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DETERMINATION OF QUINIDINE AND ITS MAJOR METABOLITES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A specific and precise assay, capable of quantitating in human plasma simultaneously but separately quinidine, dihydroquinidine and the quinidine metabolites 2′-quinidinone, 3-OH-quinidine and a third metabolite found — tentatively identified as the product formed by rearrangement of quinidine-N-oxide — is reported. The assay uses a normal phase high-performance liquid chromatographic (HPLC) system with a variable-wavelength UV detector at 235 nm and has a limit of sensitivity at approximately 20 ng/ml. The mobile phase consists of hexanes—ethanol—ethanolamine (91.5:8.47:0.03). A 2-ml plasma sample is worked up by adding primaquine base as an internal standard and extracting with ether—dichloromethane—isopropanol (6:4:1). The organic extract is evaporated and the residue reconstituted in 100—600 μl of mobile phase and an aliquot injected onto the column.

Comparison of this procedure with the Edgar and Sokolow (dichloroethane) extraction—fluorescence procedure and with the Cramer and Isaksson (benzene) double extraction—fluorescence assay indicates that both fluorescence procedures give quinidine concentrations up to 2.3 times those determined by HPLC. These discrepancies were shown to be due to carry-over of metabolites and some extraneous background fluorescence.

INTRODUCTION

Although alternative therapy is becoming available, quinidine is still among the major compounds used in the treatment of life threatening arrhythmias. Since its introduction as a cardiac depressant by Frey [1] in 1918, much effort has been expended in developing procedures to monitor its therapeutic activity in an attempt to minimize untoward reactions. Quinidine is known to have a narrow therapeutic index [2]. The definition of this range depends,

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to some extent, on the assay used to measure the drug in the blood [3, 4]. The most extensive study of clinical response vs. plasma levels is the investigation of Sokolow and co-workers [2, 5], who recommended dose adjustment until the plasma quinidine levels were greater than 2 μg/ml and less than 6 μg/ml of apparent quinidine as measured by their assay [5]. The assay involves extracting the alkalinized plasma with dichloroethane followed by measuring the fluorescence after acidification and addition of ethanol. In addition to quinidine, this method extracts dihydroquinidine and various amounts of metabolites [6].

The measurement of plasma quinidine concentration after the administration of quinidine to humans is obviously dependent on the specificity of the assay. But it is further complicated by an impurity in the commercially available quinidine. As a consequence of its method of manufacture by the isomerization of quinine, dihydroquinine is converted to dihydroquinidine. The resultant material of commerce is usually contaminated with from 5—30% of dihydroquinidine [3, 7—9]. The amount of dihydroquinidine in the commercial samples used by Sokolow and many other workers is unknown.

Dihydroquinidine has been reported to have antiarrhythmic activity similar to quinidine [3, 10], but the two compounds have not been compared as to activity in man. Both quinidine and dihydroquinidine are extensively metabolized in man via oxidative pathways. These lead to a series of hydroxylated compounds which accumulate to varying degrees in blood. The quinidine metabolites include 2'-quinidinone [11], 3-OH-quinidine [12] and O-desmethylquinidine [13]. During this study we have isolated another metabolite, tentatively identified as the rearrangement product formed from an intermediate N-oxide. Although incompletely investigated, the metabolites possess some degree of cardiovascular activity in animal studies [13, 14]. Since these metabolites might also possess some activity in man, it is far more logical to quantitate them, rather than attempt to remove them as is done in almost all presently available assays. This would be particularly important in cases of renal insufficiency where the metabolites will accrue in the plasma.

