GLC Determination of Quinidine from Plasma and Whole Blood

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Abstract □ A method for the quantitative estimation of quinidine in biological fluids is described. Plasma containing quinidine, to which a known amount of cinchonidine is added as the internal standard, is extracted at pH 12.0 with benzene. The residue from the extract is mixed with 25 μl of trimethylanilinium hydroxide in methanol, and aliquots (1–2 μl) are injected into a gas chromatograph in which the injection port is held at 350°. The methyl derivatives of quinidine and the internal standard give well-separated symmetrical peaks. Detection by flame ionization gives a linear response over the range of 0.2–12.0 μg quinidine/ml plasma. The limit of detectability is 0.05 μg/ml and the method is adequate for following blood profiles of 200-mg quinidine sulfate doses in humans.

Keyphrases □ Quinidine—GLC determination in plasma and whole blood □ GLC—determination, quinidine in plasma and whole blood

The need for quantitative studies of blood concentrations in relation to dose and therapeutic effect of quinidine has been emphasized in recent years (1–4). Fluorometric (5–9), TLC (10–12), and GLC (5, 13) methods have been reported for the estimation of quinidine in pharmaceutical preparations and biological fluids. These methods are either cumbersome and time consuming or insensitive and nonspecific. The objective of this study was to develop a sensitive, specific, GLC method suitable for pharmacodynamic studies following single doses of quinidine sulfate (100–400 mg).

EXPERIMENTAL

Reagents—Quinidine sulfate[1], cinchonidine sulfate[2], and benzene[3] were obtained commercially. Methanolic trimethylanilinium hydroxide was synthesized according to the method of Barret (14). Plasma and blood were obtained from the Red Cross Blood Bank. All other chemicals employed were analytical grade.

GLC—A gas chromatograph[4] equipped with a flame-ionization detector was employed. The column was coiled stainless steel tubing[5], 1.2 m (4 ft) long by 0.3 cm (0.125 in.) o.d., packed with phenyl methyl dimethyl silicone[5] (OV-7) on acid-washed, dimethylchlorosilane-treated, high-performance Chromosorb W[5] support, 80–100 mesh. The column was conditioned by injecting 25–50 μl of column conditioner[6] [a mixture of *N,O*-bis(trimethylsilyl)acetamide, trimethylsilyldiethylamine, and hexamethyldisilazane] and maintaining the column at 300° for 18 hr with low nitrogen flow. Operating conditions were: injection port, 350°; column, 270°; and detector, 350°. The flow rate of nitrogen was 20 ml/min. Hydrogen and compressed air flow rates were adjusted to give maximum response.

Procedure—To 1 ml plasma or whole blood samples in Teflon-lined, screw-capped centrifuge tubes (20 ml) are added 1 ml of distilled water, 1 ml of internal standard (≃2 μg cinchonidine/ml), 1 ml 1 *N* NaOH, and 10 ml benzene. The samples are extracted by shaking[7] for 10 min at 19 rpm followed by centrifugation at 2500 rpm for 10 min. Nine milliliters of the benzene layer is transferred into a custom-made evaporating tube[8] (15). The benzene extract is evaporated to dryness at 75° under a stream of dry nitrogen, and 25 μl of a methanolic solution of trimethylanilinium hydroxide (0.2 *M*) is added. The contents are dissolved by vibrating with a vortex mixer[9] before injecting 1–2 μl into the gas chromatograph. The retention times of flash-methylated quinidine and cinchonidine are 6.9 and 4.4 min, respectively.

Standards and Calibration Curve—Quinidine and cinchonidine standards were prepared by dissolving appropriate amounts of quinidine sulfate and cinchonidine sulfate in distilled water to obtain stock solutions of approximately 20 μg base/ml in each case. Appropriate dilutions of these stock solutions were made to obtain concentrations over the 0.2–12.0-μg/ml range. Peak height ratios were calculated by dividing the peak height of the quinidine methyl derivative by that of cinchonidine methyl derivative. Calibration curves were constructed from the results of spiked control plasma samples by plotting the peak height ratios against the concentration of quinidine.

