# The Determination of Quinine and the Assay of Quinine and Strychnine in Mixtures*

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Considerable difficulty has been experienced by chemists in applying the classical acidimetric titration to the determination of quinine. This may be due to the presence in this alkaloid of two amino nitrogens having widely different ionization constants. The customary procedure consists in the titration of the more basic of these amino nitrogens with the formation of the corresponding mono-acidic salt. The buffering action of this salt precludes the accurate determination of the end-point by means of an indicator.

Conant, Hall and Werner (1, 2, 3, 4, 5) found that solutions of weak bases in glacial acetic acid may be successfully titrated with a strong acid, such as perchloric or hydrobromic, made up in the same solvent. They have presented a number of potentiometric titration curves of various bases titrated with glacial acetic acid solutions of perchloric, hydrobromic, hydrochloric and sulfuric acids. Nadeau and Branchen (6) applied this titration method to the estimation of amino acids. The end-point for these titrations was determined potentiometrically or by the use of crystal violet, α-naphtholbenzein or benzoyl auramine as an indicator. Nadeau and Branchen pointed out that acetic acid has a high thermal expansion and, for this reason, they used weight burettes. Since the thermal expansion of acetic acid is a linear function of the temperature, however, titrations with a volume burette have been used in this study by applying the following formula:

$$N_1 = \frac{N_0}{1 + 0.001\,(T_1 - T_0)}$$

where

$T_0$ = Temperature at which solution was standardized.
$T_1$ = Temperature at which titration was made.
$N_1$ = Normality at $T_1$.
$N_0$ = Normality at $T_0$.

In Fig. 1 are shown the electrometric titration curves of quinine in aqueous medium with sulfuric acid, and in glacial acetic acid with perchloric acid in the same solvent. The application of the latter titration to the determination of quinine and its salts will be described later in this communication.

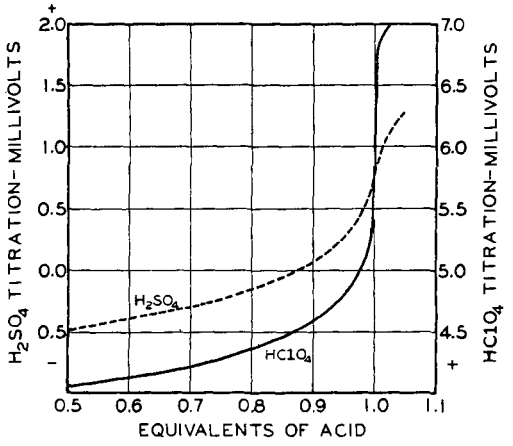


Fig. 1.—Electrometric Titration of Quinine Using a Glass Electrode.
– – – – Titration with $H_2SO_4$ in water.
——— Titration with $HClO_4$ in glacial acetic acid.

Quinine and strychnine are frequently encountered together in a wide variety of syrups, elixirs and tablets. The quantitative separation and estimation of these bases have been found to be extremely difficult, owing to the presence of a large quantity of quinine and a relatively small amount of strychnine. Several methods have been proposed for the separation of these alkaloids, all of which give widely divergent results (7, 8, 9, 10). The method most widely used is a modification of the procedure developed by Simonds (11) which was tentatively adopted by the Association of Official Agricultural Chemists (12). Recoveries by titration, as reported by collaborators in this study, varied between 74 and 126 per cent, and 77.4 and 95.9 per cent, respectively, for strychnine and quinine. The low results for quinine were claimed to be due to the difficulty in titrating this base. The gravimetric estimation of quinine, as reported by the

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collaborators, resulted in recoveries ranging between 83 and 95.9 per cent. In addition to the inconsistent results, at least three days are required to complete the assay.

The distribution coefficients of these bases between immiscible solvents did not differ sufficiently to allow their separation by extraction. It seemed possible, however, that products formed by the combination of the bases with an acid might have distribution coefficients which would permit their separation. A study was made of a number of acid salts of these alkaloids, and it was found that a mixture of quinine and strychnine in a strong sulfuric acid solution could be quantitatively separated by extracting the strychnine with a solution of dichloracetic acid in chloroform.

## EXPERIMENTAL

*Materials Used.*—

Acetic acid (glacial)—99.9%.

