Nonaqueous Titration of Sulfates of Quinine and Quinidine Using Barium Acetate

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Abstract □ A nonaqueous titrimetric method is proposed for determining the diastereomeric sulfates of quinine and quinidine. The sulfuric acid content of the alkaloid salts is precipitated, in the form of barium sulfate, with acetous barium acetate solution before the liberated alkaloid is titrated; the necessary calculations are provided. A favorable characteristic of the proposed procedure is the accuracy, speed, and ease of performance. The mean percent recoveries ($p = 0.05$) obtained with the proposed method for the sulfates of quinine and quinidine were 98.84 ± 1.00 and 99.74 ± 1.27, respectively, compared with 100.73 ± 1.44 and 100.82 ± 1.16, respectively, when the BP 1968 procedure was applied.

Keyphrases □ Quinine sulfate—nonaqueous titrimetric analysis of base after barium acetate precipitation of sulfate, pure compound and synthetic tablets □ Quinidine sulfate—nonaqueous titrimetric analysis of base after barium acetate precipitation of sulfate, pure compound and synthetic tablets □ Titrimetry—analysis, quinine sulfate and quinidine sulfate, pure compound and synthetic tablets

Sulfates of nitrogenous bases were determined by several nonaqueous titration methods. The free alkaloid may be extracted in chloroform or carbon tetrachloride from its sulfate salt and then titrated against standard *p*-toluenesulfonic acid (1). When sulfates of nitrogenous bases were titrated directly against perchloric acid, only one equivalent of the base was titrated (2); the other equivalent, being bound in the form of bisulfate, was not titratable.

BACKGROUND

The benzidine method for the determination of sulfates of nitrogenous bases (3) is based on the precipitation of the sulfuric acid content in the form of benzidine sulfate. Since any free benzidine will also be titrated, the investigators suggested that only 95% of the equivalent quantity of sulfate ions should be bound in the form of benzidine sulfate. In this method, however, a previous knowledge of the content of the base sulfate in the sample is essential for a successful determination. Almost at the same time the sulfates of streptomycin, dihydrostreptomycin, and neomycin were determined (4) by essentially the same benzidine method.

Because of the poor solubility of most alkaloid sulfates in glacial acetic acid, it was suggested (5) that titration should be performed in a mixture of phenol, chloroform, and acetone against standard perchloric acid solution in dioxane. This method was used for the determination of some alkaloid sulfates, and the end-point was determined either potentiometrically or visually using methyl red indicator. Several alkaloid sulfates were titrated (6) to the bisulfate stage in an acetic anhydride medium. Gentian violet proved to be a suitable indicator. Some alkaloid sulfates were determined (7) in the same manner with bromophenol blue as the indicator. It was reported (8) that there was a drawnout color change of Fettblau B[1] indicator in the titration of the sulfates of quinine, quinidine, atropine, and strychnine in glacial acetic acid solvent and that these alkaloids could not be satisfactorily determined.

Quinine sulfate was titrated (9) in glacial acetic acid against standard perchloric acid. The consumption of the latter was 3.00 and 2.87 equivalents/mole of quinine sulfate when α-naphtholbenzein and quinaldine red were used as indicators, respectively. Photometric titration of quinine sulfate was performed in glacial acetic acid against standard perchloric acid solution (10).

Selective determination of quinine in compound pharmaceutical preparations was carried out (11) by preliminary isolation and transformation to quinine picrate. Then the picrate was titrated visually with gentian violet indicator against standard perchloric acid solution. The acidic components of alkaloid sulfates were determined (12) in an anhydrous pyridine medium against standard sodium methoxide solution.

A method was described for the indirect determination of inorganic sulfates (13) in solutions of reactor fuels. However, this method is best suited for the determination of inorganic sulfates in liquid samples when the components are generally known.

The objective of this study was to develop a nonaqueous titration method for the determination of the sulfates of quinine and quinidine by precipitating the sulfuric acid content of the alkaloid salt with barium acetate and titrating the liberated nitrogenous base.

EXPERIMENTAL

Apparatus and Materials—The following were used: a suitable titrimeter[2] equipped with a combination electrode[3], a magnetic stirrer, and a microburet with a 10-ml capacity (graduated to 0.02 ml).

Solutions—The following were used: 0.1 *N* perchloric acid solution in glacial acetic acid, 0.3 *N* barium acetate solution in glacial acetic acid, and 0.2% (w/v) solution of gentian violet indicator in glacial acetic acid.

Acetous perchloric acid solution was standardized against potassium biphthalate[4], previously dried at 110° for 2 hr, in glacial acetic acid, and the end-point was determined potentiometrically.