At least twenty different assay procedures have been reported in the literature. These include a titrimetric method with bromine [15], a nephelometric method using a precipitant [16], colorimetric methods using ion-pair extraction with a colored anion [17—19] and many different types of fluorimetric assays [6, 20, 21]. The method of Brodie and Udenfriend [20] is still very commonly used in clinical laboratories. It involves precipitation of the plasma proteins in a diluted plasma sample using metaphosphoric acid and measuring the fluorescence of the resultant supernatant. Several modifications have been reported using different precipitating agents [9, 22—24] and extraction solvents. Kelsey and Geiling [25] used ether. Edgar and Sokolow [5, 21] used dichloroethane for the extraction, then added trichloroacetic acid and ethanol prior to determining the fluorescence of the fluids. This method has been referred to in the literature as the single extraction method. More recently Cramer and Isaksson [6] used benzene as the extraction solvent and re-extracted the basic compounds into sulfuric acid prior to fluorescence determination (double extraction method). They claimed this solvent markedly reduced the amounts of metabolites extracted. Kessler et al. [4] added 1% amyl alcohol

whereas Greenblatt et al. [26] used 1% amyl alcohol in toluene as their extraction medium. Armand and Badinand [27] published a modification involving an additional clean up step using an intermediate alkali wash. It will be shown below that even though these methods claim improved specificity, they apparently include fluorescent contributions from metabolites as well as background fluorescence from unknown constituents of the plasma. They therefore lead to spurious estimates of the quinidine concentration.

Härtel and Korhonen [28] and Ueda et al. [29] used a thin-layer procedure to separate quinidine from other fluorescent constituents. They then extracted the quinidine band and assayed the resultant fluorescence. Depending on the solvent system used [30] such a thin-layer chromatographic (TLC) method can be made very specific and allows the simultaneous determination of metabolites and parent drug, but it is tedious, time-consuming and difficult to accomplish when large numbers of samples have to be processed within a short period of time. Direct fluorimetric determination on the TLC plate has also been described [31—33]. These methods however, require a densitometer or TLC scanning devices on the fluorimeter.

Midha and Charette [34] reported on a gas—liquid chromatographic (GLC) assay that resulted in rather poor resolution of the compounds. Although these authors indicate retention times for metabolites in the system used, they do not include these substances in their assay. Other GLC assays were reported by Valentine et al. [35] and Moulin and Kinsun [36]. A comparison of gas chromatographic and two different fluorescence methods was made by Huffman and Hignite [37]. Garland et al. [38] used a GLC method coupled to a mass spectrometer. This requires, however, a stable-isotope-tagged compound to establish quantitation. The most recently published methods utilize high-performance liquid chromatography (HPLC). Conrad et al. [39] used a 100-cm reversed-phase phenyl column, solvent programming, a fluorescence detector and a heated column. Powers and Sadee [40] recommend a specific assay using an alkylphenyl column and direct sample injection after protein precipitation. Similarly to Drayer et al. [41], Crouthamel et al. [42] utilized a reversed-phase HPLC method with separation conditions of pH 2.6. Similar to quinidine and dihydroquinidine, all of the reported metabolites possess two nitrogens which will be protonated at this pH. These polar compounds may not separate from the parent compound under the conditions of the assay. They report that their assay is in good agreement with the Cramer and Isaksson method [6]. The present investigation will show that this so-called double extraction method still includes quinidine metabolites and leads to spurious quinidine levels.

Two facts speak against the use of present methods of assay of quinidine. Most of them lack specificity due to partial co-extraction of metabolites. Secondly, since the metabolites may be contributing to the therapeutic response [13, 14], it would be advantageous to attempt to quantitate not only the parent compound, but its metabolites as well.

This paper includes a new HPLC assay which measures quinidine, dihydroquinidine and their major metabolites in plasma samples after extraction. The assay will be compared with several of the commonly used fluorescence assays.

EXPERIMENTAL

Reagents and chemicals

The solvents used were hexanes (HPLC grade; Fisher, Pittsburgh, Pa., U.S.A. or UV grade; Burdick & Jackson Labs., Muskegon, Mich., U.S.A.), ethanol 200 proof (Commercial Solvents, San Jose, Calif., U.S.A.) (stored over Na_2SO_4 and filtered before use), ethanolamine (Aldrich, Milwaukee, Wisc., U.S.A.), stored over molecular sieve 3Å, benzene (nanograde) and ethyl ether (anhydrous and peroxide-free; Mallinckrodt, St. Louis, Mo., U.S.A.); all other solvents were analytical grade (Mallinckrodt).

