Colorimetric Assay of Quinine and Quinidine in Raw Materials, Formulations, and Biological Fluids

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Abstract □ The two characteristic erythroquinine and thalleioquin tests for quinine and quinidine were studied to optimize the experimental conditions for quantitative analysis. Both methods were quantitatively sensitive for either quinine or quinidine in a concentration range of 0.1–10 μg/ml with the erythroquinine method and of 3–50 μg/ml with the thalleioquin method. A TLC–colorimetric method also is described for the assay of quinine and quinidine in the presence of cinchonine, cinchonidine, and other cinchona alkaloids. The results were compared with those obtained with a spectrophotometric method.

Keyphrases □ Quinine—colorimetric analysis, pharmaceutical formulations and biological fluids □ Quinidine—colorimetric analysis, pharmaceutical formulations and biological fluids □ Colorimetry—analyses, quinine and quinidine, pharmaceutical formulations and biological fluids □ Alkaloids, cinchona—quinine and quinidine, colorimetric analyses, pharmaceutical formulations and biological fluids □ Antimalarials—quinine, colorimetric analysis, pharmaceutical formulations and biological fluids □ Cardiac depressants—quinidine, colorimetric analysis, pharmaceutical formulations and biological fluids

The separation and estimation of the major cinchona alkaloids, especially quinine (I) and quinidine (II), are complicated and difficult due to their many common properties; only a few methods are analytically useful. Several spectrometric assays for quinine and quinidine, either alone or in mixtures, were reported; the most important and widely used are UV spectrophotometry (1–3) and fluorometry (4–6).

Numerous colorimetric methods were adopted for estimating quinine and quinidine involving potassium-chromic thiocyanate (7, 8), cobalt thiocyanate (9), picric acid (10), reineckate salt (11), tropaeolin 00 (12), titanium trichloride (13), and bromthymol blue (14). TLC separation followed by UV spectrophotometry (15) or colorimetry (16), as well as other techniques such as paper (17), triple-column (18), and ion-exchange (19) chromatography, also were described.

Although the two characteristic erythroquinine (20) and thalleioquin (21) tests are most selective and specific for the detection of quinine, their quantitative application was not fully studied. Allport and Friend (22) found that the erythroquinine test suggested by Monnet (23) was very difficult to apply quantitatively due to certain uncontrollable factors; the bromine water was a critical factor and the reaction was time, temperature, and light dependent.

The purposes of this work were to: (*a*) quantify the erythroquinine and thalleioquin color tests, (*b*) develop a selective colorimetric method to assay quinine and quinidine in the presence of other cinchona alkaloids, and (*c*) apply these methods to bulk drug, dosage forms, and biological fluids.

EXPERIMENTAL

Materials—*Pharmaceuticals*—Liquid extract and tincture of cinchona[1] were used. The following also were studied: quinine ampuls containing 300 mg of quinine dihydrochloride; quinine tablets containing 150 mg of quinine sulfate; quinidine tablets containing 200 mg of quinidine sulfate; compound quinine tablets containing 20 mg of quinine hydrobromide, 15 mg of papaverine, 20 mg of phenobarbital, and 50 mg of crataegus extract; and dipyrone–quinine tablets containing 150 mg of dipyrone calcium salt and 100 mg of quinine.

Biological Fluids—Blood samples were collected from five male adult volunteers; each volunteer swallowed a single capsule containing 0.2 g of quinine sulfate and 0.2 g of quinidine sulfate 3 hr before blood was drawn. Urine samples were collected for 6 hr after drug administration.

Bromate–Bromide Solution—Prepare 0.5 *N* potassium bromate by dissolving 13.92 g of potassium bromate in distilled water. Add 60 g of potassium bromide and mix. After the salts have dissolved, adjust the volume to 1 liter with distilled water.

Potassium Ferricyanide Reagent—Prepare a 10% solution of potassium ferricyanide in distilled water.

Standard Quinine and Quinidine Solution—Dissolve a 5-mg sample of either quinine sulfate reference material[2] or quinidine sulfate reference material[3] in 10 ml of 2 *N* sulfuric acid.

Standard Solution of Mixed Cinchona Alkaloids—Dissolve 5 mg each of quinine, quinidine, cinchonine, and cinchonidine in 10 ml of 2 *N* sulfuric acid.

Apparatus—Glass plates (20 × 20 cm) coated with a slurry consisting of 30 g of silica gel G in 60 ml of distilled water to give a layer 0.25 mm thick were dried at 100° for 1 hr. A UV–visible colorimeter[4] was used.