Acetous barium acetate solution was prepared by dissolving 29.604 g of barium carbonate[5], accurately weighed, in about 500 ml of glacial acetic acid. The solution was boiled on a hot plate for 3 min and cooled, and sufficient glacial acetic acid was added to produce 1000 ml. Five milliliters of this solution, accurately measured, was diluted with 30 ml of glacial acetic acid, 2 drops of gentian violet indicator solution were added, and the solution was titrated potentiometrically against 0.1 *N* acetous perchloric acid solution. Titrations were also carried out in different solvent systems, and titration curves are illustrated in Fig. 1.

Determination of Sulfates of Quinine and Quinidine by Proposed Method—An accurately weighed amount (50–300 mg) of the alkaloid sulfate was transferred into a 100-ml titration beaker and dissolved in about 30 ml of glacial acetic acid. To the magnetically stirred solution was added slowly an accurately measured volume (2–4 ml) of 0.3 *N* barium acetate solution; then 2 drops of

[1] Ciba.

[2] Pye model 79.

[3] Pye Catalog No. 401 E07, Series 546022.

[4] Rhone-Poulenc, France.

[5] General Chemical and Pharmaceutical Co., Sudbury, Middlesex, England.

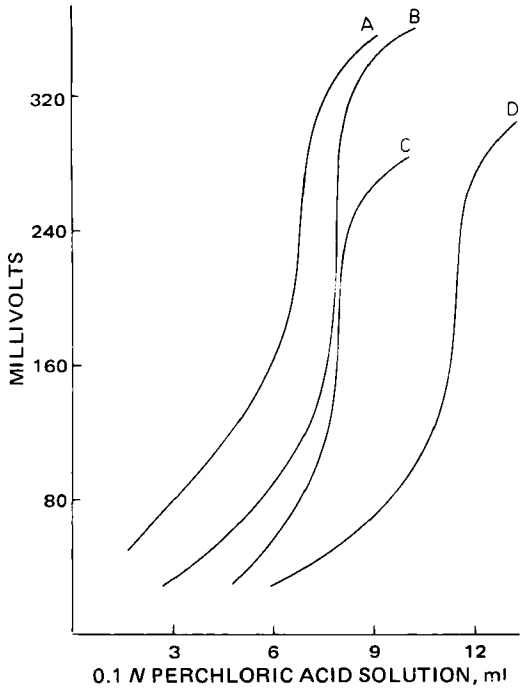

Figure 1—*Potentiometric titration curves of acetous barium acetate solution in different solvent systems. Key: A, glacial acetic acid–dioxane (1:4); B, glacial acetic acid–dioxane (1:1); C, glacial acetic acid; and D, glacial acetic acid–acetic anhydride (2:1).*

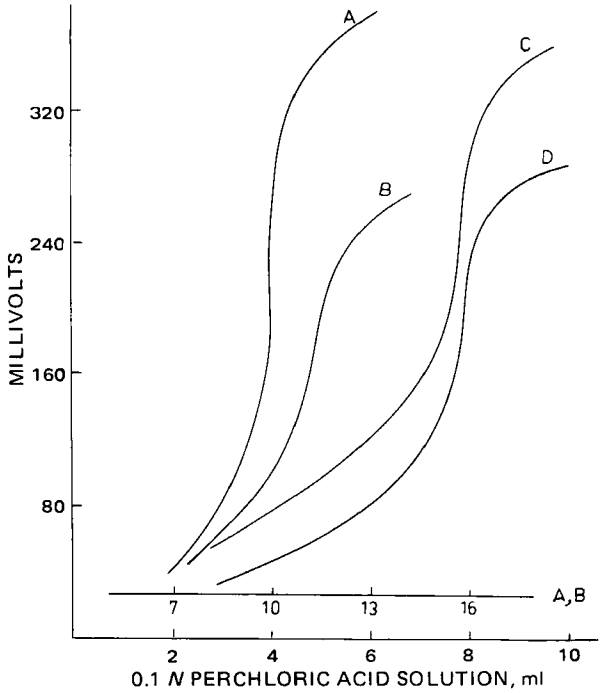

Figure 2—*Potentiometric titration curves in different solvent systems. Key: A, quinidine sulfate and barium acetate, glacial acetic acid–dioxane (1:1); B, quinidine sulfate and barium acetate, glacial acetic acid; C, quinine sulfate and barium acetate, glacial acetic acid–dioxane (1:1); and D, quinine sulfate and barium acetate, glacial acetic acid.*

gentian violet indicator solution were added, and the solution was titrated potentiometrically with 0.1 *N* acetous perchloric acid solution.

