for these compounds. A mobile phase consisting of 85:15:0.5 methylene chloride/methanol/

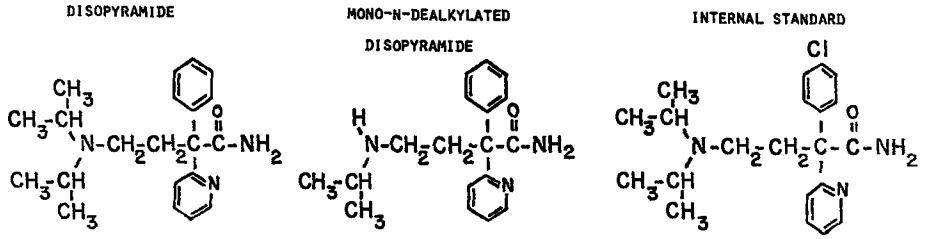

FIG. 1—*Structures of disopyramide, mono-*N*-dealkylated disopyramide, and internal standard.*

ammonium hydroxide, with the Vydac column and a flow of 1 ml/min, produced acceptable chromatography of the pure standards.

The body fluids (blood and bile) were quantitated by adding 1 ml of sample to 1 ml of internal standard (2 μg/ml) and 0.5 ml of 6*N* sodium hydroxide and extracting with 10 ml of chloroform. After the aqueous layer was aspirated, the chloroform layer was filtered through phase-separating paper into a clean tube and evaporated. The residue was reconstituted in 30 μl of mobile phase and 7 μl was injected into the liquid chromatograph

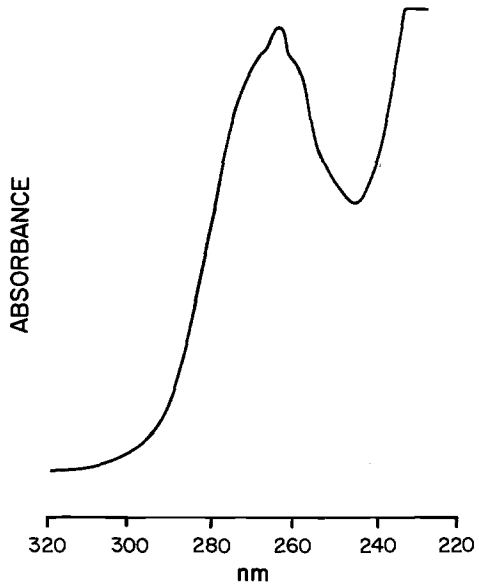

FIG. 2—*Ultraviolet spectrum (in 1*N *sulfuric acid) of disopyramide extracted from blood.*

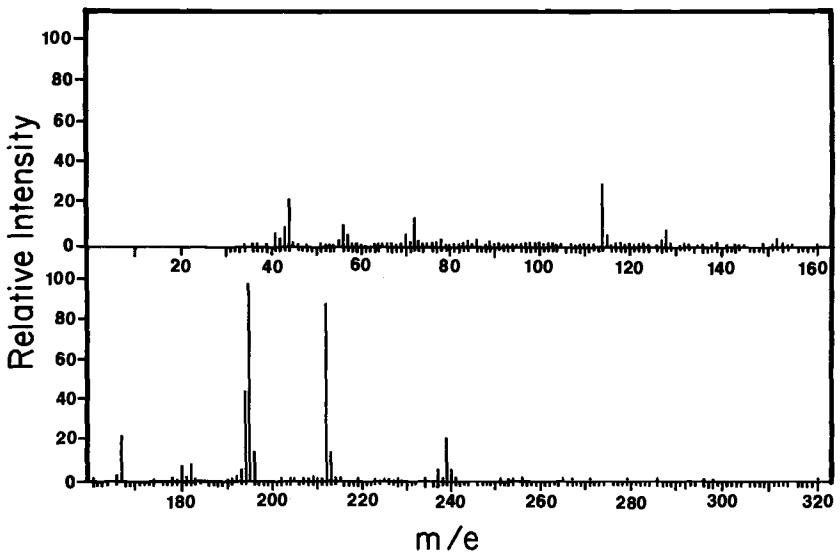

FIG. 3—*Mass spectrum of disopyramide by electron impact.*

with a detector setting of 0.02 absorbance units, full scale. Calibration curves were prepared and were linear over the range of 0.5 to 15 μg/ml for both disopyramide and its metabolite. Blood and bile specimens were diluted to fall within the linear range of the method. Figure 5 illustrates the chromatogram of the blood extract from this case.

