Rapid High-Pressure Liquid Chromatographic Determination of Quinidine and Dihydroquinidine in Plasma Samples

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Abstract □ A high-pressure liquid chromatographic assay for quinidine and dihydroquinidine was developed. A cation-exchange column was utilized with an eluting solvent pH of about 9. The internal standard was cinchonine. The reproducibility and precision of this method were evaluated by analyzing replicate samples and by comparing results with those obtained from a TLC–fluorometric procedure. In addition, several drugs were evaluated to ascertain whether they interfered with the analysis of quinidine and dihydroquinidine.

Keyphrases □ Quinidine—high-pressure liquid chromatographic analysis in plasma □ Dihydroquinidine—high-pressure liquid chromatographic analysis in plasma □ High-pressure liquid chromatography—analyses, quinidine and dihydroquinidine in plasma □ Cardiac depressants—quinidine, high-pressure liquid chromatographic analysis in plasma

Measuring plasma quinidine concentrations has proven to be a valuable aid in the evaluation and management of patients on quinidine therapy (1, 2). The first procedure for determining plasma quinidine levels was published in 1943 (3). Since that time, modifications of the original method have been proposed (4, 5), and new and improved analytical methodologies have been developed (6–9). The improvements in the analysis of quinidine relate both to increased sensitivity and better specificity.

While several procedures provide adequate sensitivity, only two are truly specific for quinidine (8, 9). These procedures allow for the separation of quinidine from dihydroquinidine and permit the quantitation of both compounds. However, these procedures involve a TLC separation followed by fluorometric analysis and are both extremely tedious and time consuming.

Since dihydroquinidine has been reported to represent as much as 24% of the total alkaloid present in some quinidine preparations (10), a rapid and simple procedure for separating and measuring both compounds concurrently would be of value for research and patient care. This report describes a new high-pressure liquid chromatographic (HPLC) procedure that facilitates the separation and analysis of both quinidine and dihydroquinidine in plasma samples.

EXPERIMENTAL

Chemicals and Reagents—Quinidine[1], dihydroquinidine[2], and cinchonine[3] were obtained as the free bases and used without further purification.

Glass-distilled benzene[4] and methanol[4] were used. A 0.01 *M* solution of trimethylamine was prepared by dissolving trimethylamine hydrochloride[3] in double-distilled water. Potassium hydroxide[5] was used in the preparation of the mobile phase, and a 5 *N* solution of sodium hydroxide[5] was prepared for the extraction.

Instrumentation—A dual-piston reciprocating pump[6] with a variable UV wavelength detector system was used[7]. Samples were injected onto the column *via* a 50-μl valve loop[8]. The column employed was a 25-cm × 4.6-mm cation-exchange column[9]. The system was operated at room temperature with a flow rate of 2 ml/min, which developed a pressure of approximately 2000 psi. The detector was set to read absorbance at 230 nm.

Mobile Phase Preparation—Trimethylamine hydrochloride (0.01 mole) was dissolved in approximately 100 ml of water. Potassium hydroxide (0.001 mole) was then added, and the solution was diluted to 1 liter with water. The pH of this solution was approximately 9. The mobile phase was then prepared by mixing this aqueous solution with methanol in a ratio of 1:4 (water–alcohol). The mobile phase was routinely degassed by application of a vacuum.

Procedure—To 1 ml of plasma was added 200 ng of cinchonine (100 μl of a methanolic solution). A 0.1-ml aliquot of 5 *N* sodium hydroxide and 3 ml of benzene were then added. The tube was shaken for 5 min and centrifuged to separate the aqueous and organic layers.

The benzene was then transferred to a clean tube and evaporated to dryness at 55° under nitrogen. The residue was dissolved in 200 μl of the mobile phase, and 50 μl was injected. All samples were analyzed in triplicate. Peak areas were determined by means of an integrating recorder[10].

RESULTS AND DISCUSSION

The described procedure is sufficiently sensitive to measure quinidine and dihydroquinidine levels as low as 100 ng/ml. To measure levels in the low nanogram region, only minor modifications are necessary. They include reducing the amount of internal standard to 100 ng, using a 100-μl injection loop, and operating at a lower attenuation. As was reported (11), the extraction of quinidine from plasma at a high pH is essentially 100% efficient. Likewise, at a high pH, dihydroquinidine is readily extracted from plasma into benzene (12).

Several compounds were evaluated for use as internal standards including cinchonidine, chloroquine, primaquine, brucine, and quinine. All of these compounds were unsuitable because of poor separation from

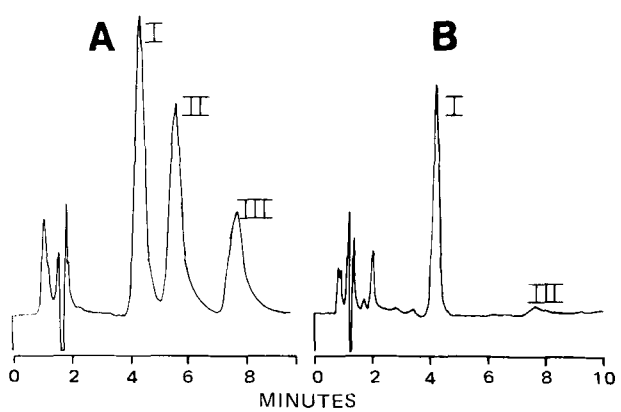

Figure 1—*Chromatograms of a plasma sample (A) spiked with 2 μg of quinidine/ml (I), 200 ng of cinchonine/ml (II), and 1 μg of dihydroquinidine/ml (III), and a sample from a patient (B) without any cinchonine added.*

[1] Supplied by C. T. Ueda, College of Pharmacy, Omaha, Neb.
[2] Tridom Chemical, Inc., Hauppauge, N.Y.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Burdick and Jackson Labs., Muskegon, Mich.
[5] ACS grade, Fisher Scientific, Fairlawn, N.J.
[6] Tracor model 995.
[7] Tracor model 970.
[8] Rheodyne model 7120.
[9] Partisil 10 SCX, Reeve Angel, Clifton, N.J.
[10] Houston Instruments model 5221-15.

