Disopyramide is determined in serum by gas chromatography with a nitrogen-selective detector, by liquid chromatography, and by gas chromatography–mass spectrometry. Comparable results are obtained with the three techniques, with a within-run and between-run precision of 5 to 10% (coefficient of variation). Least-squares analysis of data on patients’ sera, analyzed first by gas chromatography (y) and then liquid chromatography (x), gave a slope of 1.12; y-intercept, −0.31; standard error of estimate, 0.46; and correlation coefficient, 0.94. Comparison of patients’ sera by gas chromatography (y) and then by gas chromatography–mass spectrometry (x) gave a slope of 0.94; y-intercept, 0.42; standard error of estimate, 0.38; and correlation coefficient, 0.97. Interferences observed when using one technique—for example, gas chromatography—can be eliminated by analyzing the sample extract with one of the other techniques.

Additional Keyphrases: intermethod comparison - antiarrhythmic drugs

In a previous communication (1) we reported a procedure for the determination of disopyramide (Norpace; G. D. Searle and Co., Chicago, IL 60680) in serum by use of gas chromatography (GC) and nitrogen-selective detection. Although ideal for the direct determination of total disopyramide, the procedure could not determine the desalkyl metabolite of disopyramide and suffered from interference by diazepam. A practical solution to such interference problems is to use an alternative separation technique, such as liquid chromatography (LC), or a different type of detector, such as a mass spectrometer. The reason for this approach is the likelihood that an interferent would behave the same way with all three techniques. Because patients receive such a variety of medications, we find that assay with more than one kind of technique is a useful approach to overcome drug interferences with analytical procedures commonly used in the clinical laboratory.

Materials and Methods

Apparatus

We used a Model 5840A gas chromatograph with dual nitrogen–phosphorus-sensitive detectors and coiled glass columns, 1.2 m × 2 mm i.d., packed with 2% SP-2250 on Chromosorb W-HP 100/120 mesh (Hewlett-Packard, Avondale, PA 19311).

A Model 6000-A solvent delivery system, Model 600 solvent programmer, Model 440 absorbance detector, Model 450 variable wavelength detector, Model U6K universal liquid injector (Waters Associates, Inc., Milford, MA 01757), and a dual-pen recorder (Houston Instrument, Austin, TX 78753) were used for liquid-chromatographic separations. We also used a prepacked μBondapack C18 (300 × 4 mm i.d.) column from Waters Associates.

Gas chromatography–mass spectrometry (GC-MS) was performed on a Model 5985A quadrupole system (Hewlett-Packard, Palo Alto, CA 94304) with selective ion monitoring and electron impact. The system consisted of a Hewlett-Packard 5840A gas chromatograph interfaced to the mass spectrometer. The glass column was 1.2 m × 2 mm i.d. packed with 2% SP-2250 Chromosorb W-HP, 100/120 mesh. The ionization energy was 112.14 × 10⁻¹⁹ J (70 eV), the electron-multiplier voltage was set at 3000 V, and the dwell time for each ion monitored was 100 ms.

Reagents

Heptane and isobutanol were analytical reagent (AR) grade. High-purity methanol was obtained from Burdick and Jackson, Muskegon, MI 49442.

Sodium hydroxide, 0.5 mol/L.

Sulfuric acid, 1 mol/L and 0.05 mol/L.

Sulfuric acid, 0.05 mol/L in methanol: dilute 5 mL of H₂SO₄, 1 mol/L, in 35 mL of H₂O, then dilute to 100 mL with methanol.

Octane sulfonic acid, 0.05 and 0.005 mol/L, was obtained from Eastman Organic Chemicals, Rochester, NY 14650.

LC mobile phase: octane sulfonic acid, 0.005 mol/L, in methanol. Dilute 50 mL of octane sulfonic acid, 0.05 mol/L, with 150 mL of H₂O and bring to 500 mL with methanol. Adjust pH to 3.5 with H₂SO₄, 0.05 mol/L in methanol.

Standards

Disopyramide phosphate, its mono-n-dealkylated metabolite, and p-chlorodisopyramide were obtained from Searle and Co.

Standards of disopyramide, its metabolite, and the internal standard p-chlorodisopyramide were prepared at 1 g/L in absolute ethanol or in HCl, 0.1 mol/L. Dilutions of the 1 g/L standards were made for the appropriate working standards. An injection standard, which contained 0.2 g/L of disopyramide, p-chlorodisopyramide, and n-desalkylisopyramide, was prepared in H₂SO₄, 0.05 mol/L in methanol, for LC.

