GLC Determination of Quinidines in Human Plasma

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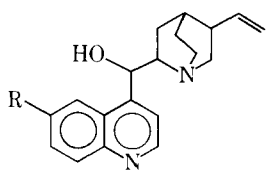
Abstract □ Human plasma was made alkaline and extracted with methylene chloride. To the extract was added the internal standard, cinchonine, followed by evaporation to dryness. The resultant residue was dissolved in a methanolic solution containing trimethylanilinium hydroxide. This solution was assayed by GLC for quinidines (quinidine and hydroquinidine). Evaluation of the method over a 0.5–10-μg/ml range in human plasma gave an overall precision and accuracy of ±4.5% (*RSD* and *RE*). Plasma of several patients was analyzed by the present method as well as by a fluorometric method for the level of quinidines. Results from the two methods were comparable.

Keyphrases □ Quinidine—GLC analysis in human plasma, compared to fluorometric analysis □ Hydroquinidine—GLC analysis in human plasma, compared to fluorometric analysis □ GLC—analysis, quinidine and hydroquinidine in human plasma, compared to fluorometric method □ Antiarrhythmic agents—quinidine and hydroquinidine, GLC analysis in human plasma, compared to fluorometric analysis

Quinidine (I*a*) is a clinically useful antiarrhythmic agent (1, 2). To achieve the proper therapeutic effect, the blood levels (3–8) and/or physiological responses (6) of quinidine must be closely monitored, especially in patients with impaired renal function (3, 9). Prior to the present work, blood levels of quinidine usually were determined by fluorometric (4, 5, 7–12) or colorimetric (13) assays.

The fluorometric assays offer extremely high sensitivity, with the lower limit of detection of blood reported to be 0.2 μg/ml (14). However, specificity may be lacking with certain fluorometric procedures since there is no prior chromatographic step and all resolution of interfering substances resides in the extraction method used. For example, Armand and Badinand (15) found that a benzene extraction of quinidine from alkaline plasma gave an interference when triamterene was present. Similarly, other investigators (8, 12, 16) showed that certain metabolites of quinidine or other substances interfere with the fluorometric assay unless specific protein precipitation and/or extraction methods are used.

A procedure using TLC to separate quinidine from its metabolites and other interfering substances, followed by fluorometric assay, was reported (17). Palmer *et al.* (18) used both TLC and GLC methods to study the metabolic fate of quinidine gluconate in humans. Their GLC method was based on precolumn derivatization to form appropriate silyl compounds.



I*a*: R = OCH_3
I*b*: R = H

Recently, Smith *et al.* (19) reported a TLC and GLC method for separating quinidine from its pharmaceutical preparation. The GLC method was based on precolumn derivatization similar to that of Palmer *et al.* (18) but used a different stationary phase. The work of Smith *et al.* (19) also clearly showed that most pharmaceutical dosage forms of quinidine contain 5–9% hydroquinidine.

Two widely used fluorometric methods (8, 12) for evaluating therapeutic plasma levels of quinidine do not differentiate between quinidine and hydroquinidine (13) but give total quinidines. Thus, the range of quinidine plasma levels used to regulate dosage regimens (3, 20, 21) of patients is actually a combined determination of quinidine and its dosage form impurity, hydroquinidine. Therefore, one objective of the present work was to develop a rapid GLC procedure for the codetermination of quinidine and hydroquinidine as an adjunct to, or in place of, the established fluorometric methods for use in monitoring plasma levels in patients receiving quinidine. The use of such a method should be extremely important for verifying either subtherapeutic or toxic plasma levels of quinidine as determined by fluorometric analysis.

In the present method, a small volume of plasma is made alkaline and extracted. To the extract is added the internal standard (cinchonine, I*b*), followed by evaporating to dryness and reconstituting in a methanolic solution of trimethylanilinium hydroxide. A portion of this solution is analyzed by GLC *via* an on-column methylation reaction.