Table I—Recovery of Quinidine and Cinchonidine from Plasma Determined by GLC Assay

Micrograms Added to 1 ml Plasma	*n*	Mean Micrograms Recovered	Mean Percent Recovery	Standard Deviation of Percent Recovery
Quinidine				
1.96	5	1.98	100.71	3.48
7.86	5	7.53	95.84	1.55
Mean 98.27 ± 3.61%				
Cinchonidine				
2.11	7	1.72	81.63	2.84

Table II—Estimation of Quinidine Added to Plasma by GLC

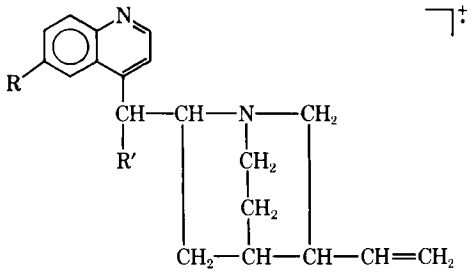
Quinidine Added, μg	*n*	Mean Peak Height Ratio	Standard Deviation	*CV*, %[a]
0.20	7	0.092	0.009	9.68
0.39	4	0.157	0.002	1.38
0.79	4	0.238	0.007	2.83
1.96	4	0.609	0.031	5.12
3.93	4	1.087	0.044	4.09
7.86	4	2.522	0.086	3.43
11.78	4	3.430	0.198	5.76

[a] Mean $CV = 4.61\%, y = mx$ where $m = 0.295 \pm 0.008$; $r = 1$.

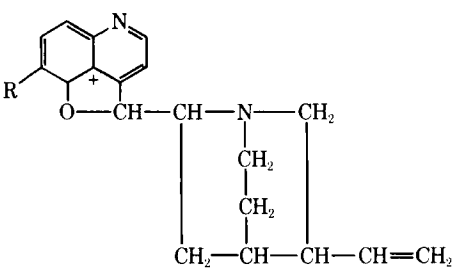
RESULTS AND DISCUSSION

The recently reported method of Smith *et al.* (5) for the measurement of impurities in quinine and quinidine preparations employs a silylation technique before GLC analysis. To adapt this

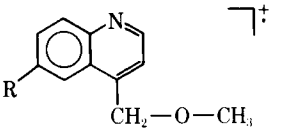
[1] J. T. Baker Laboratory chemicals.
[2] Merck Chemical Co.
[3] Caledon Laboratories, Georgetown, Ontario, Canada.
[4] Perkin-Elmer model F-11.
[5] Chromatographic Specialties, Brockville, Ontario, Canada.
[6] Silyl-8, Pierce Chemical Co.
[7] On a Roto-Rack, Fisher Scientific Co.
[8] Custom made by Canadian Laboratories Supplies Ltd., Montreal, Canada.
[9] Fisher Scientific Co.



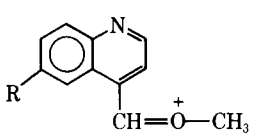
Ia: *m/e* 338, R = OCH_3, R′ = OCH_3
Ib: *m/e* 308, R = H, R′ = OCH_3
IIa: *m/e* 308, R = OCH_3, R′ = H
IIb: *m/e* 278, R = H, R′ = H



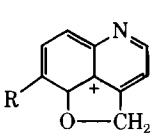
IIIa: *m/e* 323, R = OCH_3
IIIb: *m/e* 293, R = H



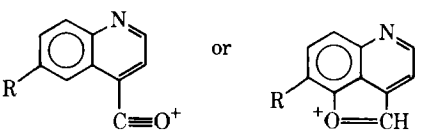
IVa: *m/e* 203, R = OCH_3
IVb: *m/e* 173, R = H



Va: *m/e* 202, R = OCH_3
Vb: *m/e* 172, R = H



VIa: *m/e* 188, R = OCH_3
VIb: *m/e* 158, R = H



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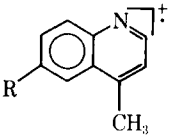
VIIb: *m/e* 156, R = H