Operating Conditions

For GC analysis, we used a column packed with 2% SP-2250 at 250 °C with a helium flow rate of 40 mL/min (1).

For LC, we used a C₁₈ reversed-phase column and a flow...
rate of 1.5 mL/min with a mixture of methanol/water (60/40 by vol) as the mobile phase.

For GC-MS, we used a 2% SP-2250 column programmed at 240 °C for 3.5 min, with the temperature then increasing to 260 °C at the rate of 20 °C/min. Masses were monitored at 195 and 212 for disopyramide and 229 and 246 for p-chlorodisopyramide.

Procedure

The procedure has been reported previously (1). Disopyramide, its mono-n-dealkylated metabolite, and p-chlorodisopyramide were extracted at a basic pH (4 mL of NaOH, 0.5 mol/L) into n-heptane/isobutanol (96/4 by vol), then extracted again into 4 mL of H₂SO₄ 1 mol/L. After the solution was made basic with NaOH, the drugs were extracted again into 10 mL of ether and evaporated.

For GC analysis we dissolved the residue in 25 μL of absolute ethanol and injected 1–2 μL for analysis. For MS the sample was diluted to 0.2 mL with methanol and 1 μL was injected for analysis. For “high-performance” liquid-chromatographic analysis, we dissolved the residue into 50 μL of methanol (600 mL/L) containing 0.05 mol of H₂SO₄ per liter, and injected 20 μL of this for analysis.

We used the ratios of the peak heights of disopyramide and its dealkylated metabolite to that of p-chlorodisopyramide (the internal standard) to calculate concentration. For GC-MS analysis, the ratio of the area counts at (m/e) 195/229 were used to calculate the disopyramide concentration.

Results

Liquid Chromatography

Chromatograms from LC of an unextracted standard mixture, a serum blank, a serum standard, and a patient’s serum are given in Figure 1. Blank sera assayed by this procedure showed no significant peaks that might interfere with the analysis. Retention times for the mono-n-dealkylated metabolite and disopyramide are 0.59 and 0.71 relative to that of p-chlorodisopyramide, which eluted at 7.85 min.

When peak heights of sera containing 2, 4, 6, and 8 mg of disopyramide per liter, determined in quadruplicate on the same day, were plotted vs concentration, the resulting line had a slope of 0.31, a y-intercept of −0.06, a standard error of estimate (Sₓᵧ) of 0.02, and a correlation coefficient (r) of 0.99. Within-run absolute percentages of analytical recovery, as determined with serum extracts (n = 4) containing 2, 4, 6, and 8 mg of disopyramide per liter, were 60 ± 7, 62 ± 2, 61 ± 4, and 64 ± 5%, respectively. Between-run absolute percentages of recovery with serum extracts containing 3, 5, and 7 mg of disopyramide per liter were 55 ± 5% (n = 11), 55 ± 7.5% (n = 9) and 52 ± 4% (n = 9), respectively.

The relative within-run percentages of recovery with serum extracts (n = 4 each) containing 2, 4, 6, and 8 mg of disopyramide per liter were 100 ± 7, 92 ± 2, 97 ± 4, and 100 ± 5%, respectively. Between-run relative percentages of recovery with serum extracts (n = 6 each) containing 3 and 7 mg of disopyramide per liter were 104 ± 11 and 99 ± 7%, respectively.

Within-run precision (coefficient of variation) of serum-based standards (n = 4 each) containing 2, 4, 6, and 8 mg of disopyramide per liter was 7, 2, 4, and 5%, respectively. Between-run precision with sera controls of 3 and 7 mg of disopyramide per liter was 11 (n = 26) and 7% (n = 6), respectively.

Extraction studies of the mono-n-desalkyl metabolite from serum (1 to 10 mg/L) gave a 24 ± 3% absolute recovery with a relative recovery of 100 ± 12%. Figure 2 illustrates serum extracts of the mono-n-desalkyl metabolite of disopyramide analyzed by LC in the presence and absence of disopyramide.

![Image](image_url)

Fig. 1. Liquid chromatograms of a mixture of pure unextracted standards, 0.2 g/L (I); a serum blank (II); a serum standard containing 5 mg of disopyramide per liter (III); and a patient’s serum with 5 mg of disopyramide per liter (IV)

The poor recovery of the metabolite relative to that of disopyramide is evident from Figure 2. Noninterference by serum constituents with the LC determination of the metabolite is also indicated in Figures 1 and 2.

Liquid and Gas Chromatography Compared

Sera from 30 patients were analyzed first by GC (y) and then by “high-performance” LC (x). The results are illustrated in Figure 3. Statistical analysis gave a slope of 1.12, a y-intercept of −0.31, Sₓᵧ = 0.46, and r = 0.94.