Previous literature reports (3, 20, 21) indicated that the therapeutic level of quinidine expected in plasma was 2–8 μg/ml. Thus, the present procedure was evaluated over the range of 0.5–10 μg/ml and gave excellent recovery values (Table I).

EXPERIMENTAL

Extraction Procedure—Whole blood was centrifuged at 2500 rpm for 10 min and the plasma was removed. To 1 ml of plasma was added 0.5 ml of 5% NaOH and 5 ml of methylene chloride. This solution was mixed thoroughly[1] for 10–15 sec and then allowed to stand for 1 min. The lower organic layer was removed, and the plasma layer was reextracted with 5 ml of methylene chloride. In general, this second extraction was centrifuged at 2500 rpm for 5 min to give separation of layers.

The lower layer was removed again and combined with the first extract, to which was added 0.5 ml of the cinchonine internal standard solution. This solution was evaporated to dryness under a stream of nitrogen at 50°. To the resultant residue was added 30 μl of 0.2 *M* trimethylanilinium hydroxide in methanol[2], followed by mixing[1]. Then 1 μl of this solution was analyzed by GLC.

GLC Determination—All experiments were performed using a gas chromatograph[3] equipped with a hydrogen flame-ionization

[1] Vortex.
[2] MethElute, Pierce Chemical Co.
[3] Packard 419.

Table I—Precision and Accuracy in Recovery of Quinidine Added to Human Plasma

Added, μg/ml	Found[a], μg/ml	±RSD, %	RE, %
0.5	0.50 (0.49–0.55)	5.42	0
1.0	1.01 (0.96–1.05)	3.61	+1.0
2.0	2.10 (2.0–2.25)	5.39	+5.0
4.0	4.11 (3.73–4.45)	3.74	+2.75
6.0	5.96 (5.50–6.30)	5.57	−0.67
8.0	7.82 (7.55–7.91)	2.24	−2.25
10.0	9.33 (9.25–9.48)	1.00	−6.7
Average percent recovered 99.9			

[a] Average of four different samples at each added concentration level (range).

detector. A 1.83-m × 2-mm glass coiled column was packed with 3% OV-17 on 80–100-mesh Chromosorb W-HP and conditioned at 340°. The column operating condition was programmed from 255 to 280° with an initial isothermal period of 4 min, a temperature rise of 5°/min, a final isothermal period of 5 min, and a nitrogen flow rate of 14.5 ml/min. The injector was operated at 220° and the detector was operated at 200° with 1.7 kg/cm^2 air and 1.0 kg/cm^2 hydrogen.

Chromatograms were recorded on a 1-mv recorder, and peak areas were determined using the method of triangulation. When using these conditions, the retention times of quinidine and cinchonine were 9.1 and 5.9 min, respectively.

Standards—Two stock solutions of quinidine and cinchonine were prepared in methanol. The quinidine standard solution contained 1 μg/ml of quinidine sulfate[4], and the cinchonine internal standard contained 4 μg/ml of cinchonine[5]. Aliquots of 0.5, 1, 2, 4, 6, 8, and 10 ml of the quinidine standard solution were added to suitable containers, each containing 0.5 ml of the cinchonine internal standard. These solutions were evaporated to dryness under a nitrogen stream at 50°.

To each residue was added 30 μl of 0.2 *M* trimethylanilinium hydroxide in methanol[2], and the resultant solution was mixed[1]. Then 1 μl of this solution was analyzed using the described GLC conditions, and peak areas were determined by triangulation. The area ratio of quinidine to cinchonine was plotted on the ordinate, and the micrograms of quinidine added was plotted on the abscissa to obtain the standard curve; this curve proved to be linear over the range evaluated.

Evaluation of Assay Method—Twenty-eight plasma samples with known amounts of quinidine added at seven different concentration levels were used in the method evaluation. The plasma used was obtained from pooled blood. A control sample of the plasma used gave no interferences with the determination of quinidine. Each plasma sample was extracted using the previously discussed extraction procedure and analyzed by the GLC determination method. The peak areas for quinidine and cinchonine were determined by triangulation, and an area ratio of quinidine to cinchonine was calculated. This area ratio was fitted to the standard curve to obtain the value for quinidine.

