Simultaneous Liquid-Chromatographic Determination of Three Antiarrhythmic Drugs: Disopyramide, Lidocaine, and Quinidine

James G. Flood,1 George N. Bowers,2 and Robert B. McComb2,3

We report a common methodology for determining three antiarrhythmic drugs: disopyramide, lidocaine, and quinidine. Alkalized serum and internal standard (p-chlorodisopyramide) are extracted into dichloromethane, the organic phase is evaporated, and the redissolved residue is injected onto a reversed-phase column (μ Bondapack C18). Quantitation is via peak-height ratios of analyte vs internal standard (as detected at 205 nm) referenced to a serum-based multiple-drug standard. A mobile phase of 30 mmol/L phosphate buffer and acetonitrile (72/28 by vol) is used. These conditions yield optimum separation and band symmetry for the analytes and some of their metabolites. Crucial factors in this simultaneous assay include pH of the mobile phase and injected solution, extraction time, and evaporation technique. Day-to-day precision (CV) for all drugs was <5%, and correlation with other assay techniques for each drug is reported. The method enables more efficient use of personnel and instrumentation without sacrificing analytical quality.

Additional Keyphrases: drug assay • monitoring therapy • enzyme-multiplied immunoassay

“High-pressure” liquid chromatography is well suited for simultaneously measuring parent drug–metabolite combinations in serum. Such simultaneous assays have obvious advantages for the clinical laboratory, including more efficient use of personnel and instrumentation. We report here the development of such a common methodology for the antiarrhythmic drugs disopyramide, lidocaine, and quinidine, which also possesses these advantages.

All of these drugs are extractable from basic aqueous solutions and have therapeutic serum ranges high enough (>1 mg/L) to allow use of ultraviolet detection. A proper choice of chromatographic and extraction conditions enables all these compounds to be assayed via a single methodology on the widely used C-18 types of column. Reversed-phase chromatographic assays for disopyramide by use of alkyl phenyl (7) and ion pairing (2) with use of C-18 stationary phases, have been reported. A C-18 reversed-phase method for lidocaine has been published (3), but the authors used an internal standard (procaine) that is unstable in native blood serum. Use of this method therefore requires meticulous timing of reagent additions to achieve acceptable precision.4 While reversed-phase chromatographic assays of quinidine (4-7) have been reported, in only two (5, 7) are C-18 columns and ultraviolet absorbance detectors used.

Materials and Instrumentation
Materials: All chemicals used were of reagent grade. Deionized water was used throughout. Acetonitrile was obtained from Burdick and Jackson Laboratories, Muskegon, MI 49442. Quinidine sulfate · 3.5 H2O was obtained from Sigma Chemicals Co., St. Louis, MO 63178. Lidocaine · HCl · H2O was a gift from Astra Pharmaceutical Products, Inc., Worcester, MA 01606. Disopyramide, mono-N-dealkylated disopyramide, and p-chlorodisopyramide were a gift from Searle Laboratories, Chicago, IL 60650.

Instrumentation: For all liquid chromatography (LC) we used a Model ALC 200 Liquid Chromatograph equipped with a Model 440 fixed-wavelength absorbance detector (254 nm), a Model 450 variable-wavelength absorbance detector, and μ Bondapack C18 reversed-phase columns (all from Waters Associates, Milford, MA 01757).

Method
Solutions and Standards
Mobile phase buffer (30 mmol/L KH2PO4), pH 4.45 (range ± 0.05) at 25 °C. Dissolve 4.08 g of KH2PO4 in water and dilute to the mark in a 1-L volumetric flask. If necessary, adjust the pH of this solution to 4.45 ± 0.05 with 0.5 mol/L H2SO4 or 1 mol/L NaOH. Filter through a 0.45-μm pore-size filter (Millipore Corp., Bedford, MA 01730) immediately before use.

Chromatographic mobile phase. With stirring, slowly add 280 mL of acetonitrile to 720 mL of the mobile-phase buffer.

Concentrated stock standards: Disopyramide. Dissolve 45.0 mg of disopyramide in a 50-mL volumetric flask with 3 mL of 10 mmol/L HCl and about 15 mL of water. Dilute to the mark with water (disopyramide may take up to 24 h to dissolve). Lidocaine. Dissolve 37.0 mg of lidocaine · HCl · H2O in a 50-mL volumetric flask with water and dilute to the mark. Quinidine. Dissolve 56.2 mg of quinidine sulfate · 3.5 H2O in a 50-mL volumetric flask and dilute with water.