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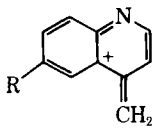
VIIIb: *m/e* 143, R = H



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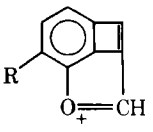
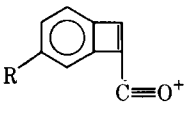
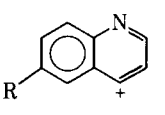
IXb: *m/e* 142, R = H



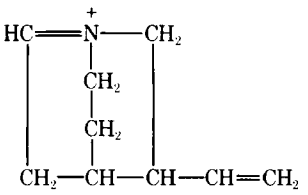
Xa: *m/e* 159, R = OCH_3
Xb: *m/e* 129, R = H



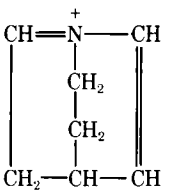
XIa: *m/e* 159, R = OCH_3
XIb: *m/e* 129, R = H



XIIa: *m/e* 158, R = OCH_3
XIIb: *m/e* 128, R = H



XIII: *m/e* 136



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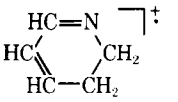
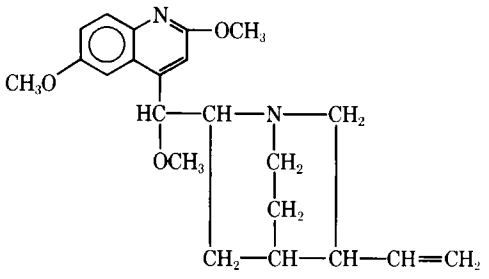
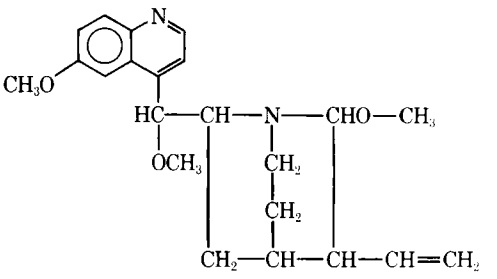


XV: *m/e* 81



XVI



XVII

Postulated mass spectral fragmentation of flash-methylated quinidine and cinchonidine

method to biological fluids, the alkaloids must be extracted and freed from any trace of moisture before silylation. This drying step can be avoided by the use of trimethylanilinium hydroxide as a methylating reagent for GLC analysis. Plasma samples do not need to be dried completely and the methylation reaction in the injection port is instantaneous at 300° (16, 17).

Flash-heater methylation of quinidine and cinchonidine with trimethylanilinium hydroxide gave sharp peaks with retention times of 6.9 and 4.4 min, respectively, under the conditions described under *General Procedure*. The structures of flash-methylated quinidine and cinchonidine were established by combined GLC-mass spectrometry[10]. The mass spectrum of methylated quinidine (Fig. 1A) showed a molecular ion at *m/e* 338. Characteristic ions at *m/e* 323, 308, 203, 202, 188, 186, 173, 172, 159, 158, 136, 129, 108, and 81 were tentatively assigned the structures illustrated. These fragmentations suggest that flash-methylated quinidine has Structure Ia. The mass spectrum (Fig. 1B) of flash-methylated cinchonidine showed a molecular ion at *m/e* 308 and other major ions at *m/e* 293, 278, 173, 172, 156, 143, 142, 136, 129, 128, 108, and 81. Structures for these ions were postulated, indicating that flash-methylated cinchonidine has Structure Ib.

Figure 2A shows a typical chromatogram obtained by processing control blank plasma as described in the procedure, in which the internal standard, cinchonidine, was omitted. The extraneous peaks I, II, V, and VI at retention times 2.8, 3.1, 14.2, and 17.2 min, respectively, were observed in the chromatograms of all human as well as whole blood samples and remain unidentified.

[10] Perkin-Elmer model 900 gas chromatograph attached to a Hitachi-Perkin-Elmer model RMSU mass spectrometer through a jet separator.