Table I gives the average micrograms of quinidine found, as well as precision and accuracy values.

Stability of Quinidine in Extraction Solvent—*Evaluation by GLC*—A quinidine[4] stock solution containing 5 μg/ml was prepared in methylene chloride. A 1-ml aliquot was placed in 24 different stoppered containers and stored at room temperature. Four samples were removed from storage each hour for 6 hr, and 0.5 ml of the stock cinchonine was added to each sample. This mixture was evaporated to dryness under a nitrogen stream at 50°.

To the resultant residue was added 30 μl of 0.2 *M* trimethylanilinium hydroxide in methanol[2] followed by mixing[1]. A 1-μl portion was analyzed by GLC, and peak areas were determined by triangulation. The amount of quinidine found was determined by fitting the area ratio of quinidine to cinchonine to the standard curve. At all time intervals, the average amount of quinidine found was 5 μg.

Evaluation by TLC—Two quinidine[4] stock solutions containing 5 μg/μl were prepared in methylene chloride and methanol, respectively. The alcoholic stock solution was used as the control, since the quinidine preparation was reported to be stable in alcohol (19). Both stock solutions were kept at room temperature.

By using the TLC methods of Smith *et al.* (19), three different plates were spotted at intervals of 0, 1, 2, 3, 4, 5, 6, and 12 hr with 20 μl of both stock solutions. When the previously described developing and visualization systems were used, no differences were noted between the two stock solutions during the first 6 hr. However, the 12-hr methylene chloride stock solution showed a small unknown spot at R_f 6.5 when developed in chloroform–acetone–methanol–ammonium hydroxide (60:20:20:1) and visualized by acid spraying and longwave UV light. In addition to quinidine and hydroquinidine, these TLC systems demonstrated that trace amounts of epiquinidine were also present in the quinidine[4] used in the method evaluation.

[4] Quinicardine, $(C_{20}H_{24}N_2O_2)_2H_2SO_4 \cdot 2H_2O$, USP grade, Merck. A weight corresponding to that needed to give 10 μg of quinidine free base/ml was used.

[5] USP grade, Fisher Scientific Co.

Table II—Assay Method Evaluation on Plasma of Male Patients Receiving Quinidine Gluconate[a] (206 mg po)

Patient Number	Postdose Blood Sample Taken, hr	Quinidine Found, μg/ml: GLC	Quinidine Found, μg/ml: Fluorometric
1	4.0	1.2	1.3
2[b]	5.5	1.9	1.7
3	6.0	3.0	2.7
4	6.0	2.9	2.8

[a] Expressed as milligrams of free base. [b] Also receiving allopurinol.

Clinical Utility of Assay Method—To evaluate the clinical utility of the present assay method, plasma from four patients receiving quinidine gluconate was assayed. These patients were being maintained on quinidine, and blood was drawn for analysis as indicated in Table II. The plasma of each patient was then assayed by the present method and by a commonly used fluorometric method (8). Results of this study are given in Table II.

RESULTS AND DISCUSSION

A GLC assay method for quinidines (quinidine and hydroquinidine) in human plasma was developed and evaluated over a concentration range of 0.5–10 μg/ml. The overall recovery of quinidines added to plasma was 99.9%. Control samples of the plasma used in the method evaluation were extracted and analyzed using the new procedure, thus demonstrating the lack of interfering components.

Plasma from four patients being maintained on quinidine was evaluated by the GLC assay method and by a commonly used fluorometric assay method. Results from the two different procedures were found to be comparable.

The present method does not distinguish between quinidine and hydroquinidine, a contaminant in commercial quinidine preparations (19); only one compound with the same retention time as an authentic sample of hydroquinidine was indicated by GLC analysis using the USP grade quinidine. Yet this same material was shown to contain hydroquinidine when examined by the TLC methods of Smith *et al.* (19). Hartel and Harjanne (13) pointed out that therapeutically it is desirable to codetermine quinidine and hydroquinidine since the antiarrhythmic properties are of the same magnitude.