Concentrated stock drug mixture: Combine 10.0-mL aliquots of each of the three concentrated stock standards.

Working standards: To three 200-mL volumetric flasks add 1, 3, and 5 mL, respectively, of the concentrated drug mixture. Dilute all the flasks to the mark with drug-free serum. Mix well and store these standards in 1- or 2-mL aliquots at −20 °C. The concentrations (mg/L) of free drug in each standard are:

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>1.0</td>
<td>3.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>1.5</td>
<td>4.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Quinidine</td>
<td>1.5</td>
<td>4.5</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Concentrated internal standard: Dissolve 8 mg of p-chlorodisopyramide in a 100-mL volumetric flask, using 2 mL of 10 mmol/L HCl and about 15 mL of water. Dilute to the mark with water and store at 4 °C.

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3 Address reprint requests to this author.
4 We have observed significant disappearance of procaine when the compound is added to serum.

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Continuously effluent concentrated mm), Elution Fig. 1. Representative chromatogram obtained with a serum-based standard
Elution order is lidocaine (2.68 min), disopyramide (3.86 min), quinidine (4.97 min), quinidine impurity, and internal standard (7.42 min)

Working internal standard (8 mg/L): Dilute 5.0 mL of concentrated internal standard to 50 mL with water. Store at 4 °C.

Instrumental conditions: Column flow rate, 2.0 mL/min. Column temperature, ambient (22–26 °C). Monitor column effluent at 205 nm (recorder sensitivity, 0.04 A full scale). Continuously stir the mobile phase to avoid air-bubble formation in the prepump tubing.

Sample preparation: Place 500 μL of standard or sample into 10 × 150 mm screw-cap centrifuge tubes. To each tube then add 500 μL of the working internal standard solution, 100 μL of 1 mol/L NaOH, and 3 mL of dichloromethane. Gently rock the tubes for 15 min on a mechanical mixer (Aliquot Mixer; Lab-Tek Products, Naperville, IL 60540). Centrifuge the tubes for 5–7 min. Transfer the bottom layer to another 10 × 150 mm tube, immerse the tubes in a beaker of water at 25 °C, and evaporate the dichloromethane in a room-temperature water bath with an air stream. Avoid exposure of the residue to the air stream for more than 30 min. Dissolve the residue in 250 μL of mobile phase and inject 15 μL or less onto the chromatograph. Figure 1 shows a chromatogram obtained from a serum-based standard.

Quantitation: Construct a calibration curve for each drug, using the analyte/internal standard peak-height ratio. This ratio for an unknown is converted to concentration by use of this calibration curve.

Results for Each Drug
Lidocaine: Within-day precision (±1 SD) was 0.08 mg/L (n = 9, x = 2.99, CV = 2.6%). Day-to-day precision was 0.18 mg/L (n = 8, x = 4.16, CV = 3.4%).

Twenty-six serum samples were analyzed by the present method (y) and the Syva EMIT technique (x). The regression equation of the results was y = 0.85x – 0.09, the correlation coefficient 0.957. Analytical recovery of lidocaine from five different human sera was 96%. The standard calibration curve was linear to at least 9.0 mg/L. The mono-N-dealkylated metabolite of disopyramide, 4-hydroxypropranolol, and an unidentified compound (presumably a metabolite of quinidine) elute near lidocaine, but will not interfere. The concentration of acetonitrile used here in the mobile phase is optimum for this separation; the disopyramide metabolite is eluted just before lidocaine. When 10 mmol/L KH2PO4, pH 4.4, and a lower concentration of acetonitrile were used, we observed a change in the elution order of lidocaine and the metabolite. Lower acetonitrile concentrations (e.g., 150 mL/L) elute lidocaine before the metabolite, and intermediate concentrations (e.g., 200–230 mL/L) do not separate the compounds at all. These compounds are even better separated at pH 5.3 (see Figure 2), but analysis time is increased.

Disopyramide: Within-day precision was 0.09 mg/L (n = 9, x = 4.24, CV = 2.1%). Day-to-day precision was 0.10 mg/L (n = 6, x = 4.96, CV = 2.0%).