Indicator—0.2 Gm. $\alpha$-naphtholbenzein per 100 cc. acetic acid.

Perchloric acid—$N/10$ in glacial acetic. The concentration of acid in reagent quality perchloric acid was determined by titration with sodium hydroxide. A weighed quantity of this acid was placed in a liter volumetric flask, an amount of acetic anhydride to react completely with water present was added and the solution diluted to one liter with acetic acid. This solution was standardized against pure, dry quinine base by the method given below for the estimation of quinine.

Perchloric acid—$N/100$ in glacial acetic. This reagent was prepared by the dilution of the above solution with acetic acid.

Sulfuric acid—$7N$ (approximate).

Dichloracetic acid in chloroform—1%. Chloroform was first washed with water to remove the alcohol.

*Determination of Quinine.*—A quantity of sample representing approximately 0.5 Gm. of quinine base was accurately weighed and transferred to a separatory funnel containing about 20 cc. of water. An excess of ammonia was added and the alkaloid extracted with five 25-cc. portions of $CHCl_3$. Each extract was filtered through a cotton pledget into a beaker and the combined extracts evaporated to dryness. The residue was dissolved in 20 cc. of acetic acid, 5 drops of indicator added and the solution titrated with $N/10$ perchloric acid to a bluish green end-point. One cc. $N/10$ perchloric acid = 0.0162 Gm. of anhydrous quinine base.

The above method was applied to several purified air-dried salts of quinine. In all cases, the amount of the anhydrous salt in these preparations was established by the determination of the acid radical, and these values were used as the criteria in determining the accuracy of the titration method. The results are given in Table I.

Table I.—Recovery of Quinine from Its Acid Salts

| Salt | Quinine Salt Present (*A*) By Determination of Acid Radical | Quinine Salt Present (*B*) By Perchloric Acid Titration | % Recovery $B/A \times 100$ |
|---|---|---|---|
| Quinine sulfate | 95.73 | 95.10 | |
| | 96.29 | 95.00 | |
| Average | 96.03 | 95.05 | 99.0 |
| Quinine hydrochloride | 93.79 | 93.92 | |
| | 93.65 | 93.83 | |
| Average | 93.72 | 93.88 | 100.2 |
| Quinine dihydrobromide | 88.94 | 89.05 | |
| | 88.93 | 88.98 | |
| Average | 88.94 | 89.02 | 100.1 |

*Separation of Quinine and Strychnine.*—Before these alkaloids could be determined, they had to be separated from interfering ingredients present in the various preparations. To separate the alkaloids from Elixir of Iron, Quinine and Strychnine, N. F., a 200-cc. sample was concentrated on a steam bath to remove alcohol, transferred to a separatory funnel, made alkaline with ammonia and extracted to completion (five times) with 25-cc. portions of chloroform. The combined chloroform extracts were extracted with three 10-cc. portions of sulfuric acid (1-10). The combined acid extracts were made alkaline and reëxtracted with chloroform as above, the chloroform extracts filtered through a pledget of cotton into a 250-cc. beaker and evaporated to dryness. This alkaloidal residue was used for the estimation of quinine and strychnine.

Tablets of quinine, strychnine and reduced iron were ground to pass through a 100-mesh screen, a weighed sample equivalent to 25–50 mg. of strychnine was placed in a separatory funnel fitted with a fritted glass disk (Ace Glass Co., 7290) and macerated for one hour with a mixture of 8 cc. of alcohol and 2 cc. of stronger ammonia T.S. The mixture was then shaken for thirty minutes in a mechanical shaker with 50 cc. of chloroform, the chloroform removed by suction and the residue reëxtracted four times with 50-cc. portions of chloroform, each addition of chloroform followed by a thirty-minute shaking period. The combined chloroform solutions were extracted with three 10-cc. portions of sulfuric acid (1-10), the combined acid solution made alkaline with ammonia and extracted with five 25-cc. portions of chloroform. The chloroform was filtered through a pledget of cotton into a 250-cc. beaker and evaporated to dryness.

