Examination of Quinidine and Quinine and Their Pharmaceutical Preparations

EDWARD SMITH[▲], SUSAN BARKAN, BRUCE ROSS, MILLARD MAIENTHAL, and JOSEPH LEVINE

Abstract ☐ Methods for the detection and measurement of possible contaminants in quinidine and quinine and their pharmaceutical preparations were investigated to establish the actual composition of quinidine and quinine now on the market. Since no single TLC or GC procedure separated all of the compounds under consideration, multiple systems encompassing them were used to characterize the samples. Chromatographic and fluorometric techniques were applied to analysis of a wide variety of samples of quinidine and quinine and their pharmaceutical formulations. The dihydro analogs were found in all of the 75 samples examined, and the desmethoxy analogs (cinchonine or cinchonidine) were found in about half of the samples. The level of the dihydro alkaloids was higher in quinidine than in quinine (usually 5–9% in quinidine and 3–6% in quinine); the level of the desmethoxy analog was higher in quinine than in quinidine (usually 0–0.5% in quinidine compared to 1–2% in quinine). No epi-alkaloid, quininone, or quinotoxine was detected in any sample.

Keyphrases ☐ Quinidine, salts, and commercial formulations—composition, identification of dihydro and desmethoxy analogs as contaminants ☐ Quinine, salts, and commercial formulations—composition, identification of dihydro and desmethoxy analogs as contaminants ☐ Cinchona alkaloids—analysis of quinidine and quinine

Palmer *et al.* (1), in a recent study of the metabolites of quinidine, reported that congeneric alkaloids were present at levels of 10–30% in the quinidine samples used. They attributed the presence of these compounds to the current method of preparing quinidine (*via* base-catalyzed epimerization of quinine), which yields a complex mixture of epimers. Because of the lower therapeutic index of some epimers, a high epimer content might be undesirable. They recognized the possible presence of dihydroquinidine, which may be present in significant amounts in commercial quinidine (1, 2).

As a consequence, a study to establish the actual status of quinidine now on the market was initiated in these laboratories. Based on its source, the compounds most likely to be associated with quinidine are quinine, cinchonine, cinchonidine, epiquinidine, epiquinine, quininone, and quinotoxine, together with their dihydro analogs.

Several workers used paper chromatography for the qualitative analysis of the various cinchona alkaloids (3–5) and for study of the decomposition of quinidine solutions (6, 7). Palmer *et al.* (1) used GC but examined only the metabolites; many other workers using GC for cinchona alkaloid detection limited their studies to the four major vinyl alkaloids: quinine, quinidine, cinchonidine, and cinchonine. TLC was used first by many workers to separate these four vinyl bases and, more recently, to separate them from their dihydro analogs.

The USP XVIII monographs for quinidine and quinine salts (8) include a TLC test for "other cinchona alkaloids." This system differentiates quinine from quinidine and detects epiquinidine and epiquinine, but it does not separate dihydroquinidine from quinidine. Recently, Bohme and Bitsch (9, 10) applied two-dimensional TLC to separate quinine, quinidine, dihydroquinine, dihydroquinidine, cinchonine, and cinchonidine, but they did not investigate the presence of the epibases, quininone, and quinotoxine. They determined the quinine content of cinchona bark by measuring the UV absorbance of the material recovered from a TLC plate after a double development of the chromatogram.

Storck *et al.* (11) also separated the main alkaloids of cinchona bark by TLC and determined the quinine and cinchonidine content spectrophotometrically. Suszko-Purzycka and Trzebny (12) made a semiquantitative determination of the dihydro content of the individual alkaloids by visual comparison of the spots. Hartel and Korhonen (13) and Hartel and Harjanne (14) separated quinidine, dihydroquinidine, and quinidine metabolites isolated from biological fluids. They removed the separated zones from the TLC plate, extracted the alkaloid from each of them, and determined the fluorescence of the extracts. They reported that the quinidine preparations used in their study contained 12–13% dihydroquinidine. Gutzwiller and Uskokovic (15) used preparative TLC to isolate quinine and quinidine epibases. Vacha *et al.* (16) described TLC systems that separated epiquinidine or epiquinine from their respective analogs but did not separate the dihydro alkaloids from the parent alkaloids.