Gas Chromatography–Mass Spectrometry

The within-run relative percentages of analytical recovery for serum standards (n = 4) at 3 and 5 mg/L were 98 ± 16 and 104 ± 15%. Between-run recovery at 3 and 7 mg/L averaged 94 ± 8% (n = 4) and 101 ± 11% (n = 3), respectively. Serum blanks analyzed by GC–MS showed no interference with the ions being monitored. Between-run analysis of a 3 mg/L sample that was determined first by GC and then by GC–MS gave 106 ± 10 and 98 ± 9% recovery (n = 4), respectively. At a concentration of 7 mg/L, the percent of recovery by GC was 102 ± 10 and by GC–MS, 104 ± 10% (n = 6), respectively. A comparison of determinations of 15 patients' samples with GC (y) and the same with GC–MS (x) was graphed as a line with a slope of 0.94, a y-intercept of 0.42, Sₓᵧ = 0.38, and r = 0.97 (Figure 4).

Interference Studies

With GC on% OV-17 and 2% SP-2250 columns, diazepam has a retention time similar to that of disopyramide (I, 2).
This interference by diazepam can be eliminated by using packed columns with less-polar liquid phases, such as 2% OV-101 or OV-1 (3, 4). However, changing the column’s liquid phase does not really eliminate the diazepam interference in GC because the active metabolite of diazepam, nordiazepam, has a retention time similar to that of disopyramide on a 2% OV-101-type liquid phase. The active metabolite of chlorodialpoxide, norchlorodialpoxide, also interferes when less-polar liquid phases are used. Diazepam does not interfere with the GC–MS determination of disopyramide.

With this LC procedure, quinidine, methaqualone, and oxazepam interfere with the internal standard, p-chlorodisopyramide. Cinchonine interferes with disopyramide, and lidocaine interferes with mono-n-desalkyl disopyramide. The following drugs did not interfere with LC: chlorodialpoxide, diazepam, flurazepam, prazepam, propranolol, n-acetylprocainamide, sulfanilamide, loxapine, propoxyphene, methyprylon, and triamterene-hydrochlorothiazide (Dyazide; Smith Kline and French). Other drugs, which are not extracted at a basic pH and thus do not interfere, include barbiturates, glutethimide, phenytoin, and salicylates. Doxepin, amitriptyline, imipramine, and their metabolites, desmethyldoxepin, nortriptyline, and desipramine, did not interfere with the LC assay. A variable-wavelength LC detector at 260 nm gave results identical to those with the fixed 254-nm wavelength detector, which indicates that there were no interferences under the eluting LC peaks.

**Discussion**

Disopyramide is an anti-arrhythmic drug commonly assayed in the clinical laboratory. Pharmacokinetic studies indicate a large variation in the β-phase half-life of the drug among individuals on the same dosage schedule (5–7). In normal patients, the drug’s half life has been reported as 7.8 ± 1.6 h with the volume of distribution being 60 ± 20 L (5). The determination of disopyramide concentrations in blood is therefore important for optimum disopyramide therapeutic effect (1, 5).

GC, LC, and GC–MS can be used to determine disopyramide. With GC, the within-run and between-run precision (CV) is 4 and 7%, respectively, with a relative percent recovery...
of 100% from serum standards (1). LC, on the other hand, has a within-run CV of 5% and a between-run CV of 9% with nearly 100% recovery from serum standards. With GC–MS, the within-run and between-run CVs are approximately 10%. The three techniques give comparable analytical results, in the present procedure, with a CV of approximately 5–10%, and a sensitivity of less than 0.25 mg/L.

Comparisons of GC with LC and GC–MS methods gave correlation coefficients of 0.94 and 0.97, respectively, with comparable standard errors of estimate. GC, GC–MS, or LC can therefore be used for the routine laboratory determination of disopyramide with comparable results being obtained by each technique. The choice of which method to use for the determination of disopyramide should be determined on the basis of the particular analytical requirements, of parent drug vs metabolite, and of possible interferents that may be present. For example, LC has the advantage that the mono-dealkylated metabolite can be determined directly without derivatization; with GC or GC–MS, however, the nonderivatized metabolite decomposes on the column (1).

With the present procedure, the absolute recovery of the mono-dealkylated metabolite is only 24%. This is probably because the secondary amine that forms is not as well extracted as the tertiary amine of the parent drug. Thus, under normal circumstances, the parent drug and metabolite cannot be simultaneously determined in the same sample extract by use of a common internal standard. Because the metabolite concentration in serum is about one-tenth that of the parent drug, the metabolite would not be detected from the liquid chromatogram of a patient with a serum disopyramide concentration of 4 mg/L. The sample would have to be reanalyzed with an appropriate internal standard and detector sensitivity.