Erythroquinine Reaction—Dilute 1 ml of either quinine or quinidine standard solutions to 10 ml with distilled water. Pipet 0.02, 0.1, 0.2, 1.0, 1.5, 2.0, and 2.5 ml of the dilute solutions in separate test tubes, dilute to ~3 ml with distilled water, and add 2 drops of hydrochloric acid. Add, dropwise, the bromate–bromide solution, shaking after each addition until a faint-yellow color appears; then shake for 20 sec, and add 10 drops of the potassium ferricyanide solution.

Make the solution just alkaline with dilute ammonium hydroxide. Extract the erythroquinine formed with chloroform (5 × 3 ml), transferring the chloroform extracts to a 10-ml volumetric flask, and dilute to volume with chloroform. Pipet 1 ml of chloroform solution, and measure the absorbance at 510 nm against a reagent blank.

A calibration curve of 0.1–10 μg of quinine or quinidine/ml *versus* color intensity obeyed Beer's law with a standard deviation of ±0.38%.

Thalleioquin Reaction—Pipet 0.04, 0.06, 0.1, 0.2, 0.4, 0.8, 1, and 1.1 ml of either quinine or quinidine standard solution into separate 10-ml volumetric flasks, dilute to ~2 ml with distilled water, and add 2 drops of hydrochloric acid. Add, dropwise, the bromate–bromide solution (~10 drops), shaking after each addition until a faint-yellow color appears. Continue shaking for 20 sec, add 10 drops of ammonium hydroxide (25%), and shake again for 15 sec. Dilute to volume with absolute methanol, and directly measure the absorbance at 450 nm against a reagent blank.

A calibration curve of 3–50 μg of quinine or quinidine/ml *versus* color intensity obeyed Beer's law with a standard deviation of ±1.2%.

TLC–Colorimetric Estimation of Quinine and Quinidine—To avoid interferences caused by cinchonine, cinchonidine, and other minor cinchona alkaloids, a TLC method for separating quinine and quinidine prior to their estimation is performed as follows. Submit 0.1 and 0.15 ml of the standard solution of mixed alkaloids and 0.01, 0.05, 0.1, 0.15, and 0.2 ml of each standard quinine and quinidine solution to chromatography on silica gel G plates, using benzene–ethyl acetate–diethylamine (2:2:1) as the solvent system (development ~2 hr). Locate the quinine and quinidine spots on the dried plate with UV light, and remove the respective spots quantitatively to separate test tubes. Add 5 ml of 0.1 *N* hydrochloric acid, heat for 10 min at 60° on a water bath, shake thoroughly for 5 min while hot, and then cool. Centrifuge or filter the contents of test tubes, and apply the proposed erythroquinine or thalleioquin

[1] Porter & Clark Ltd., London, England.

[2] N. V. Amsterdamache Chininefabriek, Amsterdam, The Netherlands.
[3] WPP, Wallace Pharmaceutical Products, Ltd., London, England.
[4] Spekol, Carl Zeiss, Jena, East Germany.

Table I—Assay of Quinine (I) and Quinidine (II) in Cinchona Preparations by the Proposed Methods

Preparation	Amount Declared, mg/ml		Amount Found[a], mg/ml: Thalleioquin Method		Amount Found[a], mg/ml: Erythroquinine Method		Amount Added, mg		Amount Found[a], mg/ml: Thalleioquin Method		Amount Found[a], mg/ml: Erythroquinine Method		Recovery, %: Thalleioquin Method	Recovery, %: Erythroquinine Method
	I	II	I	II	I	II	I	II	I	II	I	II		
Liquid extract of cinchona	40	2.0	46	1.95	45	1.92	40	2	85.8	3.92	84.8	3.9	99.5	99.2
Tincture	0.95	0.2	0.97	0.21	0.95	0.19	1	0.2	1.93	0.41	1.93	0.39	99.5	99

[a] Results are averages of five experiments.

