SUMMARY

1. Bromcresol blue at pH 6.6 forms a chloroform-extractable complex with hyoscyamine (atropine); tropine and scopolamine do not interfere.

2. Both hyoscyamine and scopolamine form chloroform-extractable complexes using bromthymol blue at pH 5.6. In this way, mixtures of both alkaloids can be determined without preliminary separation.

3. The hyoscyamine content in belladonna tincture can be accurately and selectively determined using as little as 0.1–0.2 ml. of the tincture. Bromcresol purple is used at pH 6.6 and the complex formed is treated with 0.1 *N* NaOH and measured at 580 nm. Neither tropine nor scopolamine interferes.

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NMR Quantitative Analysis of Quinidine in Mixtures of Quinidine and Hydroquinidine

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Abstract □ A new procedure for the rapid quantitative analysis of quinidine in mixtures of quinidine and hydroquinidine is described. The method is based upon measurement of the NMR spectrum of these compounds in deuterated chloroform, using 2,3,5-tribromothiophene as an internal standard. The signal chosen is from the vinyl group of quinidine. The great advantage of this procedure is that it permits the determination of the percentage of hydroquinidine in commercial bulk quinidine.

Keyphrases □ Quinidine in mixtures with hydroquinidine—NMR analysis □ Hydroquinidine as impurity in quinidine bulk—NMR analysis □ NMR spectroscopy—analysis, quinidine and hydroquinidine mixtures

Commercial bulk quinidine, the base and its salts, contains variable amounts of hydroquinidine. The USP identification test for quinidine by TLC (1) does not separate these two cinchona alkaloids (2). Many analytical procedures for quinidine have been reported, including UV spectrophotometric (3, 4), colorimetric (5), fluorometric (6), titrimetric (1), and gravimetric (7, 8) determinations. All of these procedures determine the total mixture of alkaloids: quinidine and hydroquinidine. Work in this laboratory indicates that the UV and visible absorptivity and the quantum efficiency of fluorescence are the same for quinidine and hydroquinidine.

A method for their separation and identification by TLC was described previously (2, 9). NMR spectroscopy affords a suitable method of distinguishing between the $-CH{=}CH_2$ and $-CH_2{-}CH_3$ groups present in quinidine and hydroquinidine, respectively. The quinidine spectrum (10) in deuterated chloroform possesses signals that are absent in the hydroquinidine spectrum and are thus available for use in quantitative analysis. A specific NMR quantitative analytical method for quinidine is described here.

EXPERIMENTAL

Spectra were obtained at 60 MHz. using an analytical NMR spectrometer[1]. All spectra were taken at 8 Hz., and a chart width of 72 Hz. was used for all integrals. Tetramethylsilane in deuterated chloroform[2] was used as an internal reference to measure chemical shifts.

Assay Procedure—Pure quinidine base was obtained from quinidine sulfate[3] by eliminating hydroquinidine by the method of Thron and Dirscherl (11).

Approximately 60 mg. of quinidine and 65 mg. of the internal reference 2,3,5-tribromothiophene[4] were accurately weighed and dissolved in a volumetric flask of 1 ml. of deuterated chloroform containing tetramethylsilane. The NMR spectrum was obtained and integrated five times through the region of interest. The mean of the integrated values was used for quantitative calculations.

Calculation—

$$W_{quin} = \frac{EW_{quin}}{EW_{std}} \times \frac{A_{quin}}{A_{std}} \times W_{std} \times F \qquad \text{(Eq. 1)}$$

where:

W_{quin} = weight of quinidine in milligrams
EW_{quin} = molecular weight of quinidine divided by the number of protons in the signal chosen, 324.45/1 = 324.45
EW_{std} = molecular weight of standard divided by the number of protons in the signal chosen, 320.84/1 = 320.84
A_{quin} = integral value of quinidine signal at 5.16 p.p.m.
A_{std} = integral value of standard signal at 6.93 p.p.m.

[1] Perkin-Elmer R-12B.
[2] Stohler Isotope Chemicals, Rutherford, N. J.
[3] Aldrich Chemical Co., Inc., Montréal, Québec, Canada.
[4] K & K Laboratories, Plainview, N. Y.