The quinidine primary standard used was prepared from commercially available quinidine (J.T. Baker, Phillipsburg, N.J., U.S.A.) by removing dihydroquinidine according to the method of Thron and Dirscherl [43]. The dihydroquinidine-free quinidine was crystallized from anhydrous ethanol, dried in the high vacuum for 1 h. The elemental analysis showed that the crystals contained 1 mole of ethanol. A quinidine standard solution was prepared containing 1 μg of pure quinidine base per ml of methanol and kept under refrigeration with no detectable decomposition. The internal standard used, primaquine, was obtained as primaquine diphosphate (Aldrich). The base was liberated from its salt with 2 *N* NaOH and extracted with dichloromethane. The organic extracts were washed twice with water, dried over anhydrous Na_2SO_4, evaporated under reduced pressure and dried in the high vacuum. A standard solution with 10 μg primaquine base per ml methanol was prepared and kept at 4°. Dihydroquinidine was obtained as hydrochloride (ACF Chemiefarma, Maarssen, The Netherlands) and the free base prepared in the same way as primaquine (see above). The quinidine metabolites 2'-quinidinone and 3-OH-quinidine were kindly supplied by Dr. Irwin Carroll, Triangle Research Institute*.

Instruments and chromatographic conditions

A Varian Model 8500 high-performance liquid chromatograph was used, equipped with a Varian UV detector with variable wavelength, set at λ = 235 nm, and a Varian MicroPak-Si 10 LSC column, 25 × 0.21 cm I.D. (¼ in. O.D.).

The solvent mixture used, hexanes—ethanol—ethanolamine (91.5:8.47:0.03), at a flow-rate of 60—70 ml/h gave good results and yielded a back-pressure of approx. 750 p.s.i.

Injections were made with Hamilton syringes (10—20 μl) through various high-performance injector devices (Varian Model 8500 stop-flow injector, Valco CV-6-UHPa sweep-flow injector).

Areas under the chromatogram peaks were measured by a Hewlett-Packard Model 3380 A integrator.

PROCEDURES

Sample preparation and interpretation of chromatograms

A 1.0-ml volume of methanolic primaquine standard solution containing

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10 μg/ml (internal standard) is measured into a test tube (18 × 150 mm, PTFE-lined screw cap) and evaporated to dryness at 35° under a stream of nitrogen. A 2.0-ml quantity of the plasma to be assayed is added and vortexed for a few seconds. The plasma is subsequently extracted with 10 ml of a mixture of ether—dichloromethane—isopropanol (6:4:1) by vortexing for 30 sec. After centrifugation of the sample for 5 min at 540—1200 *g* the aqueous layer is frozen in a dry-ice—acetone mixture and the organic phase decanted into another test tube. The organic layer is once more centrifuged and separated from an aqueous residue after freezing and then evaporated to dryness at 35° under a stream of nitrogen. The residue is reconstituted in 100—600 μl of elution medium and an appropriate aliquot injected onto the column.

Standard curves were prepared daily by spiking 2.0 ml of blank, drug-free plasma with 10 μg primaquine and 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 μg, respectively of quinidine (expected range of plasma concentrations in our studies), extracting them as described above.

RESULTS AND DISCUSSION

Quinidine has two basic functions with pk_a of 8.4 and 4.0. Even at the pH of plasma (7.4), quinidine is reproducibly extracted with our solvent system (see below). Two authentic samples of quinidine metabolites were made available to us, namely 2'-quinidinone and 3-OH-quinidine. A third metabolite was isolated from urine specimens during our investigations and tentatively identified as a product formed by rearrangement of an intermediate N-oxide [44]. This newly isolated metabolite will be referred to below as quinidine—oxide-R. Although O-desmethyl-quinidine has been reported [13] and would be expected to be extracted in our solvent system, we did not detect any other quinidine metabolite in our single dose bioavailability studies, either by HPLC or by TLC.

Using the chromatographic conditions described under Experimental, quinidine, dihydroquinidine, internal standard and three quinidine metabolites could be separated as shown in Fig. 1, where a test mixture has been injected. The complex mixture can be separated within 20 min from plasma samples (Fig. 2).