Table I—Evaluation of Assay Precision and Accuracy for Quinidine (n = 7)

Quinidine Added, μg/ml	Mean Quinidine Measured, μg/ml	*SD* of Measured Concentration
1	0.96	0.07
5	4.90	0.46
10	11.0	1.0

quinidine or too long retention times. Cinchonine was selected because it eluted fairly rapidly and was readily separated from quinidine and dihydroquinidine.

Figure 1A shows a chromatogram of a spiked plasma sample containing quinidine (2 μg/ml), cinchonine (200 ng/ml), and dihydroquinidine (1 μg/ml). The retention times were 4.4, 5.7, and 7.8 min, respectively. Chromatograms of extracted blank plasma samples showed no peaks. No plasma constituents eluted with retention times near those of the compounds being measured.

Figure 1B shows a chromatogram from a patient sample without any cinchonine added and demonstrates that none of the metabolites of quinidine eluted under the cinchonine peak. The small amount of dihydroquinidine detected is typical of what has been observed with most patient samples. The concentration of quinidine measured in the sample shown was 1.2 μg/ml, while the concentration of dihydroquinidine was estimated to be 50 ng/ml. The dihydroquinidine detected here represented approximately 4% of the total alkaloid measured. This result agrees with the percentage of the impurity found in the drug product administered. It has been reported that the disposition kinetics of quinidine and dihydroquinidine are essentially the same (12). Therefore, one would anticipate that the ratio of quinidine to dihydroquinidine in the plasma would not change with time.

Standard curves were prepared by adding known amounts of drug to blank plasma samples with known concentrations of quinidine and dihydroquinidine and then determining the peak area ratios, relative to the internal standard, at various concentrations. Either water or plasma could be used for preparing the standard curves. The relative extraction efficiencies of these two compounds and the internal standard were not affected by plasma components.

The quinidine standard curve was linear over a range of 0.5–10 μg/ml and extrapolated through the origin. The slope of the regression line was 0.62 with a correlation coefficient of 0.99. The standard curve for dihydroquinidine was linear over a range of 0.5–5.0 μg/ml and also extrapolated through the origin. The slope of the regression line was 0.62 with a correlation coefficient of 0.99.

The precision and accuracy of this assay procedure were evaluated by analyzing several plasma samples with known concentrations of quinidine and dihydroquinidine. Quinidine was evaluated at three concentrations, and dihydroquinidine was evaluated at four levels. The results of the quinidine samples are summarized in Table I. The standard deviations ranged from 7% at 1 μg/ml to 9% at 10 μg/ml.

The results from the evaluation of the dihydroquinidine measurements are reported in Table II. The standard deviations ranged from 15% at 0.5 μg/ml to 3% at 5 μg/ml. To facilitate more accurate measurements of smaller quantities of dihydroquinidine, it is necessary to utilize a smaller quantity of the internal standard and to operate the detector at a lower attenuation.

The procedure was further evaluated by comparing the results of the analysis of patient samples by both the HPLC method and a TLC–fluorometric procedure (8). Blood samples were obtained from six patients who were receiving oral quinidine sulfate for ventricular arrhythmias.

Table II—Evaluation of Assay Precision and Accuracy for Dihydroquinidine

Dihydroquinidine Added, μg/ml	n	Mean Dihydroquinidine Measured, μg/ml	*SD* of Measured Concentration
0.5	6	0.53	0.08
1.0	7	0.98	0.08
3.0	7	3.0	0.22
5.0	7	4.9	0.17

Table III—Comparison of HPLC and TLC–Fluorometric Assay Procedures Using Patient Samples

Patient	Quinidine Concentration, μg/ml	
	HPLC	TLC–Fluorometric[a]
C.K.	0.90	1.0
B.M.	1.8	1.8
J.M.	1.9	2.0
J.M.	2.2	2.3
W.G.	0.90	1.0
D.F.	0.57	0.66
R.T.	0.84	0.88

[a] Reference 8.

Samples were drawn using heparinized syringes to avoid possible problems with commercially available blood-drawing tubes (13). The results are shown in Table III. The two methods agree quite favorably with only minor variability, which ranged from 0.1 to 0.4 μg/ml.

The samples analyzed by the TLC–fluorometric procedure were inadvertently thawed and left at room temperature for approximately 48 hr. They were then refrozen and stored for several days before analysis. The observed results indicate that plasma samples containing quinidine are stable at room temperature for at least 48 hr.

Since patients receiving quinidine usually are concurrently taking other medications, it was necessary to evaluate the possible interference by other drugs with the assay procedure. Plasma samples were obtained from patients on various medications and were then extracted and analyzed according to the described protocol. No interfering peaks were observed when plasma samples containing the following drugs were analyzed: propranolol, lidocaine, procainamide, digoxin, warfarin, chlorothiazide, spironolactone, furosemide, isoniazid, allopurinol, flurazepam, triamterene, and dipyridamole. All of these drugs were being administered in therapeutic doses, and blood samples were drawn, when possible, to reflect peak levels.

In summary, an HPLC procedure for measuring plasma concentrations of quinidine and dihydroquinidine has been developed. The procedure is sufficiently rapid to provide clinical usefulness in that it requires, on the average, only 20 min to extract and analyze a sample. However, it is sufficiently sensitive to be of value for research purposes.

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