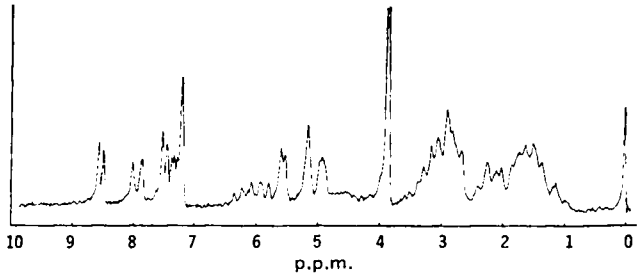


Figure 1—*NMR spectrum of quinidine in $CDCl_3$.*

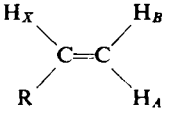
W_{std} = weight of standard in milligrams
F = correction factor

RESULTS AND DISCUSSION

The NMR spectra of quinidine and hydroquinidine[4] in deuterochloroform solutions are shown in Figs. 1 and 2, respectively. The solvent contains about 1% tetramethylsilane, which produces the reference signal at zero on the parts per million or δ scale on the charts. The concentrations of quinidine and hydroquinidine are 50.72 and 57.60 mg./ml., respectively.

The positions of the observed chemical shifts are located at lower fields than reported by Dega-Szafran and Mitura (10). The results were confirmed by recording these spectra on another spectrometer[5]. Peaks at 5.88 and 6.13 p.p.m. (δ) for quinidine and hydroquinidine, respectively, were also reported in saturated solutions and attributed to the hydroxylic proton (10). In this study, saturated solutions at room temperature also presented these peaks at δ values of 6.24 and 6.70 p.p.m. for quinidine and hydroquinidine, respectively. The rise in concentration of these solutes in $CDCl_3$ increases the extent of intermolecular hydrogen bonding. Hydrogen bonding decreases the electron density around the proton and thus moves the proton absorption to lower field. For instance, when the concentration of hydroquinidine is weak, the proton shift is presumably located at a δ value of 5.60 p.p.m. However, when this concentration is increased above 125 mg./ml., work in this laboratory shows that the peak starts moving gradually to lower field.

Quinidine is an *ABX* system:



in which the protons H_A, H_B, and H_X are presumably located in Fig. 1 at 4.90, 5.14, 5.76, 5.90, 6.05, 6.20, and 6.32 p.p.m. (10). The 2,3,5-tribromothiophene (12) was chosen as an internal standard for quantification of quinidine, because its NMR spectrum in deuterated chloroform presents a singlet at 6.90 p.p.m. and does not interfere with the spectrum obtained from a mixture of quinidine and hydroquinidine (Fig. 3).

The singlet at 5.16 p.p.m. was chosen for quantification of quinidine because of its high intensity of integration and its absence in the hydroquinidine spectrum. A correction was made on the integral value of this peak, because a resonance peak (or peaks) of some protons on the substance studied having a chemical shift in the close proximity of the chosen resonance signal for integration interferes in a constant ratio with this signal. The relative intensity of the integrals of each peak depends on the couplage of the protons (13). In the NMR analysis of mixtures of aspirin, phenacetin, and caffeine, a correction was made by Hollis (14) for the carbon-13 sideband from the aspirin peak which falls beneath the analytical methyl peak of caffeine at 3.4 p.p.m. Such carbon-13 sidebands could also contribute to the interference with the integral of the singlet at 5.16 p.p.m. Furthermore, in some cases, spinning sidebands can interfere with integration. In this work, the spinning rate of the sample tube was adjusted between one-fifth and three-fifths of the maximum speed position of the spinner air control valve on the spectrometer. For this range of spinning rate, the integral value of the chosen signal is constant. It does appear then that, in these conditions, spinning sidebands do not interfere with the integral of the chosen signal, because the intensity and displacement of these sidebands from a resonance peak are recognized to be proportional to the spinning speed (15). The correction factor that must be applied to the integral of the chosen analytical resonance signal is determined experimentally with the internal standard.

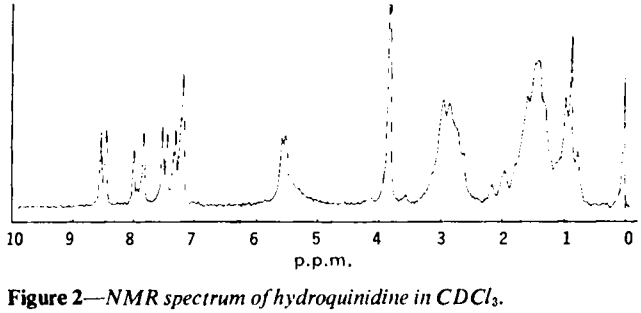


Figure 2—*NMR spectrum of hydroquinidine in $CDCl_3$.*

[5] Varian A-60.