Recently, Roder *et al.* (17) reported the determination of the quinine, quinidine, cinchonidine, and cinchonine content of the extract of cinchona bark by densitometry of the fluorescent compounds on thin-layer chromatograms. They treated the developed chromatogram with a mixture of ether and concentrated sulfuric acid to activate cinchonidine and cinchonine. Although the four alkaloids were not completely resolved, the two pairs of alkaloids could be quantitated by the use of selective activation and emission wavelengths.

The scope of the present study included quinine in order to obtain a complete picture of the quality of the cinchona alkaloids now marketed as pharmaceuticals. Since no single TLC or GLC system would separate all of the compounds under consideration, multiple systems that encompassed all of them were used to characterize the samples.

EXPERIMENTAL

Materials—Cinchonine, cinchonidine, quinine, and quinidine free of their respective dihydro analogs were prepared from the commercial products *via* their mercury addition compound, essentially by the method of Thron and Dirscherl (18). Typically, a solution of 5 g. of quinidine sulfate in 50 ml. of 10% sulfuric acid is added to a solution of 7.5 g. of mercuric acetate in 75 ml. of 5%

Table I—Purified Cinchona Alkaloids

Compound	Solvent for Recrys-tallization	Melting Point Observed[a]	Melting Point Reported	Reference
Cinchonine	Alcohol	258–260°	264°	20, 21
Cinchonidine	Alcohol	216°	205,210°	20, 22
Dihydrocin-chonine	Alcohol	280°	270.5–273°	23
Dihydrocin-chonidine	Alcohol-water	232–234°	232°	20
Dihydroquinine	Benzene-hexane	173°	173°	20
Dihydro-quinidine	Alcohol	174°	169°	20
Quinine	Benzene-hexane	176°	177°	20
Quinidine	Alcohol	176°	173°	20
Quininone	Ether	109°	107–108.5°	19

[a] Melting points were determined with a DuPont model 900 differential thermal analyzer.

Table II—hR_f Values of Cinchona Alkaloids

Alkaloid	SG Layer[a], Solvent 1[b]	SH Layer, Solvent 2	SH Layer, Solvent 3
Quininone	76	74	64
Epiquinidine	68	43	32
Epiquinine	64	41	31
Cinchonine	54	38	43
Dihydrocinchonine	48	23	30
Quinidine	45	46	52
Quinotoxine (quinicine)	45	15	12
Cinchonidine	40	40	48
Dihydroquinidine	39	34	39
Dihydrocinchonidine	37	27	35
Quinine	24	42	50
Dihydroquinine	23	32	39
Quinidine thioglycerol adduct	0	11	38

[a] SG = silica gel G or GF, 0.25 mm. SH = silica gel H, 0.25 mm. [b] Solvent 1 (8) = chloroform–acetone–diethylamine (50:40:10). Solvent 2 = chloroform–acetone–methanol–ammonium hydroxide (60:20:20:1). Solvent 3 = methanol–ammonium hydroxide (100:1).

acetic acid. The solution is heated 4 hr. at 40–50°, cooled to room temperature, and made basic with ammonium hydroxide. The dihydroquinidine is extracted with ether. The aqueous layer is acidified with dilute sulfuric acid, and the mercury addition compound is reduced by adding sodium sulfite portionwise until no more mercury is precipitated. After mercury is removed by repeated filtration, the filtrate is made basic with ammonium hydroxide and the free base is extracted with ether. The ether is evaporated under vacuum, and the quinidine is recrystallized from alcohol. The other vinyl alkaloids were prepared in an analogous manner and recrystallized with the solvent noted in Table I.