At the time of this study, alternative methods for disopyramide analysis were not available to us. However, we were able to re-assay 21 specimens from a single patient that had been analyzed (2) in another study from this hospital (9). The data fit the regression equation y = 1.02x + 0.07; the correlation coefficient was 0.982. Analytical recovery of disopyramide from different human sera averaged 97%. Standard curves were linear to at least 13 mg/L.

Quinidine: Within-day precision was 0.13 mg/L (n = 9, x = 4.29, CV = 3.0%). Day-to-day precision was 0.17 mg/L (n = 8, x = 4.26, CV = 4.0%). Twenty-five patients’ samples were analyzed by this method (y) and a double-extraction fluorescence method (x) (10). The results are shown in Figure 3. The data fit the regression equation y = 0.730x + 0.020 and the correlation coefficient was 0.943. Analytical recovery of quinidine from different patients’ sera was >95%. Standard curves were linear to at least 11 mg/L.

Interferences
During this study we monitored all analyses simultaneously at 205 and 254 nm to check for compounds co-eluting with the

![Fig. 2. Capacity factors (K') as a function of mobile phase buffer pH](image link)
analytes or internal standard. No significant interferences were found. Propranolol is not completely resolved from the internal standard under the conditions stated. However, as the serum concentration of propranolol is generally much lower than the drugs and internal standard in this study (11), this compound ordinarily does not interfere. Propranolol does separate from the internal standard at lower buffer concentration (10 mmol/L) and higher temperatures (30°C). Procaïnamide, N-acetyl procaïnamide, and N-propionyl procaïnamide elute with or close to the solvent front.

Analytical recovery of these compounds from lipemic, icteric, or hemolyzed sera was quantitative.

Results

Development of Method

Extraction. Initial experiments suggested that the analytes could be extracted from serum by using a procedure similar to that of Rocco et al. (8). Modification of this technique led to the procedure reported here. Study showed that: (a) The extraction must be carried out for at least 10 min to obtain maximum absolute recovery of the analytes and internal standard. (b) Conditions for the evaporation of the dichloromethane phase affect the recovery of lidocaine. Heating the dichloromethane to 60°C results in a definite loss of lidocaine, as does excessive exposure of the residue to the air stream (we recommend evaporation at room temperature and that the evaporation interval be less than 30 min). (c) Use of excess amounts of base in the extraction procedure is to be avoided. Sodium hydroxide from the aqueous phase may be entrapped in the dichloromethane layer and be present in the evaporation residue; this causes erratic analyte peak widths and retention times.

The absolute analytical recovery of analytes and internal standard was >90%.

Chromatography. Preliminary experiments showed that retention times for analytes and internal standard were similar when a 70/30 (by vol) pH 4.0 phosphate buffer (10 mmol/L)/acetonitrile mobile phase was used at 2 mL/min flow rate and 30°C. Also, the separation depends markedly on the pH of the mobile phase. The elution times for lidocaine and, especially, for quinidine depend much more on the pH of the mobile phase than do those for the other compounds (Figure 2). Mobile-phase buffer pH's of >4 and <8 provide a good separation of the three analytes and internal standard.

Table 1 shows that the peak shapes of the analytes are also greatly affected by mobile-phase pH. At pH's of <4.6, all analytes exhibit good efficiency with minimal band asymmetry. At pH values >4.5 there is a decrease in chromatographic efficiency, especially for quinidine. We evaluated other commercial C-18 columns as to mobile-phase pH effects, but the one we used showed the best peak shapes for quinidine in the pH 4–5 range and the most constant bandshape factors for all analytes over the entire pH 3–5 range. The optimal pH for this analytical separation appears to be 4.0–4.5.

A 30 mmol/L phosphate buffer is used in the method finally adopted. At 10 mmol/L buffer, an unidentified peak co-elutes with the internal standard in about 10% of patients' samples. Use of a 30 mmol/L buffer resolves this peak from the internal standard and provides better pH control in the mobile phase and the reconstituted sample.

Detection. We monitored effluents simultaneously at 254 and 205 nm throughout this study. Lidocaine must be quantitated at 205 nm, owing to its low absorbity at 254 nm. Should a 205-nm detector be unavailable, disopyramide and quinidine exhibit sufficient sensitivity to be monitored at 254 nm, but 205 nm is recommended because (a) we have occasionally observed small unidentified peaks at the same elution time as these analytes when using 254-nm detection that were not seen using 205-nm detection, and (b) the absorbity of quinidine at 205 nm is rather pH-insensitive as compared to 254 nm.