A blank determination was performed in the same manner using the same volume of barium acetate solution. The proper color change of the indicator at the point of maximum inflection in the titration curves, obtained by plotting the volume of titrant in milliliters against the millivolt readings, was noted. Potentiometric titrations were also performed in a glacial acetic acid–dioxane solvent system.

Analysis of Tablets—Twenty tablets of quinine sulfate or quinidine sulfate were weighed and reduced to a fine powder. An amount of powdered tablets equivalent to about 200 mg of alkaloid sulfate was accurately weighed and placed in a 100-ml titration beaker containing 30 ml of glacial acetic acid. The solution was heated gently for a few minutes, and 4.0 ml, accurately measured, of 0.3 *N* barium acetate solution was added slowly with continuous stirring. Two drops of gentian violet solution were added, and the solution was titrated with 0.1 *N* acetous perchloric acid solution to a pure blue color change. A blank experiment was carried out using the same volume of barium acetate solution.

RESULTS AND DISCUSSION

In glacial acetic acid and glacial acetic acid–dioxane solvent systems, barium acetate behaves as a moderately strong base. Therefore, when it is titrated with acetous perchloric acid solution, the consumption of the latter is 2 moles/mole of barium acetate (Scheme I).

$$(CH_3COO)_2Ba + 2HClO_4 \rightarrow Ba(ClO_4)_2 + 2CH_3COOH$$

Scheme I

Because of its basic properties in nonaqueous media, barium acetate has not been used to liberate nitrogenous bases from their sulfate salts, in a manner similar to the use of mercuric acetate reagent which binds halide ions (2) and prevents their interference in the nonaqueous titration of halides of nitrogenous bases, since the liberated base and excess barium acetate will consume perchloric acid. However, in the determination of sulfates of quinine and quinidine by the proposed method, the sulfuric acid content of the alkaloid is precipitated in the nonaqueous solvent in the form of barium sulfate by the addition of excess barium acetate solution; 2 moles of quinine or quinidine is liberated for each mole of barium acetate consumed (Scheme II).

$$(C_{20}H_{24}N_2O_2)_2H_2SO_4 + (CH_3COO)_2Ba \rightarrow 2C_{20}H_{24}N_2O_2 + \text{excess } (CH_3COO)_2Ba + BaSO_4 + 2CH_3COOH$$

Scheme II

The liberated alkaloid and the unconsumed barium acetate, being moderately strong bases in glacial acetic acid and glacial acetic acid–dioxane solvent systems, cannot be differentiated when potentiometrically titrated with perchloric acid solution, and only one inflection can be observed in the titration curve of the mixture. Consequently, this single inflection makes the assignment of an equivalent volume of standard perchloric acid to the liberated alkaloid or the remaining excess of barium acetate impossible, so the concentration of alkaloid sulfate in the sample cannot be calculated. However, since 1 mole of barium acetate is equivalent to 2 moles of perchloric acid (Scheme I), as in the blank titration, and since 2 moles of quinine or quinidine is produced in the precipitation reaction (Scheme II) and then consumes 4 moles of perchloric acid (Scheme III), there is a quantitative double increase in the consumption of the titrant for each mole of barium acetate reacting in the precipitation reaction.

$$2C_{20}H_{24}N_2O_2 + 4HClO_4 \rightarrow 2C_{20}H_{24}N_2O_2 \cdot 2H^+ + 4ClO_4^-$$

Scheme III

Advantage was taken of this quantitative increase in the consumption of titrant, as a result of the precipitation reaction, to calculate the percent recovery of quinine sulfate or quinidine sulfate as follows. If $HClO_{4(1)}$ and $HClO_{4(2)}$ are the milliequivalents of perchloric acid utilized in blank and experimental titrations, respectively, then:

$$HClO_{4(1)} = \text{mEq } (CH_3COO)_2Ba \qquad \text{(Eq. 1)}$$

and:

$$HClO_{4(2)} = \text{mEq alkaloid sulfate} + \text{mEq excess } (CH_3COO)_2Ba \qquad \text{(Eq. 2)}$$

Table I—Nonaqueous Titration of Diastereomeric Sulfates of Quinine and Quinidine by Proposed Method