The dihydro alkaloids were prepared by catalytic hydrogenation of the corresponding vinyl alkaloid over platinum oxide in ethanol. The isolated alkaloid was then recrystallized with the solvent noted in Table I. Quininone was prepared by oxidizing quinine with benzophenone in the presence of potassium *tert*-butoxide by the procedure of Woodward *et al.* (19). It was recrystallized twice from ether.

All of the isolated recrystallized alkaloids were dried under vacuum in a drying pistol at the temperature of refluxing xylene. The solvent of crystallization is removed in this process.

Sample Preparation—*Capsules, Tablets, and Powdered Quinidine or Quinine Salts*—Dissolve a portion of the sample in enough 50% alcohol to provide a solution equivalent to about 5 mg. of alkaloid base/ml. (The dissolved excipients do not affect the subsequent procedures.) Filter if necessary.

Quinidine or Quinine Base—Prepare a solution of about 5 mg./ml. in alcohol.

Injections—Transfer an aliquot of the injection containing about 80 mg. of the alkaloid salt to a separator containing 25 ml. of water, acidify with a few drops of diluted sulfuric acid, and extract with 50 ml. of ether. Discard the ether. Make the aqueous layer alkaline with ammonia and extract with 50 ml. of ether. Wash the ether layer with 25 ml. of distilled water, transfer the ether solution to a conical flask, and evaporate just to dryness under a stream of air. Dissolve the residue in enough alcohol to make a solution of about 5 mg. of alkaloid base/ml.

Assay Preparation—Dilute an aliquot of the sample preparation with enough alcohol to make a final dilution of 0.5 mg. of alkaloid base/ml.

Preparation of Thin-Layer Plates—Spread a slurry of 30 g. of silica gel H and 70 ml. of water over five 20 × 20-cm. plates with a suitable spreader[1] to obtain a 0.25-mm. layer. Air dry the plates and store in a dust-free atmosphere. Prepare silica gel G or GF plates (0.25 mm. thick) in an analogous manner, using a slurry of 30 g. of substrate and 60 ml. of water. (Commercial silica gel G or GF plates are also suitable.)

TLC System 1 (8)—This system separates quinine, quinidine, epiquinidine, epiquinine, cinchonidine, cinchonine, and quininone. It does not separate the dihydro alkaloids from the parent alkaloids.

The plates are 0.25-mm. silica gel G or GF, and the developing solvent is chloroform-acetone-diethylamine (50:40:10).

TLC System 2—This system gives the best separation of the dihydro alkaloids from their respective vinyl alkaloids. It does not separate the epi-alkaloids, nor quinine from quinidine. The plates are 0.25-mm. silica gel H, and the developing solvent is chloroform-acetone-methanol-ammonium hydroxide (60:20:20:1).

TLC System 3—This system separates the epi-alkaloids and the dihydro alkaloids from the parent alkaloids, but it does not separate quinine from quinidine. The plates are 0.25-mm. silica gel H, and the developing solvent is methanol-ammonium hydroxide (100:1).

Qualitative TLC Identification—(Use TLC Systems 1 and 3.) At a point about 2 cm. from the bottom edge of the plate, spot 2 μl. of the sample preparation and each of the standard solutions of the respective alkaloids. Add the developing solvent to a trough in the bottom of a tank, one side of which is lined with a sheet of filter paper (Whatman No. 1 or equivalent). Introduce the plate immediately after adding the developing solvent to prevent presaturation of the tank. Let the solvent migrate 10–14 cm. from the point of spotting, and air dry the developed plate. Observe the plate under long wavelength UV light to locate the quinine series. To visualize the desmethoxy series, spray the plate with either 50% sulfuric acid, which converts them into fluorescent compounds, or iodoplatinic acid, which produces colored spots with all of the alkaloids. (Because of the diethylamine used in TLC System 1, these plates must be sprayed with acid prior to visualization.)