Of the solvent systems tested, the one described above gave best results in respect of relative retention times of the different compounds. According to the status of the column, slight adjustments in the ethanol content might be necessary to yield the separation shown in Figs. 1 and 2. In addition, the ethanolamine concentration can be varied to get different retention times; however, long equilibration times (1—2 h) are needed to achieve stable conditions. Ethanolamine is added because it apparently suppresses ionization, reduces the retention times and eliminates tailing. It yields symmetrical peaks with maximal theoretical plates. Ethanolamine is apparently superior to ammonia because the former provides a more stable solvent mixture, a prerequisite for automation of sample injection, such as was used in our routine analyses.

The HPLC method described in this paper has been used to quantitate

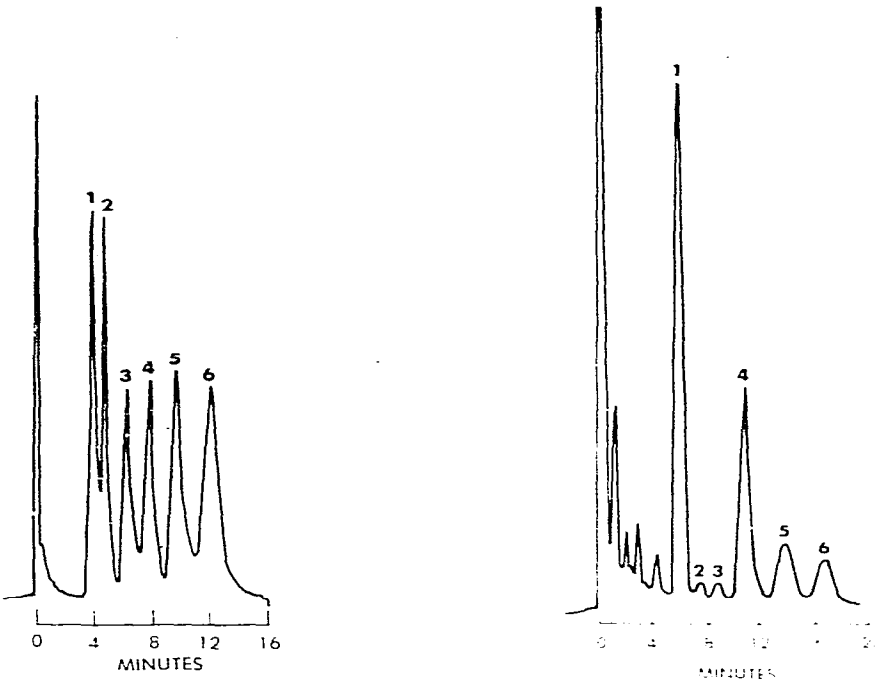

Fig. 1. HPLC separation of quinidine and metabolites in a test mixture. Column: Varian MicroPak-Si 10, 25 × 0.21 cm I.D.; mobile phase: hexanes—ethanol—ethanolamine (91.5:8.47:0.03); flow-rate: 1 ml/min; UV detection at 235 nm; peaks: 1 = quinidine, 2 = dihydroquinidine, 3 = 2'-quinidinone, 4 = primaquine, 5 = quinidine—oxide-R, 6 = 3-OH-quinidine.

Fig. 2. HPLC separation of quinidine and metabolites in patient plasma. Column: Varian MicroPak-Si 10, 25 × 0.21 I.D.; mobile phase: hexanes—ethanol—ethanolamine (92.97:7.0:0.03); flow-rate: 1 ml/min; UV detection at 235 nm; peaks: 1 = quinidine, 2 = dihydroquinidine, 3 = 2'-quinidinone, 4 = primaquine, 5 = quinidine—oxide-R, 6 = 3-OH-quinidine.

quinidine and metabolites in more than 5000 samples of blood or saliva. The limit of its sensitivity is at 20 ng/ml plasma. For plasma levels expected in the range of 20—100 ng/ml use of less internal standard is recommended.

Quantitation of quinidine was achieved on the basis of a standard curve, where known concentrations of drug have been plotted against the peak height ratio quinidine—primaquine. From these values a least square unweighted regression line was calculated.