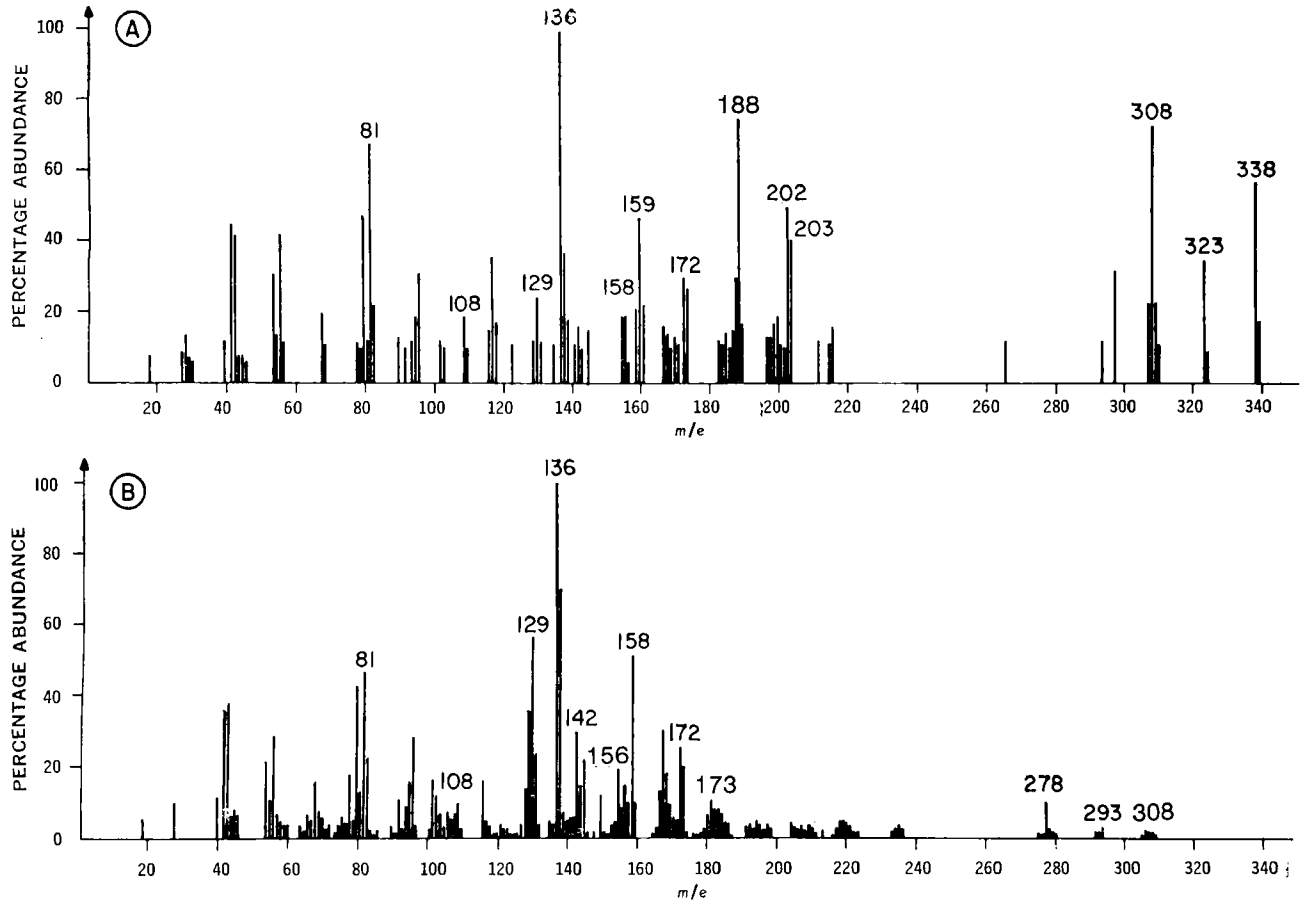


Figure 1—*GLC–mass spectrometry of flash-methylated quinidine (A) and cinchonidine (B).*