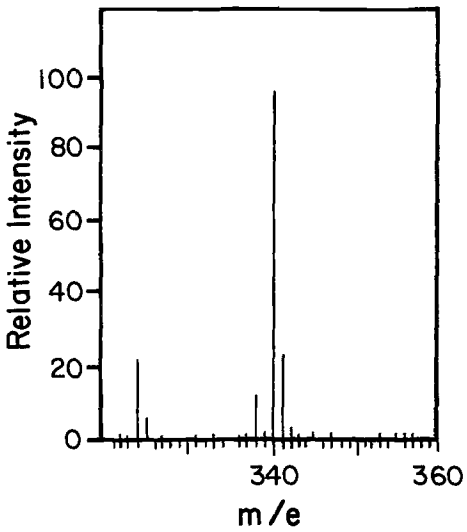

FIG. 4—*Mass spectrum of disopyramide by chemical ionization.*

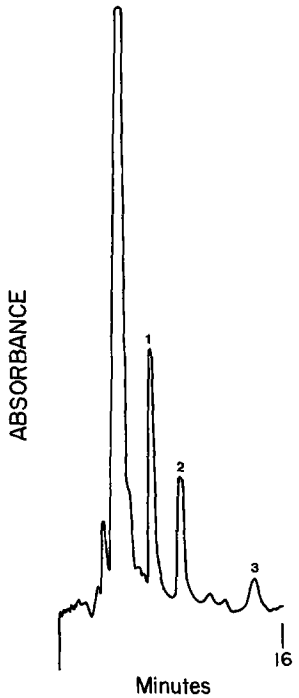

FIG. 5—*Blood extract (diluted 1:10) containing 2.66 mg disopyramide/dl by liquid chromatography.*

Analysis of drug-free blood samples indicated no peaks corresponding to the retention times of the three peaks of interest. Tissue samples were analyzed by a similar procedure that also produced no interfering peaks. A 5-ml aliquot of a 1:9 tissue slurry along with 70 μg of the internal standard was extracted with 40 ml of chloroform/isobutanol 80:20. The organic layer was filtered through phase-separating paper and extracted with 5 ml of 1*N* sulfuric acid. After a cyclohexane wash, 1 ml of the acid layer was made basic with 6*N* sodium hydroxide and extracted with 10 ml of chloroform. The chloroform was evaporated, the residue reconstituted with 30 μl of mobile phase, and 7 μl injected into the liquid chromatograph. Figure 6 presents the chromatogram of the liver extract from this case.

Linear calibration curves, similar to those of blood, were prepared by using the drug-free tissues. The quantitative results of these analyses are summarized in Table 1.

The eluting peaks from the liquid chromatographic separation of the blood and tissue samples were collected and analyzed by using the direct insertion probe of the mass spectrometer. Peak 2 on the liquid chromatogram gave electron impact and chemical ionization mass spectra identical to standard disopyramide (see Figs. 3 and 4). Peak 3

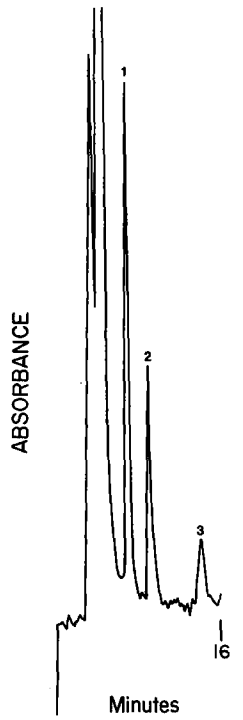

FIG. 6—*Liver extract containing 3.58 mg disopyramide/dl by liquid chromatography.*

TABLE 1—*Tissue concentrations.*

Sample	Disopyramide, mg/dl	Mono-*N*-Dealkylated Disopyramide
Blood	2.66	0.59
Liver	3.58	1.12
Kidney	14.7	0.67
Bile	34.9	14.5