Quantitative TLC Separation—At a point 2 cm. from the bottom of the edge of the silica gel H plate, spot three 2-μl. aliquots of each assay preparation. If the qualitative procedure shows the presence of epi-alkaloids, use TLC System 3; otherwise, use TLC System 2. Develop as described under *Qualitative TLC Identification.*

Direct Scanning Fluorescence Measurement—The following conditions were found to be optimal for the apparatus used[2]: excitation wavelength, 345 nm.; and emission barrier filter, 430 nm. Set the source slit so the maximum intensity of the observed fluorescence is obtained and the entire width of the spot is scanned. (These conditions were provided by an 8-mm. slit in the instrument.) Set the variable slit to give a maximum of 90% of the 5-mv. range of the recorder for the most intense spot when the following settings of the amplifier[3] are used: right-hand step switch on 10, sensitivity key on 1, high tension switch on 1, amplification selector switch on F, and precision setting control knob on 5.

Align the TLC plate so that the centers of the spots pass under the center of the scanning slit. Scan the plate at 50 mm./min. with a chart rate of 200 mm./min. Use baseline corrected disk integrations[4] or electronic integration to calculate the ratio of the al-

[1] Desaga standard adjustable applicator (Brinkmann Instruments Inc., Westbury, NY 11590) was used for the preparation of TLC plates.

[2] Zeiss chromatogram spectrophotometer, Carl Zeiss Inc., New York, NY 10018
[3] PMQ II, Carl Zeiss, Inc.
[4] Simpson model 2741 recorder with disk integrator, Simpson Electric Co., Chicago, IL 60644

Table III—Relative Retention Times of Trimethylsilyl Derivatives of Cinchona Alkaloids

Alkaloid	Relative Retention Time[a]
Cinchonidine	0.55
Cinchonine	0.55
Dihydrocinchonidine	0.50
Dihydrocinchonine	0.50
Epiquinidine	0.87
Epiquinine	0.95
Dihydroquinidine	0.93
Dihydroquinine	0.93
Quinidine	1.00
Quinine	1.00
Quininone	0.83
Quinotoxine (quinicine)	1.52
Thioglycerol adduct of quinidine	13.4

[a] The retention times are relative to the appearance of quinidine, with the use of a glass column 6.1 m. × 3 mm. (20 ft. × 0.125 in.) packed with 3% OV-225 on Gas Chrom Q. (Quinidine elutes at approximately 21 min. with the column at 225°.)

kaloids. The following settings on the electronic integrator[b] provided optimal background correction with the silica gel H plates (settings should be checked on each instrument with standard mixtures of alkaloids): baseline tracking up 600, down 2000 μv./min.; input noise rejection, 5; minimum peak rate, 30 sec.; slope sensitivity, 2; count rate, 4 kc./mv.; minimum peak counts, 2000/sec.; and threshold level, 100 μv./min.

Solution Fluorescence Method—View the dried chromatogram under long wavelength UV light, and mark off areas of the alkaloids on the TLC plate. Scrape off the area of the separated alkaloids and a blank area of the plate corresponding in R_f to the alkaloid spot. Quantitatively transfer each scraped portion to a 15-ml. glass-
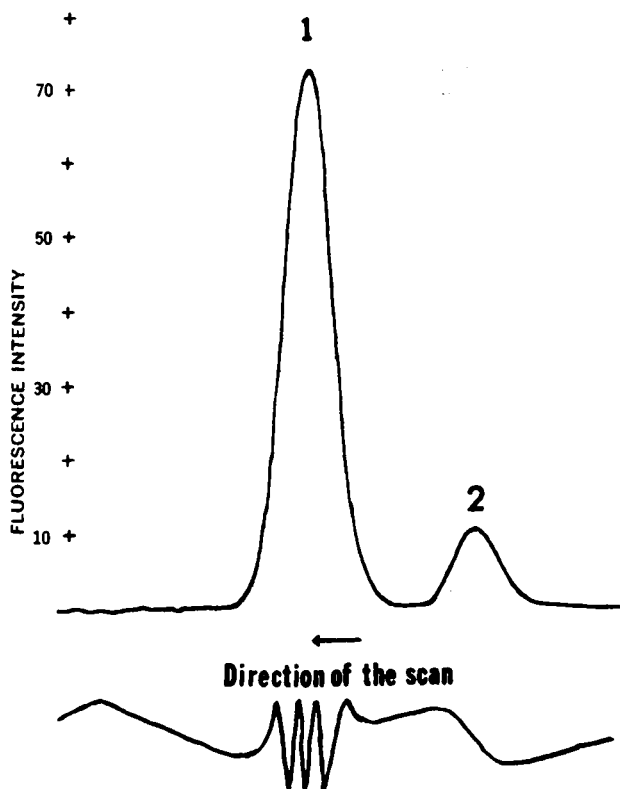
Table IV—Direct Scanning Fluorescence Method: Comparison of Methods of Integration of Repetitive Scans[a]

