concordant titrations. The solution of unknown concentration was then titrated in a similar manner and the calculations made by dividing the number of cubic centimeters of the standard solution necessary for the decolorization of 0.5 cc. of the diluted bromine water by the corresponding number for the unknown. The resulting figure represented the amount of alkaloid, in mg. in 1 cc. of the unknown solution. By employing such a procedure it was possible to estimate within 5 per cent of the concentration of unknown solutions.

The test is also applicable to tissue extracts upon which quantitative studies as to alkaloidal content are desired. In addition to quinine and quinidine mentioned by Weiss and Hatcher it was found that the following alkaloids, when present in a concentration of about 1–1000, lend themselves well to the test: ethylmorphine, diacetylmorphine, methylmorphine, cinchonidine, pilocarpine, strychnine, procaine, theobromine, caffeine, histamine, brucine, and to a limited extent, epinephrine. The following were found to be without influence upon the color of bromine water, and hence not suitable for the test: atropine, scopolamine, cocaine, betaine, sparteine, hydrastine, ephedrine and morphine. Physostigmine and papaverine first produced a white cloudiness which cleared upon the addition of a slight excess of the solution; whether practical use could be had in the case of these two is questionable. Apomorphine gave rise to a cherry-colored solution which did not decolorize. Nicotine had no decolorizing effect when used in a 1–1000 solution, but was quite effective in a concentration of 1 per cent.

REFERENCE.

(1) Soma Weiss and Robert A. Hatcher, *Proc. Soc. Exptl. Biol. Med.*, 23 (1925), 33–35.

A METHOD OF DETERMINING A SMALL AMOUNT OF STRYCHNINE IN THE PRESENCE OF LARGER AMOUNTS OF QUININE AND CINCHONIDINE.*

BY GUSTAVE A. STICHT.

When the cinchona alkaloids are dissolved in an excess of hydrochloric acid, and these salts evaporated on a water-bath to dryness, until no more excess of acid is present, the so-called *di* salts are formed, and as is known, one-half of their acid radical is acid to methyl red when they are in solution, and both acid radicals are acid to phenolphthalein. Most other alkaloids, as strychnine, under the same treatment, only form salts that are neutral to methyl red, and acid to phenolphthalein. If a *di* acid and a *mono* acid alkaloidal salt are present together, then using methyl red and phenolphthalein indicators, the respective amounts of alkaloids can be estimated.

If the proportion of cinchona alkaloids to strychnine is not very large, and their acid radicals are combined as stated, then titrated as described below, the amounts of acid due to the cinchona alkaloid and strychnine, respectively, can be estimated with a fair degree of accuracy.

But in elixirs and such preparations, the amount of cinchona alkaloids is usually much larger than the strychnine, hence the bulk of the cinchona alkaloids must be separated. This is best done in the case of quinine and cinchonidine as a

* Scientific Section, A. PH. A., Toronto meeting, 1932.

tartrate. In the presence of the four cinchona alkaloids, quinine, cinchonidine, cinchonine, quinidine, and small amounts of strychnine, the estimation of the strychnine could be accomplished by successively precipitating quinine and cinchonidine as tartrate, cinchonine by its insolubility in ether, and quinidine as iodide. But the precipitations must be carried out in somewhat dilute solutions, to keep the strychnine from being co-precipitated. The method is rather tedious, because after each separation, the alkaloids in solution must be separated from the preponderance of salts, by chloroform and ammonia before being further manipulated. The precipitated alkaloids must likewise be treated with chloroform and ammonia if their determination is desired.

For convenience it is best to have at least one grain in the sample to be assayed, though smaller amounts have been determined.

The alkaloids are extracted from the measured or weighed sample by chloroform and ammonia, which solvent is evaporated or distilled off. When nearly dry, the residue is dissolved in an excess of dilute hydrochloric acid, with the aid of heat, the solution with its washings transferred to a dish of about 300 cc. capacity, and the rest of the chloroform is evaporated on a water-bath. The dish with its contents of about 100 cc. is placed over a small flame, some methyl red indicator added, and from a burette, dilute ammonia, about 5%, is very slowly added to the hot liquid, stirring at the same time, until the liquid is yellowish.

