Fluorometric Determination of Quinidine

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**Introduction**

Quinidine, a naturally occurring alkaloid found in cinchona bark, is widely used for the treatment and prevention of most types of atrial and ventricular arrhythmias. Reasons for the therapeutic monitoring of quinidine include its narrow therapeutic range (1); the demonstrated correlation between clinical effect and serum quinidine concentrations (1); interpatient variability in absorption, metabolism, and elimination (2); and the similarity between quinidine toxicity and ineffectively controlled arrhythmias.

Several procedures for measuring quinidine have been used, and the apparent therapeutic range depends on the method used. Factors complicating the interpretation and comparison of results by different procedures include the fact that all standard quinidine formulations contain a small amount of dihydroquinidine, which apparently has antarrhythmic activity, and the fact that some of the quinidine metabolites may possess some activity, although most of them are apparently inactive. The most commonly used methods have been fluorometric, the protein precipitation method of Brodie and Udenfriend (3) being one of the earliest such assays. This nonspecific assay measures nonactive metabolites as well as quinidine and dihydroquinidine; it was improved upon by the procedure of Sokolow and Edgar (4), in which a single organic solvent extraction of quinidine with dichloroethane eliminates some of the metabolites before fluorescence. The double-extraction method of Cramer and Isaksson (5), with which most of the metabolites are eliminated, measures both quinidine and the active dihydroquinidine, and is the fluorometric procedure most commonly used in clinical laboratories. It is the one presented here. Certain "high-performance" liquid chromatographic methods (6, 7) can differentiate between quinidine, dihydroquinidine, and the inactive metabolites, but their use in the clinical laboratory is limited by the instrumentation required and the generally longer analysis time. Another procedure available is a homogenous enzyme assay procedure (EMIT®, Syva Corp.), the results of which appear to correlate well with those by the double-extraction procedure of Cramer and Isaksson presented here.

**Principle**

Quinidine is first extracted from alkalized serum or plasma into benzene. It then is re-extracted into a sulfuric acid solution, in which quinidine fluoresces with an intensity proportional to its concentration.

**Materials**

**Reagents**

\[ \text{NaOH, 100 mmol/L}: \text{Dissolve 4.0 g of NaOH pellets in 800 mL of water in a 1-L volumetric flask and dilute to volume. This solution is stable for a year when stored in a brown-glass bottle at room temperature} \]

\[ \text{H}_2\text{SO}_4, 50 \text{mmol/L}: \text{Dilute 2.7 mL of concentrated H}_2\text{SO}_4 \]

\[ \text{to 1 L with water in a volumetric flask. Stable for one year when stored in a brown-glass bottle at room temperature} \]

**Quinidine stock standard, 308 \mu\text{mol/L} (100 mg/L):** Dissolve 120.7 mg of quinidine sulfate \([\text{C}_{20}\text{H}_{24}\text{O}_{3}\text{N}_2\text{H}_2\text{SO}_4}\cdot 2\text{H}_2\text{O}\) in 800 mL of water in a 1-L volumetric flask and dilute to volume. This solution is stable for one year when stored in a brown-glass bottle.

**Note:** Each F.M.S. recommends dilution with 0.5 mol/L H_2SO_4 instead of water.

**Quinidine working standard, 15 \mu\text{mol/L} (5 mg/L):** Dilute 5.0 mL of the stock standard to 100 mL with water, in a volumetric flask. Prepare freshly each day.

**Apparatus**

Any spectrofluorometer may be used with which the excitation wavelength can be set at 360 nm and the emission wavelength at 450 nm (see the note accompanying step 13 of the procedure for filters used with a Turner Model 111 Filter Fluorometer). The Turner Model 430 Spectrofluorometer and the Amino-Bowman Model J4-8960 Spectrofluorometer have been used in the Submitter’s laboratory. The Perkin-Elmer Model 204 Fluorometer has been used in the Evaluation F.M.S.’s laboratory.

**Specimen**

Serum.

**Procedure**

1. Into 15-mL glass-stoppered centrifuge tubes, or 16 x 125 mm screw-top tubes, place 1.0 mL of the NaOH solution.
2. Add 0.50 mL (volumetric pipet) of quinidine-free serum to one of the tubes (blank).
3. Add 0.50 mL (volumetric pipet) of working standard to the second tube (standard).
4. With a volumetric pipet, add 0.50 mL of unknown serum to the third tube (unknown).
5. Add 8.0 mL (volumetric pipet or calibrated dispenser) of benzene to each tube.
Warning: Benzene is classified as a probable carcinogen. Avoid contact with the skin or inhalation of the vapor.