Aliquot	Electronic Integration				Disk Integration		
1	9.1	8.9	9.0	9.2	8.8	8.9	8.5
2	8.6	8.6	8.8	8.6	8.4	9.1	8.2
3	8.4	8.5	8.6	8.5	8.8	8.7	8.4

[a] All results are given in percent of dihydroquinidine.

stoppered centrifuge tube, add 10.0 ml. of 0.1 N H_2SO_4 (prepared from fluorescent grade sulfuric acid) to each tube, stopper, shake for 30 sec., and centrifuge at 20,000 r.p.m. for 30 sec. Transfer a portion of the supernatant liquid to a 1-cm. fluorescence cell, and measure the fluorescence directly with the excitation at 350 nm. and the emission at 455 nm.[6]. Calculate the ratio of the alkaloids in each sample from the fluorescence readings corrected for the contribution of the blank.

GLC Procedure—The system described separates the vinyl alkaloids from their dihydro analogs. It does not resolve the quinidine-quinine, dihydroquinidine-dihydroquinine, cinchonidine-cinchonine, and dihydrocinchonidine-dihydrocinchonine pairs, but it does resolve these pairs from each other. The following conditions are used: column, 6.1-m. × 3-mm. (20-ft. × 0.125-in.) glass, packed with 3% OV-225 on Gas Chrom Q; injector temperature, 235°; column temperature, 225°; and carrier gas, nitrogen at 30 ml./min. A suitable gas chromatograph[7] with a flame-ionization detector and an electronic integrator[b] is used. Evaporate 0.2 ml.of the assay preparation, containing the equivalent of 0.1 mg. of alkaloid under nitrogen, in a 0.3-ml. conical vial[8] and then dry under vacuum for 30 min. Add 0.1 ml. of N-methyl-N-trimethylsilyl-trifluoroacetamide or bistrimethylsilyl-trifluoroacetamide, cover with a Teflon-lined septum secured with a screw cap, and heat at 60° for 45 min. In-
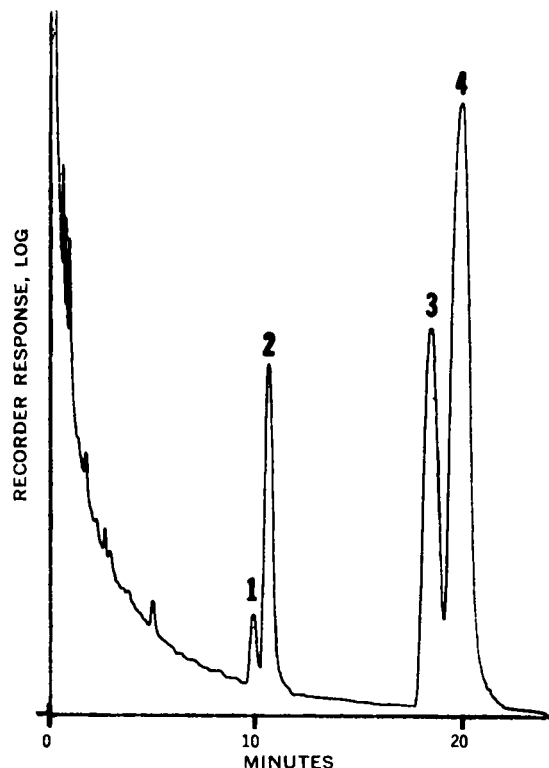