To accommodate small variations in the chromatographic system which may occur from one day to another, standard curves were prepared daily. Due to insufficient amounts, no standard curve for 3-OH-quinidine, or quinidine—oxide-R could be prepared. Similarity of their extinction coefficients with that of quinidine was verified

Recovery and reproducibility of the HPLC method

In order to check the recovery and reproducibility of quinidine in the

extraction procedure with the solvent mixture ether—dichloromethane—isopropanol (6:4:1) plasma samples spiked with 0.1, 0.25, 0.5, 1.0, 1.5 and 2.0 μg/ml, respectively have been extracted according to the extraction procedure described above. The peak heights measured after extraction were compared with peak heights obtained after injecting exact volumes of a quinidine solution of known concentration onto the HPLC column by means of an injector loop. The result shows that quinidine is extracted over the range of 0.1—2.0 μg/ml by 91.5—93.6% with coefficients of variation (C.V.) between 0.9—3.8% and that 91.5% of primaquine internal standard is extracted at a concentration of 5.0 μg/ml (C.V. = 5%). The metabolites were shown to be extracted reproducibly with a C.V. of approx. 5%.

The slopes and intercepts of eight standard curves obtained over a period of about 3 months were compared in an analysis of variance [45] in order to assess their variation with time. As can be seen from Table I, the slopes but not the intercepts of the standard curves are found to vary with time ($p \leq 0.05$). Such changes are not unusual considering gradual changes in column performance.

TABLE I

ANALYSIS OF VARIANCE ON SLOPES AND INTERCEPTS DERIVED FROM THE STANDARD CURVES FOR QUINIDINE IN PLASMA

F Statistic (slope) (7, 32) = 3.370 S ($P \leq 0.05$); F statistic (intercept) (7, 32) = 2.026 NS ($P > 0.05$).

Curve No.	r^2	Slope	S.E. of slope	S.E. of slope (%)	Intercept	S.E. of intercept	S.E. of intercept (%)
1	0.9991	0.8379	0.0124	1.48	0.0312	0.0139	44.63
2	0.9991	0.8514	0.0131	1.54	−0.0025	0.0147	578.35
3	0.9977	0.8713	0.0209	2.40	0.0198	0.0235	118.69
4	0.9995	0.8982	0.0100	1.11	−0.0026	0.0112	432.05
5	0.9962	0.9591	0.0298	3.10	−0.0236	0.0334	141.85
6	0.9918	0.9350	0.0426	4.55	−0.0270	0.0478	177.04
7	0.9986	0.8739	0.0161	1.84	0.0141	0.0181	128.37
8	0.9987	0.9095	0.0162	1.78	−0.0110	0.0182	165.91

Comparison with other quinidine determination methods

Quinidine levels in patient blood samples, obtained by HPLC, were compared to results obtained by the single extraction method according to Edgar and Sokolow (Table II) and to the so-called double extraction method of Cramer and Isaksson (Table III)*.

Both of these methods yield higher results when compared to the results of the specific HPLC assay. The two sets of plasma samples were obtained from single oral doses of 400 mg quinidine sulfate administered to healthy adults. The only detectable metabolite at these concentrations was the quinidine—oxide-R. The values listed in column 4 of Tables II and III are the sums of the metabolite plus the quinidine concentration as measured by HPLC.

*The Edgar and Sokolow assay [5] will be referred to as the ES assay and the Cramer and Isaksson assay [6] as the CI assay.

TABLE II

COMPARISON OF QUINIDINE LEVELS MEASURED BY HPLC METHOD AND BY ES FLUORESCENCE ASSAY

Time after administration of quinidine (min)	Quinidine by HPLC (μg/ml)	Quinidine—oxide-R* by HPLC (μg/ml)	Quinidine + quinidine—oxide-R by HPLC (μg/ml)	Quinidine by ES (μg/ml)
0	0	0	0	0
5	0	0	0	0
10	0.07	0.04	0.11	0.15
15	0.19	0.07	0.26	0.39
25	0.54	0.14	0.68	0.98
30	0.67	0.15	0.82	1.12
40	0.84	0.14	0.98	2.36
46	0.91	0.14	1.05	1.46
51	0.92	0.20	1.12	1.88
75	0.69	0.14	0.83	1.32
91	0.69	0.17	0.86	1.02
105	0.66	0.17	0.83	0.88
122	0.53	0.14	0.67	0.74
181	0.46	0.14	0.60	0.82
242	0.41	0.08	0.49	0.63
307	0.41	0.09	0.50	0.50
360	0.32	0.06	0.38	0.51
421	0.26	0.07	0.33	0.43
480	0.24	0.07	0.31	0.37

*Quinidine—oxide-R refers to a newly isolated quinidine metabolite formed by rearrangement of an intermediate N-oxide.