6. Shake the mixture for 30 s on a mechanical shaker.
7. Centrifuge to separate the layers.
8. With a volumetric pipet, transfer 5.0 mL of the benzene (upper) layer to a 15-mL glass-stoppered centrifuge tube containing 5.0 mL of the H2SO4 solution.
9. Shake the mixture for 30 s on a mechanical shaker.
10. Centrifuge to separate the layers.
11. Aspirate and discard the benzene (upper) layer.
12. Transfer about 2.0 mL of the lower layer to a cuvet.
13. Measure the fluorescence of each solution, using an excitation wavelength of 360 nm and an emission wavelength of 450 nm. The spectrofluorometer is blanked with the blank solution.

Note: The following filters have been satisfactorily used in the Turner Model 111 (Filter) Fluorometer: Primary (right hand), 110-811 (7-60). Secondary (left hand), 110-816 (2A) +110-823 (2ND).

Note: Evaluators F.M.S. recommends that all glassware be washed in 2.5 mol/L H2SO4 and rinsed at least seven times with distilled water.

Calculation

\[
\frac{F_{\text{unknown}}}{F_{\text{standard}}} = 15 = \text{micromoles of quinidine per liter,}
\]

or

\[
\frac{F_{\text{unknown}}}{F_{\text{standard}}} = 5 = \text{mg of quinidine per liter}
\]

where \( F \) = fluorescence intensity, in arbitrary units.

Note: It is convenient to set the fluorescence intensity of the standard at 15 (corresponding to 15 \( \mu \)mol/L) or 50 (corresponding to 5.0 mg/L); this allows the concentration (fluorescence intensity) of the unknowns to be read directly from the scale.

Discussion

Linearity and sensitivity: A plot of five concentrations of aqueous standards taken through the procedure results in a linear curve to a least 45 \( \mu \)mol/L (15.0 mg/L) quinidine. The correlation coefficient was 1.000. Least-squares linear regression analysis yielded a slope of -0.0231 and a standard error of estimate of 0.259. Quinidine can be reproducibly detected and quantitated in concentrations as low as 1.5 \( \mu \)mol/L (0.5 mg/L).

Note: Evaluators T.W.F. and L.P. report a linear curve to at least 60 \( \mu \)mol/L (20 mg/L) quinidine, with either benzene or toluene as the extracting solvent. With toluene, the correlation coefficient was 0.999 and least-squares regression analysis yielded a slope of 1.000 and a standard error of estimate of 0.126.

Analytical recovery: Quinidine at concentrations of 3, 15, 30, and 45 \( \mu \)mol/L (1, 5, 10, and 15 mg/L) was added to drug-free serum and the average recoveries (as compared with the aqueous standard taken through the procedure) were 96, 94, 99, and 95%, respectively. Cramer and Isaksson (5) reported a mean recovery of 99.9% (range 95–110%) for 88 plasma samples.

Note: Evaluators T.W.F. and L.P. report an average recovery of 101% (range 94–104%) for eight quinidine concentrations ranging from 1.5 \( \mu \)mol/L (0.5 mg/L) to 60 \( \mu \)mol/L (20 mg/L) when toluene was used instead of benzene as the extracting solvent. Evaluators R.D.M. substituted methylene chloride for benzene and found the linearity, sensitivity, and analytical recovery to be comparable. There appears to be a slight positive bias of approximately 0.3–0.9 \( \mu \)mol/L (0.1–0.3 mg/L) per sample when methylene chloride is used instead of benzene, and R.D.M. recommends toluene, which does not show this bias.

Precision: Based on routine analyses of portions of a pooled specimen by at least six technologists, the day-to-day precision (CV) of the method is 9.6 (n = 36) at a mean concentration of 20 \( \mu \)mol/L (6.4 mg/L).

Therapeutic range: The therapeutic range (through values) for quinidine determined by this method is 6–15 \( \mu \)mol/L (2–5 mg/L).

Specificity: This method measures both quinidine and the active contaminant dihydroquinidine, but does not measure to the same extent the inactive metabolites measured by the protein-precipitation method of Brodie and Udenfriend (3) and the single-solvent extraction method of Sokolow and Edgar (4). Quinidine concentrations as determined by the more specific “high-performance” liquid-chromatographic methods are about 80% as large as by the method presented here (6, 7). This difference is ascribable to the measurement of dihydroquinidine by the fluorometric method. Because this compound has pharmacological activity, its measurement as if it were quinidine does not affect the acceptability of the method presented here as a useful clinical assay for the therapeutic monitoring of quinidine.

References


Editor's note: The reader is reminded that Selected Methods do not bear the official imprimatur of the Association. They are methods that seem durable and generally useful, and that have been checked by several evaluators. As detailed elsewhere (Clin. Chem. 19, 1207 [1973]), these methods are offered here for criticism by the world community of users, and will be revised appropriately before being collected into a bound volume, Selected Methods of Clinical Chemistry. The last such volume was published by the Association in 1977.