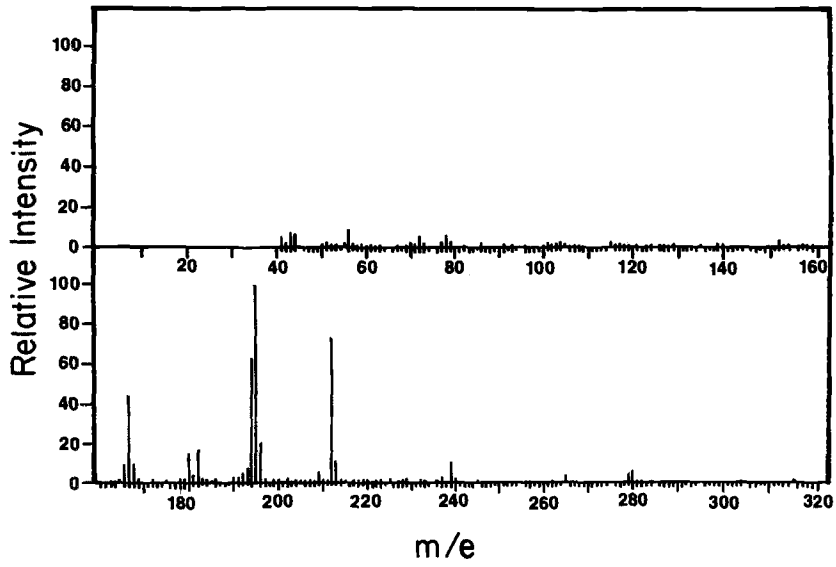

FIG. 7—*Mass spectrum of mono-*N*-dealkylated disopyramide by electron impact.*

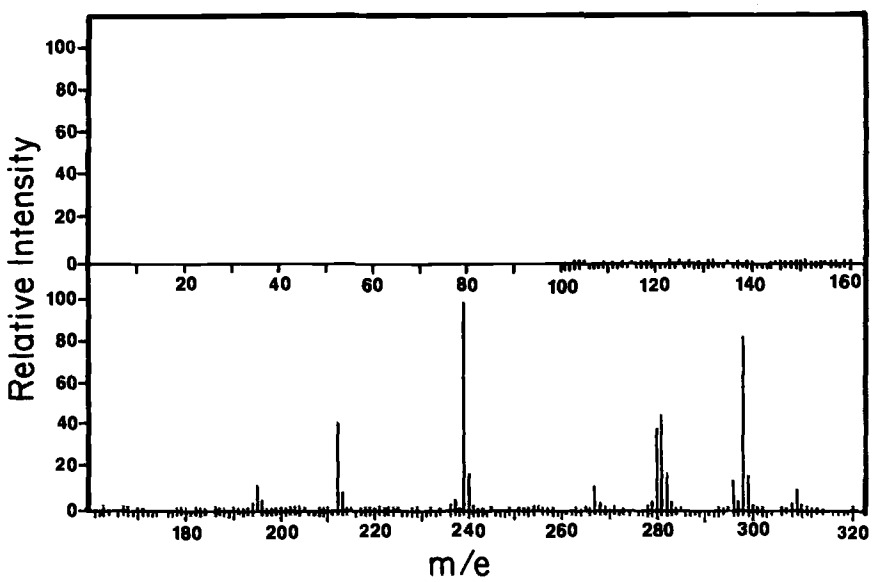

FIG. 8—*Mass spectrum of mono-*N*-dealkylated disopyramide by chemical ionization.*

gave the electron impact and chemical ionization spectra represented by Figs. 7 and 8. These spectra are identical to known standard mono-*N*-dealkylated disopyramide.

Discussion

The blood concentration of disopyramide in this case was 26.6 μg/ml, which is many times the reported normal therapeutic level of the drug (2 to 4 μg/ml) [*7,8*]. Toxicity is

estimated to occur at 9 μg/ml or above [7,9]. Although diazepam was indicated in the history, none was found, nor were any other drugs or alcohol. Based on history and laboratory findings the cause of death was determined to be cardiopulmonary arrest caused by ingestion of disopyramide.

The procedures developed for these determinations were very reproducible with no deterioration of the liquid chromatographic column even though 0.5% ammonium hydroxide was employed. The column used in this case has been in continuous use for six months with no decline in performance. This longevity is attributed to the practice of pumping 30 to 50 ml of methanol through the column each night before shutdown, which serves the purpose of removing ammonium hydroxide from the column and leaving the packing in a state that makes reproducibility of the liquid chromatographic conditions possible.

Tissue specimens, which were hydrolyzed prior to extraction, produced no significant difference in quantitative values from those specimens that were not hydrolyzed; therefore, hydrolysis offers no advantage.

Acknowledgments

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