Figure 1—*TLC scan of a quinidine sample. Key: 1, quinidine; and 2, dihydroquinidine.*

Figure 2—*GLC chromatogram of the trimethylsilyl derivatives of the alkaloids in a quinidine sulfate sample. Key: 1, dihydrocinchonidine; 2, cinchonine; 3, dihydroquinidine; and 4, quinidine.*

[b] Infotronics model CRS-104 electronic integrator with digital readout, The Infotronic Corp., Houston, TX 77042

[6] Aminco-Bowman spectrophotofluorometer, American Instrument Co., Silver Spring, MD 20901
[7] Varian Aerograph model 1200 gas chromatograph, Varian Aerograph, Walnut Creek, Calif.
[8] Kontes K-749000, Kontes Glass Co., Vineland, N. J.

Table V—Comparison of Methods of Determination of Dihydroquinidine in Quinidine[a]

Aliquot	Electronic[b]	Disk[b]	Solution Fluorescence	GLC
		Method of Determination		
1	6.8	6.5	6.3	6.3
2	7.1	6.4	6.0	6.4
3	7.1	6.5	—	6.4
4	7.0	6.5	6.9	—
5	6.8	6.7	6.5	—
Average	7.0	6.5	6.4	6.4

[a] All results are given in percent of dihydroquinidine. [b] Each value is an average of repetitive scans of the same spot.

ject 3 μl. of sample. Record the response and integrate the peak areas electronically. Calculate the ratio of the alkaloids in the samples from the integrated areas.

RESULTS AND DISCUSSION

Forty samples of quinidine and 35 samples of quinine in the form of the alkaloid, its salts, and pharmaceutical dosage forms were examined. The dihydro analogs were found in all samples, and the desmethoxy analogs (cinchonine or cinchonidine) were found in some samples. No epi-alkaloid, quininone, or quinotoxine was detected in any of the samples. The level of the dihydro alkaloids was higher in quinidine than in quinine; the level of the desmethoxy analogs was higher in quinine than in quinidine.

In preliminary work the alkaloids were extracted from the sample with methylene chloride. These solutions degraded rapidly to generate several additional TLC spots, one of which corresponded to quininone. The simple dissolution of the samples in alcohol gave stable sample preparations. No changes were observed in solutions standing in the dark for over a month.

Initial qualitative TLC is needed to verify the identity of the sample[9] and to select the quantitative TLC system to be used. TLC System 1 shows the presence of the epi-alkaloids. If they are present, TLC System 3, which resolves them from the natural bases, is used for quantitative measurement; if they are absent, TLC System 2, which gives a better resolution of the natural analogs, is preferred. The R_f values of the alkaloids are listed in Table II.

The GLC procedure permits the simultaneous quantitation of the desmethoxy alkaloids as well as the dihydro and vinyl alkaloids. The relative retention times of the trimethylsilyl derivatives of the alkaloids are listed in Table III. Since the GLC procedure does not separate the quinine series from the quinidine series, it is necessary to examine the sample by TLC, using TLC System 1 to verify the identity of the sample[9].

The most rapid and simple method for the examination of the chromatogram for the naturally fluorescent alkaloids uses the TLC scanner, preferably with a digital integrator for the quantitation. A typical scan is illustrated in Fig. 1. Since these instruments are probably not in widespread general use at this time, the method based on the fluorescence of the solution of the resolved alkaloids may be the most widely applicable procedure. The latter has greater specificity, since both the specific emission and the excitation wavelengths characteristic of these compounds can be set with the spectrophotofluorometer.

In scanning the thin-layer plates on the chromatogram spectrophotometer, occasional response was observed, indicating the presence of very minor spots not visible to the eye by their fluorescence nor after the chromatogram was sprayed with iodoplatinate reagent. These did not correspond to any of the alkaloids listed in Table II. If they were due to alkaloids having the same relative fluorescence as quinidine, they would amount to less than 0.2%.