Sample	Quinine Sulfate			Quinidine Sulfate		
	Weight, mg	Weight Found, mg	Recovery, %	Weight, mg	Weight Found, mg	Recovery, %
			Pure Compound			
1	285.0	281.6	98.89	204.2	203.3	99.56
2	255.3	256.2	100.35	198.0	196.7	99.34
3	228.0	222.5	97.59	132.2	132.3	100.07
4	205.7	205.6	99.95	102.3	101.7	99.41
5	146.0	146.5	100.34	102.2	101.8	99.61
6	102.1	103.6	101.47	101.0	100.9	99.99
7	99.3	97.8	98.49	99.9	99.5	99.60
8	94.0	92.1	97.98	70.3	69.7	99.15
9	57.3	57.5	100.35	57.4	57.9	100.90
	Mean percent recovery (p = 0.05)		98.84 ± 1.00	Mean percent recovery (p = 0.05)		99.74 ± 1.27
			Synthetic Tablets			
1	200.2	199.5	99.65	200.2	203.3	101.55
2	200.1	198.8	99.35	200.2	199.5	99.65
3	200.0	197.9	98.95	200.2	199.5	99.65
4	200.0	195.6	97.80	200.0	201.4	100.70
5	199.8	195.4	97.79	200.0	199.4	99.70
	Mean percent recovery (p = 0.05)		98.71 ± 1.08	Mean percent recovery (p = 0.05)		100.25 ± 0.38

Table II—Determination of Quinine Sulfate and Quinidine Sulfate by BP 1968 Procedure

Sample	Quinine Sulfate			Quinidine Sulfate		
	Weight, mg	Weight Found, mg	Recovery, %	Weight, mg	Weight Found, mg	Recovery, %
1	502.7	510.0	101.45	501.9	509.0	101.41
2	500.0	504.0	100.80	500.5	508.0	101.49
3	500.0	498.0	99.60	500.0	501.6	100.32
4	501.0	499.2	99.64	500.0	500.4	100.08
	Mean percent recovery (p = 0.05)		100.37 ± 1.44	Mean percent recovery (p = 0.05)		100.82 ± 1.16

Also, according to the stoichiometries expressed by reactions shown in Schemes I–III, it can be stated that:

$$\text{mEq alkaloid sulfate} = 2\{[\text{mEq } (CH_3COO)_2Ba] - [\text{mEq excess } (CH_3COO)_2Ba]\} \quad \text{(Eq. 3)}$$

Substituting Eq. 1 into Eq. 3 and dividing by 2 yield:

$$\tfrac{1}{2}\text{mEq alkaloid sulfate} = HClO_{4(1)} - \text{mEq excess } (CH_3COO)_2Ba \quad \text{(Eq. 4)}$$

On rearranging, Eq. 4 becomes:

$$\text{mEq excess } (CH_3COO)_2Ba = HClO_{4(1)} - \tfrac{1}{2}\text{mEq alkaloid sulfate} \quad \text{(Eq. 5)}$$

Then substituting Eq. 5 into Eq. 2 and rearranging give:

$$\text{mEq alkaloid sulfate} = 2(HClO_{4(2)} - HClO_{4(1)}) \quad \text{(Eq. 6)}$$

Therefore, the percent recovery of quinine sulfate or quinidine sulfate is:

$$\% \text{ recovery} = \frac{2(HClO_{4(2)} - HClO_{4(1)}) \times 195.74 \times 10^2}{W} \quad \text{(Eq. 7)}$$

which simplifies to:

$$\% \text{ recovery} = \frac{2(E - B) \times N \times 195.74 \times 10^2}{W} \quad \text{(Eq. 8)}$$

where E = milliliters of perchloric acid equivalent to total basicity in the experimental titration, B = milliliters of perchloric acid equivalent to barium acetate in the blank titration, N = normality of perchloric acid, W = weight of sample (milligrams), and 195.74 = equivalent weight of quinine or quinidine sulfate dihydrate.

Potentiometric titration curves for barium acetate alone and mixed with either quinine or quinidine sulfate in different solvent systems are shown in Figs. 1 and 2, respectively. In all solvent systems studied, the appearance of the blue color of gentian violet indicator signaled the potentiometric end-point. Glacial acetic acid–dioxane (1:1) mixture was the solvent of choice, since a greater break in the titration curve and a more satisfactory indicator color change were obtained.

Recovery data for quinine sulfate and quinidine sulfate, in pure form and in synthetic tablets, using the proposed method are listed in Table I. The results were quantitative and reproducible. The overall mean percent recoveries (p = 0.05) for the assay of samples of quinine sulfate and synthetic tablets, prepared to contain 150 mg of the drug, were 98.84 ± 1.00 and 98.71 ± 1.08, respectively. Recoveries (p = 0.05) for quinidine sulfate and its synthetic 200-mg tablets were 99.74 ± 1.27 and 100.25 ± 0.38, respectively. Starch and talc, the common excipients of tablets, do not interfere with the proposed method; however, titratable lubricants such as magnesium stearate do interfere.