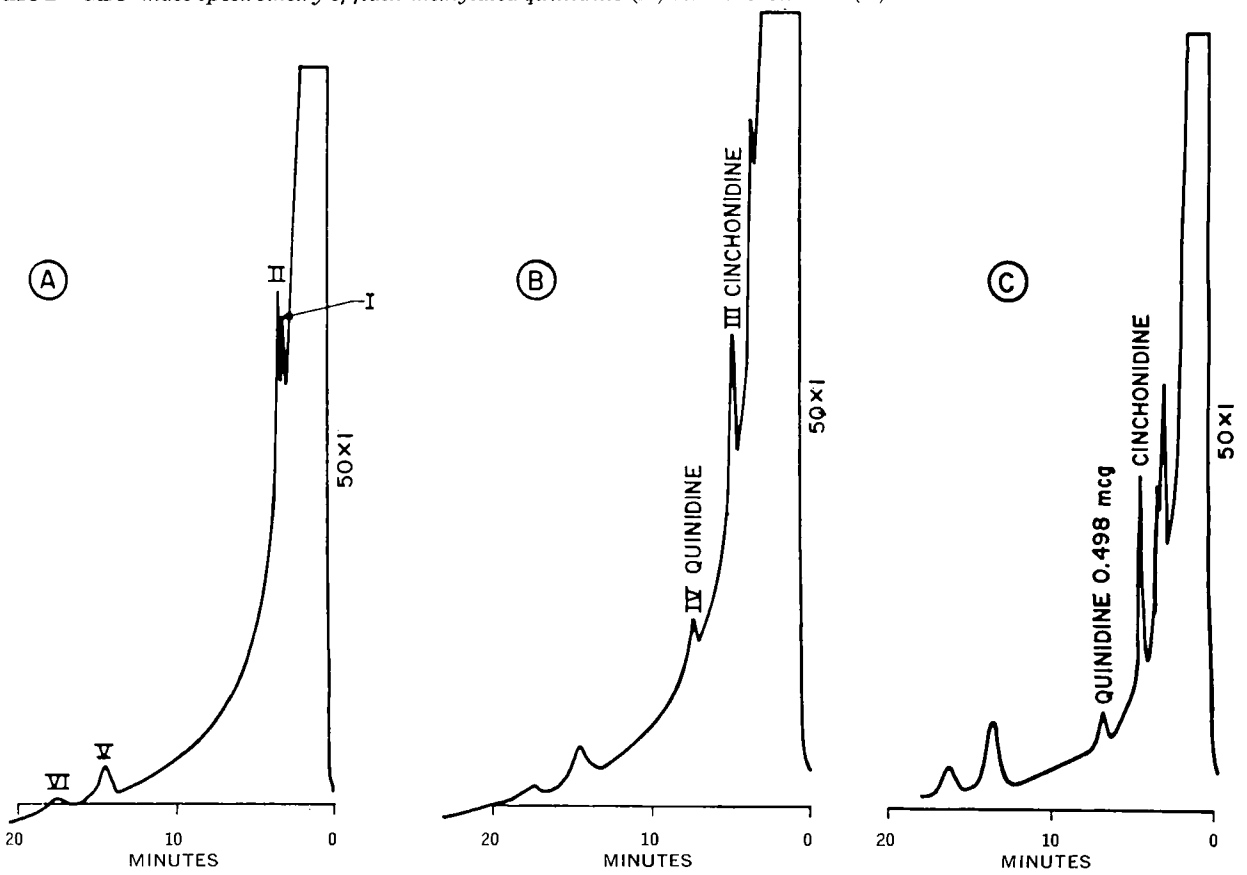


Figure 2—*Chromatograms obtained from flash methylation of plasma blank (A), plasma spiked with 0.79 μg quinidine and 2.11 μg cinchonidine (B), and plasma from volunteer receiving 200 mg quinidine sulfate (C).*