Mixtures of known ratios of quinidine and dihydroquinidine were subjected to TLC by TLC Systems 2 and 3 and were quantitated by direct scanning and by solution fluorescence. They were

---

[9] One sample labeled quinidine hydrochloride was found to be quinine hydrochloride, and one sample labeled quinidine was found to be cinchonidine.

Table VI—Other Alkaloids in Quinidine Preparations

Sample	Dihydroquinidine, %	Cinchonine, %
Sulfate tablets	6.1	—
	6.5	0.9
	10.7	0.4
	7.6	1.3
	8.5	0.1
	6.7	0.2
	4.3	—
	6.7	0.1
	4.7	—
	6.9	0.1
	6.6	—
	6.8	—
	5.5	—
	6.5	—
	7.0	0.1
	11.8	0.5
Sulfate capsules	9.0	—
	21.7	—
	7.2	0.6
Gluconate injection	10.1	—
	4.3	—
	8.5	—
Sulfate injection	8.2	—
	10.6	—
Alkaloid powder	5.9	—
	6.1	—
	5.6	—
	7.3	0.5
Gluconate powder	6.1	—
	6.4	—
	5.5	0.6
Sulfate powder	3.0	—
	4.9	—
	5.6	—
	8.9	—
	16.5	7.1[a]
	14.2	—
	18.7	0.5
	22.1	—
	9.0	—

[a] This sample also contained 0.7% dihydrocinchonine.

also examined by the GLC procedure. Response was linear by all systems.

Table IV illustrates the results of repetitive scans of the same spot of a single sample by disk integration and electronic integration. Table V shows averages of scans of replicate chromatograms of individual solutions compared with results by the GLC procedure and the solution fluorescence of the same spot of the same chromatogram. With the disk integrator, graphic correction of the background is necessary. With the electronic integrator, accurate compensation of the background can be obtained by setting the baseline tracking characteristics with standards of known ratios of quinidine or quinine and its dihydro analog. In general, the closest agreement was found with the GLC and the solution fluorescent procedures, but on the average all methods of quantitation gave good agreement.

The amount of dihydroquinidine found in the various samples is summarized in Table VI. Dihydroquinidine was found in all samples, mostly in the 5-9% range with extremes of 3 and 22%. No epiquinidine, epiquinine, quinine, quininone, or quinotoxine was detected in any sample by the TLC systems that would detect them at 0.2%. The thioglycerol adduct of quinidine reported by Levine and Maienthal (24) was found in quinidine injections containing thioglycerol as a preservative.

Cinchonine and cinchonidine and their dihydro analogs may be detected by the qualitative TLC procedures; but because of their fluorescence properties, they do not interfere in the quantitative determination. Cinchonine was absent or was present at very low levels in the quinidine samples. It was quantitated by the GLC procedure. The results are shown in Table VI. An exception was one sample of quinidine sulfate powder which contained 7% cinchonine and 0.7% dihydrocinchonine. The GLC curve for this sample is illustrated in Fig. 2.

Table VII—Other Alkaloids in Quinine Preparations

Sample	Dihydroquinine, %	Cinchonidine, %	Dihydrocinchonidine, %
Sulfate capsules	4.1	1.5	—
	4.8	0.9	0.2
	5.1	1.0	—
	5.2	0.7	—
	6.3	0.4	—
	5.1	—	—
	3.2	0.8	—
	8.4	0.3	0.7
	4.2	1.1	1.5
	4.1	1.1	0.6
	2.7	0.3	0.5
	5.6	1.0	0.8
	6.2	0.1	1.1
	7.9	0.3	0.1
	7.6	0.6	0.1
	3.5	1.2	0.1
	0.2	—	—
	3.2	1.2	—
	7.6	1.3	—
	4.5	—	—
	4.0	0.1	0.1
	1.9	—	—
	4.3	—	—
	8.5	—	—
Sulfate tablets	5.1	1.1	—
	4.1	1.7	0.1
Alkaloid powder	8.7	1.6	0.1
	8.1	0.6	—
Hydrobromide powder	3.3	0.7	0.1
	1.5	—	—
Dihydrochloride powder	3.4	1.6	—
Phosphate powder	6.7	0.7	—
Sulfate powder	5.1	0.1	1.3
	6.6	1.9	—
	4.8	1.9	0.1