Due to the unavailability of the metabolites of quinidine, these compounds were not evaluated by the present method. Earlier work (17) demonstrated that a benzene extract of urine gave the smallest amount of metabolites, apparently due to their low solubility (13). Thus, the authors recommended the use of the benzene extraction method of Cramer and Isaksson (8) due to its specificity and accuracy for codetermination of quinidine and hydroquinidine.

The proposed GLC method was evaluated clinically by comparing it to the Cramer and Isaksson method (Table II). Due to the excellent comparison between the two methods, the extraction of metabolites by the two methods is assumed to be aproximately the same. Indeed, no other components were noted on the chromatograms of patients receiving quinidine that were not on the chromatograms of plasma to which quinidine had been added.

A study was made of quinidine stability at room temperature in the extraction solvent methylene chloride by two chromatographic methods. This study was prompted by the report (19) that pharmaceutical dosage forms extracted with methylene chloride degrade rapidly to give several additional TLC spots. First, a stock solution of methylene chloride was evaluated by the proposed GLC method using multiple samples at 1-hr intervals for 6 hr. No decomposition of the quinidine was noted, as evidenced by complete recovery of the quinidine added. Second, a previously reported (19) TLC system was used to evaluate two stock solutions of quinidine in methylene chloride and methanol at 1-hr intervals for 6 hr, followed by a final 12-hr sample. A small unknown spot appeared in the 12-hr sample of the methylene chloride stock solution.

Thus, some decomposition of quinidines apparently can occur in methylene chloride, but it is not rapid at room temperature. Any potential decomposition of the quinidines can be avoided easily by immediately evaporating the extracting solvent and placing the enclosed residue under refrigeration until analysis.

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Synthesis of Histamine Analogs

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Abstract □ Nineteen histamine analogs were synthesized, and their biological actions were compared to those of histamine in blood pressure, gastric secretion, and nasal decongestant screens. The analogs include *N*-substituted 4-aminoethylimidazoles, *N*-substituted 2-pyridylethylamines, 2-pyridylcyclohexylamines, and 2-pyridylcyclopropylcarbamates. None of the compounds showed appreciable histamine agonist or antagonist properties.

Keyphrases □ Histamine—19 analogs synthesized, biological actions compared, blood pressure, gastric secretion, and nasal decongestant screens □ Structure–activity relationships—19 histamine analogs □ Imidazole derivatives—synthesis of 19 histamine analogs, structure–activity relationships □ Pyridylethylamine derivatives—synthesis of 19 histamine analogs, structure–activity relationships

The biological properties of the naturally occurring autacoid, histamine, are well known. A molecule that selectively mimics or antagonizes a single action of histamine is a potentially useful agent in a broad range of pharmacological activities, including effects on vascular, bronchial, and intestinal musculature as well as inflammatory and secretory mechanisms. The classical antihistamines have only a narrow spectrum of histamine antagonism, blocking some histamine-stimulated secretions and intestinal smooth muscle contractions.

An excellent review of the actions of certain histamine analogs was reported (1), and the effect of structural modification on histamine agonist and antagonist actions was reviewed (2). A qualitative assessment suggests that the histamine molecule can be modified in many ways with retention of agonist activity so long as the length of the side chain remains unchanged. These modifications include replacement of the imidazole ring with other heterocyclic rings, alkyl substitutions on the side chain, and various substituents on the basic nitrogen of the side chain. The cyclopropyl derivative, 2-(4-imidazolyl)cyclopropylamine, was reported (3) to be almost devoid of activity.

The work reported here was designed to determine whether the selectivity of action could be increased by altering any of the three structural components of histamine: the ring, side chain, or *N*-substituent. The imidazole ring was substituted by a pyridine ring