Discussion

We attribute the significant bias observed between the liquid-chromatographic and fluorescence methods (10) for quinidine to the specificity of the liquid-chromatographic technique. Previous investigators using C-18 (7) and alkylphenyl (6) columns also found lower results by chromatographic methods than by nonspecific fluorometric ones, because the latter also evidently measure quinidine metabolites. Samples from patients on quinidine usually had a peak eluting shortly before the retention time of lidocaine; and we also saw this peak on using 330-nm detection, suggesting it might be a quinidine metabolite. There was a correlation (n = 14, r = 0.824) between the discrepancy in liquid chromatography and fluorescence methods and the unknown peak/internal standard peak height ratio. Standard additions of quinidine to patients' samples that showed the largest discrepancies between the two methods yielded quantitative recoveries by both liquid chromatography and fluorescence, further suggesting that the observed bias is mostly due to lack of specificity in the fluorescence method.

Drayer (4) also reported a significant bias (about 20%) between the double-extraction fluorescence method (11) and a more specific liquid-chromatographic technique. “Therapeutic ranges” should be adjusted downward if a more nearly specific chromatographic technique is used to replace one of the nonspecific fluorometric assays.

The impurity present in commercial quinidine preparations (presumably dihydroquinidine) eluting just before the internal

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**Table 1. Band-Shape Factors**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>pH 3.1</th>
<th>pH 3.8</th>
<th>pH 4.6</th>
<th>pH 5.3</th>
<th>pH 6.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disopyramide</td>
<td>137</td>
<td>133</td>
<td>112</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>120</td>
<td>102</td>
<td>51</td>
<td>23</td>
<td></td>
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<tr>
<td></td>
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<tr>
<td>Internal standard</td>
<td>128</td>
<td>124</td>
<td>128</td>
<td>103</td>
<td></td>
</tr>
</tbody>
</table>

* Retention time^2/(peak width at 10% peak height)^2

^2 28/172, acetonitrile/30 mmol/L KH2PO4 buffer; pH adjusted with 0.2 mmol/L H2SO4 or 1 mol/L NaOH.

^c Not determined.
standard (see Figure 1) will cause a standardization error in analytical techniques for quinidine. In the liquid-chromatographic method reported here, quinidine is separated from this impurity; the method will thus have a positive bias equal to the weight percent of impurity in the quinidine standard. The impurity peak height is only about 5–6% of the quinidine peak at 205 nm; the bias it introduces into the LC method is presumably small, probably <10%. This bias is opposite to the specificity bias discussed above. The quinidine standards used should be checked to ensure that the proportion of impurities is low.

Disopyramide results by our technique showed no obvious bias when compared with those by the liquid-chromatographic method used at an independent laboratory. The mono-N-dealkylated metabolite of disopyramide can be identified and quantitated by this method in the serum of patients receiving disopyramide (9), but quantitation of this metabolite has not yet been shown to be therapeutically useful.

The correlation data obtained for lidocaine suggest a proportional bias between our method and the enzyme-multiplied immunoassay technique (results for both methods are reported in milligrams of amine form of the drug per liter). We have not been able to assign a cause for this discrepancy. Our technique provides >96% analytical recovery of lidocaine when standard additions (from the same lot of lidocaine used in making up the standards) are made to human sera. In addition, absolute recovery of lidocaine and the internal standard from pooled serum exceeds 90%.

We also analyzed lidocaine samples with use of N-propionyl procainamide as the internal standard and a different chromatographic mobile phase; the results were similarly lower than those obtained by the immunoassay. Furthermore, immunoassay calibrators yield low results when analyzed by LC, and LC serum standards are overestimated by the immunoassay. These additional experiments suggest that the liquid chromatographic assay is internally consistent and that the bias with respect to the immunoassay is due to a standardization difference between the two methods.

This procedure has been used for 18 months in this laboratory and is currently used by at least two other hospital laboratories. We have not yet encountered interferences with the peak height estimation of these analytes.

We thank Mr. Burton Deane for performing some of the assays.

References