Table II—Assay of I and II in Formulations by Proposed and AOAC Methods

Preparation, Label I or II Claim	Amount Found[a], mg: Thalleioquin Method	Amount Found[a], mg: Erythroquinine Method	Amount Found[a], mg: AOAC Method	Amount Added, mg	Amount Found[a], mg: Thalleioquin Method	Amount Found[a], mg: Erythroquinine Method	Amount Found[a], mg: AOAC Method	Recovery, %: Thalleioquin Method	Recovery, %: Erythroquinine Method	Recovery, %: AOAC Method
Quinine tablets, 150 mg	152	150.5	155	50	202	200	204.8	100	99	99.6
Quinidine tablets, 200 mg	201	200	202	50	250	249	253	98	98	102
Quinine ampul, 300 mg	299	298	302	50	348	347	352	98	98	100
Compound quinine tablets, 20 mg	19.5	19.2	21	10	29.4	29	31	99	98	101
Dipyrone–quinine tablets, 52 mg	51.9	51.2	—	50	101.6	100.4	—	99.4	98.4	—
SD								±0.38	±1.2	±0.85

[a] Results are averages of five experiments.

method to the filtrates against a reagent blank. Construct calibration curves for both quinine and quinidine.

Estimation of Quinine and Quinidine in Raw Materials—*Cinchona Extract*—The application of the proposed procedures to cinchona extract or tincture involves the extraction of alkaloids prior to the TLC–colorimetric estimation.

Extraction—Transfer 1.0 ml of liquid extract of cinchona into a 50-ml volumetric flask, add 6 ml of 10% formic acid, heat on a water bath for 30 min, and cool. Add 40 ml of tetrahydrofuran, shake the mixture vigorously for 10 min, filter, and dilute to 50 ml with tetrahydrofuran.

Carry out the TLC–colorimetric estimation of quinine and quinidine using 0.1 and 0.2 ml of tetrahydrofuran extract. Calculate the amount of quinine and quinidine from their respective calibration curves.

Estimation of Quinine and Quinidine in Formulations—*Quinine Ampuls*—Dilute 0.1 ml of injectable solution with distilled water to 5 ml, make alkaline with ammonium hydroxide, and extract with chloroform (3 × 10 ml). Evaporate the chloroform extracts to dryness on a water bath, dissolve the residue in 100 ml of 1% H_2SO_4, transfer to a 1-liter volumetric flask, and adjust to volume with distilled water. Perform any of the proposed colorimetric assay methods on 1 ml of this solution.

Simple and Compound Quinine Tablets—Weigh accurately a quantity of finely powdered tablets equivalent to 5 mg of quinine, and extract exhaustively with 1% H_2SO_4 (3 × 10 ml). Filter the acid extract, make alkaline with ammonia, and extract with chloroform (3 × 10 ml). Evaporate the chloroform extract to dryness on a water bath, and dissolve in 100 ml of 1% H_2SO_4 in a volumetric flask. Perform any of the proposed procedures on 1 ml of this solution.

Dipyrone–Quinine Tablets—Weigh accurately a quantity of the finely powdered tablets (10 mg of dipyrone–quinine salt), extract with 1% H_2SO_4 (3 × 10 ml), filter the acid extract, make distinctly alkaline to litmus with sodium hydroxide solution, and extract with chloroform (3 × 10 ml). Evaporate the chloroform on a water bath, and dissolve the residue in 100 ml of 1% H_2SO_4 in a volumetric flask. Carry out the erythroquinine or thalleioquin assay procedures on 1 ml.

Estimation of Quinine and Quinidine in Biological Fluids—*Blood*—Mix 5 ml of blood with an equal volume of hydrochloric acid, and heat on a boiling water bath for 1 hr. Cool, dilute with 5 ml of distilled water, and filter. Wash the residue with distilled water (3 × 5 ml). Combine the filtrate and washings, make the solution alkaline with ammonium hydroxide, and extract with chloroform (2 × 30 ml). Dry the combined chloroform extracts with anhydrous sodium sulfate, filter, and dilute to 100 ml with chloroform. Evaporate an aliquot of the chloroform extract to dryness, dissolve in the least amount of 2 *N* sulfuric acid, and estimate quinine and quinidine by the TLC–colorimetric method using the erythroquinine reaction.

Urine—Prepare the 6-hr sample exactly as described for blood, omitting the dilution with water. Estimate quinine and quinidine by the TLC–colorimetric method using either the erythroquinine or thalleioquin method.