The residues thus prepared, or other mixtures of quinine and strychnine free of interfering ingredients, were dissolved in 10 cc. of the dichloracetic-chloroform reagent, transferred to a separatory funnel and the beaker rinsed with small portions of the

Table II.—Separation of Quinine and Strychnine

| | Strychnine | | Quinine | | Ratio of Strychnine to Quinine |
|---|---|---|---|---|---|
| | Present, Gm. | Recovered, % | Present, Gm. | Recovered, % | |
| Mixtures of Quinine and Strychnine | 0.0800 | 97.9 | 0.8338 | 100.0 | 1–10 |
| | 0.0800 | 98.8 | 1.0528 | 99.3 | 1–13 |
| | 0.0800 | 98.0 | 0.9451 | 98.7 | 1–12 |
| | 0.0600 | 97.7 | 0.8829 | 99.2 | 1–15 |
| | 0.0600 | 97.9 | 1.1243 | 99.2 | 1–19 |
| | 0.0600 | 98.0 | 1.2172 | 99.2 | 1–20 |
| | 0.0300 | 96.0 | 1.3486 | 99.3 | 1–45 |
| | 0.0300 | 95.8 | 1.5823 | 99.0 | 1–53 |
| Elixir of Iron Quinine and Strychnine N. F. VI | 0.0273 | 98.1 | 1.3076 | 99.7 | 1–48 |
| | 0.0273 | 97.5 | 1.3076 | 99.8 | 1–48 |
| | 0.0273 | 98.1 | 1.3076 | 99.3 | 1–48 |
| Reduced Iron, Quinine and Strychnine | 0.0293 | 96.3 | 1.2429 | 99.6 | 1–42 |
| | 0.0293 | 97.4 | 1.2429 | 99.0 | 1–42 |

reagent until the total volume was approximately 25 cc. This solution was shaken for two minutes with 10 cc. of 7*N* sulfuric acid. The chloroform layer was filtered through a pledget of cotton into a second separatory funnel. The acid solution was repeatedly extracted with three 20-cc. portions of the reagent and two 20-cc. portions of washed chloroform. The combined chloroform extracts were shaken through four successive separatory funnels, each containing 5 cc. of 7*N* sulfuric acid. Three 20-cc. portions of the chloroform-dichloracetic acid reagent and two 20-cc. portions of washed chloroform were shaken through the acid solution remaining in these four separatory funnels. The combined chloroform extracts and washings were shaken in a separatory funnel with 20 cc. of ammonia (1–4), the chloroform layer was filtered through a pledget of cotton in a 400-cc. beaker. The ammoniacal solution was washed with a 20-cc. portion of chloroform, the latter filtered and added to the above beaker. The chloroform was evaporated on the steam bath to dryness, care being taken to prevent decrepitation. A 5-cc. portion of acetic acid, to which 5 drops of α-naphtholbenzein indicator had been added, was neutralized with *N*/100 perchloric acid and added to the residue. The resulting solution was titrated with *N*/100 perchloric acid.

The acid solutions and washings from the strychnine separation were combined and carefully neutralized with concentrated ammonia and cooled. The quinine was extracted with five successive 25-cc. portions of chloroform, the extracts filtered through cotton into a 250-cc. beaker. The chloroform was evaporated to dryness, the residue dissolved in acetic acid, transferred to a 100-cc. volumetric flask and diluted to volume. A 50-cc. aliquot of this solution was titrated with *N*/10 perchloric acid using five drops of α-naphtholbenzein solution as an indicator.

1 cc. *N*/100 perchloric acid = 0.00334 Gm. strychnine

This procedure has been applied to various mixtures containing a known amount of quinine and strychnine, and the results are given in Table II.

In the separation of these alkaloids, the sulfuric acid extracts showed considerable fluorescence due to the presence of quinine. The last washing, which ordinarily contained a negligible amount of this alkaloid, usually exhibited a noticeable fluorescence and, with a little experience, this property may be used by the analyst as an indication of the complete extraction of quinine.

As indicated in Fig. 1, the electrometric titration curve of quinine in glacial acetic acid rose quite rapidly at the neutralization point. This property is exhibited by a number of alkaloids and should be of considerable value in their estimation.

## SUMMARY

(1) The titration of quinine in glacial acetic acid has been studied and a method for its assay, based on this procedure, has been proposed.

(2) An accurate and convenient method for the separation and determination of quinine and strychnine in various mixtures has been described.

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