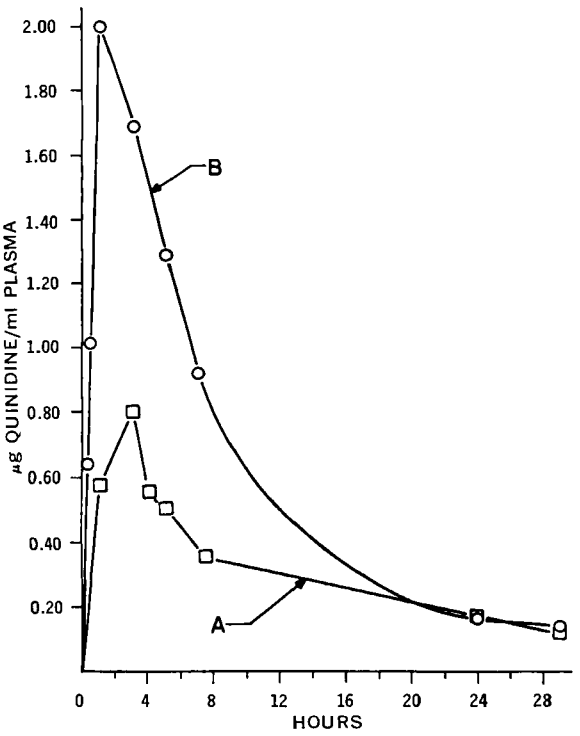


Figure 3—*Plasma profile of quinidine. Key: (A), healthy male volunteer (73 kg) receiving a 200-mg tablet of quinidine sulfate; and (B), dog receiving 10 mg quinidine sulfate/kg.*

A chromatogram obtained when the method was applied to spiked plasma containing 0.79 μg of quinidine and 2.11 μg of cinchonidine as the internal standard is shown in Fig. 2B, where it is clear that the extraneous peaks I, II, V, and VI do not interfere with the peak of quinidine IV (6.9 min) and the internal standard peak III (4.4 min). Figure 2C shows a chromatogram from a 1-ml plasma sample of a male volunteer who received a 200-mg tablet of quinidine sulfate[11]. An analysis time of 20 min was achieved. The metabolites of quinidine, *i.e.*, 2-hydroxyquinidine and monohydroxyquinidine (18), do not interfere with the assay, because on flash methylation (XVI and XVII) they gave retention times of 12.1 and 10.6 min, respectively.

The response of the flame-ionization detector to quinidine was linear with concentrations in the 0.2–12-μg/ml range (Table II). The overall recoveries of quinidine and cinchonidine from plasma at pH 12.0 by extraction with benzene are 98.27 ± 3.61 and 81.63 ± 2.84%, respectively (Table I). The ratio of the peak heights of quinidine to the internal standard plotted against concentration in the 0.2–12.0-μg/ml range gave a straight line passing through the origin (r = 1). A mean slope value of 0.295 ± 0.008 was obtained. The overall coefficient of variation was 4.61% (Table II).

The GLC procedure was compared with the spectrofluorometric method of Cramer and Isaksson (6). Routine analysis by both methods of duplicate plasma samples from a dog administered 10 mg/kg of quinidine sulfate[1] gave good agreement with an overall difference of 2% (Table III).

Application of the method to plasma level determinations was demonstrated (Fig. 3). A 200-mg dose of quinidine sulfate[11] was given to a healthy male volunteer (73 kg), and plasma was withdrawn at intervals over 29 hr and analyzed (Fig. 3A). A plasma profile of a dog (29.6 kg) receiving 10 mg quinidine sulfate[1]/kg is shown in Fig. 3B.

[11] Rougier Inc., Montreal, Canada.

Table III—Comparison of Plasma Levels by GLC and Spectrofluorometric Methods following Administration of Quinidine Sulfate (10 mg/kg) to a Dog (29.6 kg) (n = 2)

Hours after Administration	Concentration Determined by: GLC, μg/ml	Concentration Determined by: Spectrofluorometry, μg/ml
1	2.00	2.04
2	1.80	1.84
3	1.64	1.67

The described GLC method is simple, sensitive, and specific in that it distinguishes quinidine from its metabolites and can be employed for single- as well as multiple-dose pharmacodynamic studies.

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