In comparison, the mean percent recoveries (p = 0.05) for the determination of the diastereomers, quinine and quinidine sulfate, by the gravimetric method of BP 1968 (14) were 100.37 ± 1.44 and 100.82 ± 1.16, respectively (Table II). Statistical analysis of the two sets of results obtained by applying the proposed and BP 1968 procedures indicated that the difference between the two mean percent recoveries obtained for each compound was statistically insignificant and that the results obtained by the two methods were almost equally accurate and precise.

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Quantitative GLC Determination of Codeine in Plasma

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Abstract □ A sensitive and accurate GLC method for quantitating codeine in plasma at levels of 50 ng/ml, with limits of detection as low as 5 ng/ml, is described.

Keyphrases □ Codeine—GLC analysis, *n*-butyl chloride extraction, plasma □ GLC—analysis, codeine in plasma

Codeine has long been used as an analgesic in pharmaceutical preparations. Because of the recent interest in drug efficacy and bioavailability, a sensitive method for determining low nanogram quantities in plasma is needed. Several methods for the analysis of codeine in urine have been developed for the study of its metabolism and for forensic purposes (1–3). However, the levels found in urine are many times greater than those found in plasma, so these methods are generally not applicable in plasma analysis.

Schmerzler *et al.* (4) reported a GLC method for codeine in serum (and urine) of adequate sensitivity. This method has two serious drawbacks: the tendency for chloroform to form emulsions, especially with serum or plasma, and a variable recovery of codeine in the extraction procedure.

In this study, *n*-butyl chloride, which does not readily form emulsions with plasma, was used in place of chloroform. Dihydrocodeinone was added to the plasma to serve as an internal standard for both the extraction procedure and GLC quantitation.

EXPERIMENTAL

Apparatus—A gas chromatograph[1] with a flame-ionization detector was used.

Reagents—*n*-Butyl chloride[2] saturated with water was used for all extractions. Dihydrocodeinone was synthesized in this laboratory from thebaine by a two-step procedure (5). The water used to prepare aqueous reagents was checked to ensure low GLC background; all other solvents and chemicals were reagent grade.

Glassware was oven cleaned at 400° (6), silanized with 5% dimethyldichlorosilane in toluene, and rinsed with toluene and methanol. Immediately before use, the glassware was rinsed with chloroform to maintain a low GLC background. Chromatographic grade carbon disulfide[3] was used for GLC.

Procedure—To 2.0 ml of plasma was added 1 μg of dihydrocodeinone internal standard in aqueous solution. The plasma was made alkaline with 0.10 ml of 1 *N* NaOH and extracted twice with 2 ml of butyl chloride on a reciprocal shaker at 60 cpm for 20 min. The organic fractions were combined, a 0.5-ml aliquot of 0.05 *N* H_2SO_4 was added, and the solution was shaken for 20 min. Then the organic layer was discarded, and the aqueous phase was washed with an additional 1 ml of butyl chloride. The aqueous layer then was made alkaline with 0.05 ml of 1 *N* NaOH and extracted twice with 1 ml of butyl chloride.

The organic layers were combined in a conical vial, and the solvent was evaporated immediately before GLC analysis. The residue was taken up in 10 μl of carbon disulfide, and 2 μl was injected for GLC analyses. The column used was a 1.8-m (6-ft), 2.5-mm i.d., silanized glass column with 2% (w/w) XE-60 on acid-washed Anakrom A (90–100 mesh).

The temperatures were: injector, 250°; column, 230°; and detector, 250°. The nitrogen carrier gas flow rate was 20 ml/min. The ratio of the codeine to dihydrocodeine peak areas was compared to a standard curve; peak areas were measured by peak height times width at half-height.

Table I—Recoveries of Codeine Added to Plasma

Codeine Added to Plasma, ng/ml	Codeine Found[a], ng/ml
0	ND[b]
50	48 ± 2 (n = 2)
100	105 ± 7 (n = 3)

[a] Corrected for the calculated extraction recovery. [b] Not detected.

RESULTS AND DISCUSSION

The objective of this study was to devise a method that was accurate and precise without requiring inordinate amounts of time for evaluation of recoveries. Several codeine analogs were considered as possible internal standards. Of these, morphine and norcodeine could not be used since they are major metabolites of codeine (7). Dihydrocodeine and codeine were unresolved by GLC on OV-17 or XE-60. Since dihydrocodeinone was well resolved on both columns and had a relative retention time of 1.67 (codeine = 1.0) on the XE-60 column, it was the choice as an internal standard. With the use of dihydrocodeinone, this procedure is nearly

[1] Fisher Victoreen series 4400.
[2] Burdick and Jackson.
[3] Fisher.