Parent drug and metabolite can be determined simultaneously if one uses two internal standards, for example, p-chlorodisopyramide and loxapine, at two different concentrations. Loxapine has a retention time about twice that of p-chlorodisopyramide. The following drugs tested did not interfere with loxapine: quinidine, propranolol, chlorzepoxide, flurazepam, diazepam, oxazepam, prazepam, doxepin, desmethyldoxepin, amitriptyline, and nortriptyline. Using the present LC procedure, one would first determine disopyramide, then change the detector sensitivity to determine the metabolite. Use of an acetate derivative for the determination of the mono-n-alkylated metabolite by GC has also been reported (8).

Interference studies with GC indicate that diazepam interferes with disopyramide when SP-2250 and OV-17 columns are used (1, 2) but not when less-polar OV-1 and OV-101 columns are used (3, 4). However, the diazepam metabolite nordiazepam interferes with disopyramide on the less-polar columns. It is therefore important in reporting interference studies to consider not only the parent drug but also active metabolites that attain a significant serum concentration.

Diazepam does not interfere with disopyramide by GC–MS on an SP-2250 column. Even though both drugs are coming off the column at the same time, the selectivity of the MS eliminates the diazepam interference. Because diazepam does not have the same mass fragments as disopyramide, i.e., m/e 195 and 212, it will therefore not interfere with disopyramide. By selectively tuning the instrument to look only at selected masses, interferences from other drugs can be eliminated. MS is therefore the ultimate selective detector for GC.

Quinidine, metaxaqualone, and oxazepam interfere with p-chlorodisopyramide, and lidocaine interferes with the mono-n-alkyl metabolite of disopyramide with LC. Thus the apparent great advantage of LC over GC (the direct metabolite determination by LC) is lost if any of these drugs are in the sample. GC with a nitrogen-selective detector therefore appears to have somewhat of an advantage over LC for the routine determination of disopyramide. An advantage of LC over GC has been reported to be the noninterference of n-acetylpromethazine with the internal standard p-chlorodisopyramide (9). Although the two have a similar retention time on a 2% SP-2250 column, N-acetylpromethazine is not extracted in this procedure and will therefore not interfere.

Because it is not affected by the above interferences, GC–MS would therefore appear to be the method of choice. However, the complexity, cost, and sophistication of the instrumentation make it unlikely that most laboratories will be able to have GC–MS for routine use. In addition, to use GC–MS as a substitute for GC or LC analysis in the mg/L range seems quite inappropriate, because simpler and less expensive techniques such as GC and LC give comparable results.

Procedures involving GC (1–4, 8), LC (9, 10), and thin-layer chromatography (11) have been reported for the determination of disopyramide. A reversed-phase ion-pair LC procedure with methanol/water (53/47 by vol) and heptane sulfonic acid as the ion-pairing reagent has been reported (10). The LC procedure we used here differs in having sodium octylsulfonate as the ion-pairing reagent with a methanol/water (60/40 by vol) eluting mixture. Although individual chromatographic procedures have been reported for the determination of disopyramide, a comprehensive comparative study of GC with nitrogen-selective detection, LC, and GC–MS has not been reported. To our knowledge, a GC–MS procedure with non-isotopically labeled disopyramide as internal standard for the determination of disopyramide has not been reported before this study. Use of nonisotopically labeled internal standards for GC–MS is a more practical and fruitful approach for the routine application of GC–MS in the clinical laboratory.

Interferences by drugs in procedures commonly used in the clinical laboratory is an ever-present problem we all confront every day. Ultimately, no simple analytical procedure will exhibit absolute specificity for every patient’s sample. A sound approach for dealing with analytical interferences in drug assays is to set up a scheme whereby at the end of the sample preparation the analysis can be performed by different chromatographic techniques, GC, LC, or GC–MS. Even though each chromatographic technique will have its specific interferences, the likelihood that the same interference will behave the same way in two different chromatographic systems is reduced.

The application of this approach for the determination of disopyramide—with GC with a nitrogen-selective detector, “high-performance” LC, and GC–MS—indicates that good correlation exists among the three methods of analysis. After extracting the drugs in heptane/isobutanol, re-extracting into H2SO4 and then ether, assay can be by GC, LC, or GC–MS. The choice of technique depends on the magnitude and type of interference problem to be solved. The haphazard choice of one technique because of convenience or fashion should be avoided.

References