Column 5 includes the results obtained directly using the ES or CI fluorescence assay. The quinidine values obtained by the ES assay (Table II, column 5) exceed the HPLC quinidine values (column 2) often by more than 100%. They usually exceed the combined quinidine plus metabolite concentrations, possibly indicating a changing blank value. On the other hand, the CI assay values (Table III, column 5) are reasonably explained in this set of samples by the sum of quinidine plus metabolite concentration (column 4). Both fluorescence assays therefore overestimate the intact quinidine.

In order to evaluate the degree of metabolite carryover, several sets of plasma samples were assayed by HPLC and fluorimetrically according to the CI method. The CI extracts were re-extracted with ether—dichloromethane—isopropanol (6:4:1) after alkalinizing to pH 9 with 3% NH_4OH. The content of quinidine and its metabolites in the CI extracts were then determined according to the HPLC method as described in the experimental section. Due to the instability of the internal standard in this procedure, amounts of quinidine, dihydroquinidine and metabolites in the samples were quantitated by comparison of their relative area under the chromatogram peaks to the areas obtained from injection of absolute amounts of quinidine.

Tables IV and V include data from one subject at steady state. Tables IV and VII include data from repeated assay of pooled patient plasma samples.

TABLE III

COMPARISON OF QUINIDINE LEVELS MEASURED BY HPLC METHOD AND BY CI FLUORESCENCE ASSAY

Time after administration of quinidine (min)	Quinidine by HPLC (μg/ml)	Quinidine—oxide-R by HPLC (μg/ml)	Quinidine + quinidine—oxide-R by HPLC (μg/ml)	Quinidine by CI (μg/ml)
0	0			0
6	0			0
15	0.37	0.11	0.48	0.47
19	0.65	0.12	0.77	0.76
28	0.95	0.18	1.13	1.24
34	1.14	0.21	1.35	1.48
43	1.28	0.24	1.52	1.54
48	1.27	0.29	1.56	1.74
54	1.24	0.22	1.46	1.57
61	1.21	0.21	1.42	1.50
76	1.28	0.31	1.59	1.49
92	1.20	0.26	1.46	1.49
106	1.16	0.22	1.38	1.31
124	1.09	0.24	1.33	1.01
183	0.87	0.19	1.06	1.06
242	0.84	0.21	1.05	0.88
306	0.73	0.08	0.81	0.78
363	0.60	0.12	0.72	0.82
426	0.46	0.10	0.56	0.55
487	0.47	0.09	0.56	0.47
860	0.24			
1390	0.10			

The direct HPLC determination indicated that the two sets of samples differed in the amounts and ratios of the constituents. The CI fluorescence assay in each instance overestimates the quinidine concentration. In Table IV the values range from 194 to 228% of the quinidine content measured by direct

TABLE IV

COMPARISON OF QUINIDINE LEVELS IN A PATIENT AT STEADY STATE, MEASURED BY HPLC METHOD AND BY CI METHOD

Plasma sample (time after last dose, min)	Plasma levels by HPLC method (μg/ml)			Plasma levels by CI method (μg/ml)
	Quinidine	Quinidine—oxide-R	3-OH-quinidine	
29	0.60	0.11	0.35	1.37
46	0.66	0.11	0.33	1.46
60	0.69	0.11	0.39	1.56
120	0.80	0.13	0.39	1.57
184	0.69	0.10	0.41	1.34

TABLE V

IDENTIFICATION AND QUANTITATION OF COMPOUNDS EXTRACTED BY CI ASSAY

Plasma sample (time after last dose, min)	Plasma levels by CI method (μg/ml)	Quantitation of quinidine and metabolites in CI samples by HPLC (μg/ml)		
		Quinidine	Quinidine—oxide-R	3-OH-quinidine
29	1.37	0.63		0.21
46	1.46	0.60		0.17
60	1.56	0.69	*	0.20
120	1.57	0.99		0.24
184	1.34	0.62		0.16

*Insufficient amounts for quantitation.