The examination of quinine samples showed dihydroquinine contents ranging from 0.2 to 9%, with the majority of the samples containing 3-6% (Table VII). The cinchonidine content ranged from none to 2%, with most of the samples containing about 1%. The level of cinchonidine found in quinine was much higher than that of cinchonine in quinidine.

The current USP monographs have no limit tests for the dihydro analog in the natural alkaloid. In the light of the findings, it has been recommended to the USP that the standards for quinidine and quinine and their salts should be redefined to reflect their actual composition.

REFERENCES

(1) K. H. Palmer, B. Martin, B. Baggett, and M. E. Wall, *Biochem. Pharmacol.*, **18**, 1845(1969).

(2) T. A. Henry, "The Plant Alkaloids," 4th ed., Blakiston, Philadelphia, Pa., 1949, p. 424.

(3) D. J. Lussman, E. R. Kirch, and G. L. Webster, *J. Amer. Pharm. Ass., Sci. Ed.*, **40**, 368(1951).

(4) J. Reichelt, *Pharmazie*, **11**, 718(1956).

(5) H. Von Auterhoff and K. Kalpathy, *Pharm. Acta Helv.*, **38**, 491(1963).

(6) E. Pawelczyk and R. Wachowisk, *Chem. Anal.*, **11**, 885 (1966).

(7) E. Pawelczyk, R. Wachowisk, and I. Pluta, *Diss. Pharm. Pharmacol.*, **22**, 165(1970).

(8) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, pp. 579, 581, 583.

(9) H. Bohme and R. Bitsch, *Arch. Pharm.*, **303**, 418(1970).

(10) *Ibid.*, **303**, 456(1970).

(11) J. Storck, J.-P. Papin, and D. Plas, *Ann. Pharm. Fr.*, **28**, 25 (1970).

(12) A. Suszko-Purzycka and W. Trzebny, *J. Chromatogr.*, **17**, 114(1965).

(13) G. Hartel and A. Korhonen, *ibid.*, **37**, 70(1968).

(14) G. Hartel and A. Harjanne, *Clin. Chim. Acta*, **23**, 289(1969).

(15) J. Gutzwiller and M. Uskokovic, *J. Amer. Chem. Soc.*, **92**, 204(1970).

(16) V. P. Vacha, P. Cuba, V. Preininger, L. Hruban, and F. Santavy, *Planta Med.*, **12**, 406(1964).

(17) K. Roder, E. Eich, and E. Mutschler, *Pharm. Ztg.*, **115**, 1430 (1970).

(18) H. Thron and W. Dirscherl, *Ann.*, **515**, 252(1935).

(19) R. B. Woodward, N. L. Wendler, and F. J. Brutschy, *J. Amer. Chem. Soc.*, **67**, 1425(1945).

(20) H. G. Boit, "Ergebnisse Der Alkaloid-Chemie Bis 1960," Akademie-Verlag, Berlin, Germany, 1961, pp. 574-576.

(21) B. T. Cromwell, in "Modern Methods of Plant Analysis," vol. IV, K. Paech and M. V. Tracey, Eds., Springer-Verlag, Berlin, Germany, 1955, p. 394.

(22) "The Merck Index," 8th ed., Merck & Co., Inc., Rahway, N. J., 1968, p. 262.

(23) J. F. Mead, M. M. Rapport, and J. B. Koepfli, *J. Amer. Chem. Soc.*, **68**, 2704(1946).

(24) J. Levine and M. Maienthal, *J. Pharm. Sci.*, **58**, 770(1969).

ACKNOWLEDGMENTS AND ADDRESSES