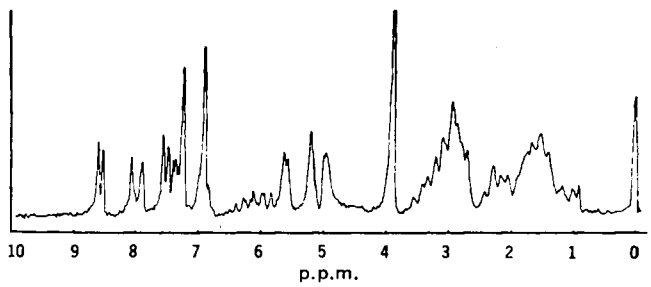


Figure 3—*NMR spectrum of quinidine and hydroquinidine mixture in $CDCl_3$.*

The results obtained from the analysis of 11 known samples of purified quinidine are shown in Table I. The correction factor, F, was calculated from Eq. 1 for each sample. The mean value of F was then used to evaluate the amount of quinidine found by Eq. 1. The mean of percent found is 100.04 and the standard deviation from the mean is 0.972. Synthetic mixtures of purified quinidine and hydroquinidine were made to detect the influence of hydroquinidine on the quinidine spectrum, particularly on the singlet at 5.16 p.p.m. Table II shows five different mixtures containing up to 40.97% of hydroquinidine. The mean of percent found and the standard deviation from the mean are not statistically different from those of Table I. For Student's t test and Fisher's F test, the level of significance, p, is less than 0.01 for differences of the means and standard deviations from the mean, respectively.

Table I—Analysis of Quinidine by NMR

Amount Weighed of Quinidine, mg.	Amount Weighed of Standard, mg.	F	Amount Found, mg.	Percent of Amount Found	Deviation from Mean, %
41.51	83.42	0.925	42.29	101.88	1.84
49.46	73.75	0.947	49.18	99.44	0.60
52.82	83.82	0.954	52.11	98.66	1.38
52.99	80.18	0.946	52.75	99.55	0.49
54.96	53.02	0.948	54.60	99.34	0.70
57.55	54.24	0.947	57.25	99.49	0.55
59.15	66.09	0.936	59.55	100.68	0.64
61.78	64.74	0.944	61.66	99.81	0.23
61.87	55.50	0.943	61.80	99.89	0.15
73.15	83.31	0.928	74.28	101.54	1.50
78.10	81.40	0.940	78.28	100.23	0.19
Mean F: 0.942			Mean percent found:		100.04
SD: 0.0079			*SD:* 0.972		
Average deviation from mean: 0.0063			Average deviation from mean: 0.75%		

Table II—Analysis of Quinidine by NMR in Synthetic Mixtures of Quinidine and Hydroquinidine

Amount Weighed of Quinidine, mg.	Amount Weighed of Hydroquinidine, mg.	Percent Hydroquinidine in Mixtures	Amount Weighed of Standard, mg.	Amount Found of Quinidine, mg.	Percent of Amount Found	Deviation from Mean, %
52.72	6.00	10.22	73.77	52.14	98.89	1.21
49.60	14.29	22.37	55.77	50.00	100.81	0.71
65.71	20.83	24.07	89.03	65.93	100.33	0.23
55.08	26.06	32.12	77.61	54.77	99.44	0.66
47.24	32.79	40.97	58.85	47.73	101.04	0.94
					Mean percent found: 100.10%	
					SD: 0.912	
					Average deviation from mean: 0.748%	

The precision, reproducibility, and specificity of this NMR method make it useful as a rapid procedure for the determination of quinidine in the absence or presence of hydroquinidine. However, because of the displacement of the chemical shift of the hydroxyl proton in the region of the internal standard at very high concentration, the weight of the sample must be kept as low as possible. Excellent results were obtained for samples of 40–80 mg./ml. Preliminary work indicates that this method can also be applied to the analysis of quinidine in mixtures of the salts of these two alkaloids.

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