RESULTS AND DISCUSSION

The modifications proposed for the thalleioquin and erythroquinine reactions proved to be simple and specific in comparison with the AOAC (1970) method (24). The latter method depends upon measuring the UV absorption of the 0.1 *N* hydrochloric acid extract of the alkaloid at 347.5 nm and is liable to interferences caused by any substance absorbing at 347.5 nm, *e.g.*, aloin, podophyllin, anthraquinones and their derivatives, other cinchona alkaloids, and yellow dyes (24). The color reactions, however, seem very selective. During this work, these reactions could be

Table III—Assay of I and II in Blood and Urine Sample by the Proposed Methods

Sample	Volunteer	Amount Found, mg: Thalleioquin Method		Amount Found, mg: Erythroquinine Method		Amount Added, mg		Amount Found, mg: Thalleioquin Method		Amount Found, mg: Erythroquinine Method		Recovery, %: Thalleioquin Method	Recovery, %: Erythroquinine Method
		I	II	I	II	I	II	I	II	I	II		
Blood	1			0.04	0.04	0.1	0.1	—	—	0.14	0.140	—	100
	2			0.04	0.04	0.2	0.1	—	—	0.239	0.138	—	98.78
	3			0.044	0.44	0.15	0.15	—	—	0.194	0.194	—	100
	4			0.04	0.04	0.1	0.2	—	—	0.138	0.238	—	98.5
	5			0.38	0.035	0.1	0.1	—	—	0.138	0.138	—	100
Urine	1	10	10	10	9.5	5	5	14.9	15.05	14.7	14.6	99.5	98
	2	10.5	10	10.2	9.2	7.5	7.5	17.7	17.75	17.1	17.1	99.6	98.5
	3	9.5	9	9	9	1	1	10.26	10.22	10.0	9.97	99.0	98.5
	4	11	8.5	10	8	2.5	2.5	12.23	12.23	11.3	11.5	99.2	99
	5	8.8	9	8.5	8.5	5	5	13.72	13.73	15	15	99.5	98.9

safely carried out in the presence of crataegus extract and other alkaloids such as papaverine, strychnine, morphine, and atropine.

Although the proposed reactions are specific for quinine and quinidine, a TLC procedure is necessary for cinchona preparations to separate quinine and quinidine and to eliminate other interfering constituents. The four main cinchona alkaloids showed good separation; the R_f values of quinine, quinidine, cinchonine, and cinchonidine were 0.18, 0.44, 0.55, and 0.41, respectively.

Ammonium hydroxide was not capable of releasing quinine base from the dipyrone–quinine salt, while sodium hydroxide solution was satisfactory.

The proposed methods also were applied successfully to the recovery of quinine and quinidine from blood and urine with reproducible results. With blood, because of the small concentration of the alkaloids present, only the more sensitive erythroquinine method was adopted.

Good recoveries at different concentrations and spiking and reasonable standard deviations were obtained with dosage forms and biological fluids by the erythroquinine and thalleioquin methods (Tables I–III).

To obtain protein-free aqueous filtrates from blood and urine, the samples were digested with hydrochloric acid to liberate strongly conjugated alkaloids. Hydrochloric acid digestion was preferable to other procedures.

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High-Pressure Liquid Chromatographic Determination of Tetracyclines in Urine

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Abstract □ The quantitation of oxytetracycline, tetracycline, and chlortetracycline was accomplished by high-pressure liquid chromatography using an anion-exchange column. The tetracyclines were extracted from urine as their calcium complexes. Concentrations as low as 12 μg of oxytetracycline/ml and 4 μg of tetracycline and chlortetracycline/ml were quantitated accurately. The relative standard deviation of the method varied from 0 to 5%.

Keyphrases □ Oxytetracycline—high-pressure liquid chromatographic analysis, urine □ Tetracycline—high-pressure liquid chromatographic analysis, urine □ Chlortetracycline—high-pressure liquid chromatographic analysis, urine □ High-pressure liquid chromatography—analyses, oxytetracycline, tetracycline, and chlortetracycline in urine □ Antibacterials—oxytetracycline, tetracycline, and chlortetracycline, high-pressure liquid chromatographic analyses, urine

To establish the urinary excretion rate of tetracyclines in the urine of cattle, sheep, and swine following intravenous drug administration, an accurate method for the quantitation of oxytetracycline, tetracycline, and chlortetracycline was required. TLC (1–5) and paper chromatographic (6–9) methods were laborious and lacked sufficient sensitivity and accuracy. One GLC method (10) required the formation of trimethylsilyl derivatives and sometimes resulted in the formation of tetracycline degradation products. None of the previously reported fluorometric methods (11–14) could be used due to large and variable amounts of fluorescent material in urine obtained from untreated control animals.

Several high-pressure liquid chromatographic (HPLC) methods for the separation and determination of tetracyclines have been reported. A low efficiency column packing was used for the separation of tetracyclines, but the method lacked the sensitivity required for analysis of biological samples (15). The experimental conditions required for the qualitative separation of several tetracyclines were discussed (16), but the quantitative utility of this method was not reported. Several reports (17, 18) did not include oxytetracycline. The purpose of this study was