TABLE VI

COMPARISON OF QUINIDINE LEVELS IN POOLED PATIENT PLASMA, MEASURED BY HPLC METHOD AND BY CI METHOD

Sample	Plasma levels by HPLC method (μg/ml)					Plasma levels by CI method (μg/ml)
	Quinidine	Dihydro-quinidine	2'-Quini-dinone	Quinidine—oxide-R	3-OH-quinidine	
1	1.51	0.08	0.07	0.30	0.34	2.13
2	1.45	0.08	0.08	0.35	0.28	2.17
3	1.57	0.08	0.09	0.38	0.28	2.17

TABLE VII

IDENTIFICATION AND QUANTITATION OF COMPOUNDS EXTRACTED BY CI ASSAY

Sample	Plasma levels by CI method (μg/ml)	Quantitation of quinidine and analogues in CI samples by HPLC (μg/ml)				
		Quinidine	Dihydro-quinidine	2'-Quini-dinone	Quinidine—oxide-R	3-OH-quinidine
1	2.13	1.39	0.11		0.03	0.18
2	2.17	1.51	0.11	*	0.03	0.19
3	2.17	1.44	0.09		0.04	0.17

*Insufficient amounts for quantitation.

HPLC. In Table VI the corresponding values average at 143%. Apparently, virtually all of the quinidine is extracted by the benzene extraction as shown by the subsequent HPLC determination (see quinidine concentration in Tables IV + V and VI + VII, respectively). This is probably also true for dihydro-quinidine. In addition, the studies indicate that the metabolites also are partly

extracted by benzene, 3-OH-quinidine to 50—60% and quinidine-oxide-R to 10%. The extraction efficiency of benzene for 3-OH-quinidine of 50—60% correlates well with the observation of Drayer et al. [41]. Insufficient concentrations of 2'-quinidinone were present to permit estimation of the benzene extraction efficiency for this metabolite. Since the concentrations of the metabolites vary from patient to patient, the error introduced by use of the CI assay is unpredictable and no correction factor can be applied. This is particularly important in patients with renal insufficiency.

Although limited studies were done, the method of Armand and Badinand [27] as applied by Huynh-Ngoc and Sirois [3] was investigated in the same fashion as with the CI method. It was noted that the repeated extraction of the samples with the 0.1 *N* NaOH led to large discrepancies in the fluorescence assay as shown in Table VIII.

TABLE VIII

COMPARISON OF QUINIDINE LEVELS IN A PATIENT AT STEADY STATE, MEASURED BY HPLC METHOD AND BY ARMAND—BADINAND METHOD

NA = Not available.

Specification of plasma sample (time after last dose, min)	Plasma levels by HPLC method (μg/ml)			Plasma levels by Armand—Badinand method (μg/ml)
	Quinidine	Quinidine—oxide-R	3-OH-quinidine	
301	0.72	NA	NA	1.16
471	0.51	0.05	0.34	0.74
0	0.56	0.07	0.36	0.88
38	0.74	0.13	0.44	1.19

From these experiments it can be concluded that in spite of its superiority over protein precipitation methods and single extraction methods the double extraction CI method still lacks specificity for quinidine even when an additional clean-up step with alkaline washings is introduced. It is obviously impossible to predict a priori quinidine and metabolite ratios in humans. Therefore, no correction factor can be devised to estimate true quinidine levels from data obtained by unspecific methods as has been suggested for data obtained from precipitation methods [37]. In order to attempt to assess the specificity, several new assays [35, 37, 42, 46] have been compared to the CI method or its modification according to Armand et al. If a good correlation between the two assays in question was found, the authors assumed them to be specific and reliable. The results of the present study, however, show that their standard of comparison is nonspecific and unreliable. Therefore these new assays must be re-assessed as to their claims of specificity.

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