Sometimes, upon adding the dilute ammonia, small lumps of alkaloid are formed, mixed with the precipitated tartrate, which formation must be avoided. It is best then to re-dissolve the precipitate while solution is kept hot, by the careful addition of stronger acid, until all is dissolved again, and then once more adding the dilute ammonia as described above. The lumps are apt to form if the ammonia is added too rapidly, the solution of alkaloids is too concentrated, or the solution was not stirred sufficiently while neutralizing. Then from another burette, dilute acid, about $N/10$, is added, until the liquid is red.

To the clear liquid add Rochelle salts, approximately one Gm. for each Gm. alkaloid present, and while the liquid is still hot, more of the dilute acid is added until the liquid is a decided red, not a pink. Allow to stand in a cool place at least two hours, or better over night. This does not precipitate all the quinine, but should reduce the ratio of quinine to strychnine. At this stage there is a possibility of some strychnine being carried down with the quinine, if not acid enough.

The tartrate precipitate and solution is filtered by suction as rapidly as possible, and washed from the dish onto the filter, using about 50 cc. of water in all, then dropwise the edge of filter is washed with about 10 cc. more of water. The filtrate from this tartrate with its washings are placed in a large enough separator, and the alkaloids in the solutions extracted with chloroform and ammonia, the chloroform evaporated or distilled off, the residue of alkaloids with its small amount of solvent dissolved with sufficient excess of dilute hydrochloric acid; this acid solution with its washings placed in a dish, and evaporated on a water-bath. When nearly dry, a fair amount of free hydrochloric acid should be given off. Evaporate to dryness, mixing with a stirring rod, if need be, then add a little water and evaporate again to dryness. This should drive off all excess of hydrochloric acid. It should not be kept on the water-bath longer than necessary, and if possible, have the water-bath a little below boiling.

Ammonia fumes must be kept away from the acid solution of the alkaloids while evaporating to dryness.

The dry residue in the dish is taken up with a small amount of water, washed into a 100-cc. flask or cylinder, brought up to mark, mixed and 50 cc. of this transferred to a titrating vessel; a wide mouth bottle with a clear bottom is very good to see the color changes. A few drops of methyl red indicator are added and then the liquid is titrated to a pure yellow with $N/50$ sodium hydrate, and cc. used noted. A white surface placed under the bottle shows the color changes very well.

The other 50 cc. of liquid is transferred to another titrating vessel, then 50 cc. of 95% neutral alcohol added, then some phenolphthalein indicator, and titrated with the $N/50$ soda to a faint pink which remains so for at least one-fourth minute, and number of cc. noted. The error by measuring this way is practically negligible for this case.

Then make a blank test, using 50 cc. of same alcohol with 50 cc. of water and phenolphthalein indicator and note the number of cc. of $N/50$ required to obtain same slight pink.

From the number of cc. of second titration subtract the number of cc. for the blank test, then from this subtract twice the number of cc. used in the first titration, and this difference multiplied by two, should give the amount of $N/50$ for the strychnine.

To reiterate, it is important that the hot acid solution, wherein the tartrate is precipitated, is made sufficiently acid again while hot after the addition of the Rochelle salt, but not acid enough so as to dissolve too much of the precipitate. If not enough free acid present, some strychnine, or many other alkaloids, if present, will be carried down with the tartrate, about 0.020 Gm. more or less of strychnine when about 2 Gm. of quinine is precipitated, the amount of liquid not having such a large influence.

ASSAY METHOD FOR THE DETERMINATION OF AN ALKALOID OR TOTAL ALKALOIDS IN COATED OR UNCOATED TABLETS.*

BY LESTER C. DICK.

Coated tablets, except sugar-coated, white, are placed in a beaker and the interfering color removed by washing with a solvent.

The tablets are dried at 100° C. and fifty or more are weighed, the average weight noted and the tablets reduced to a fine powder.

Uncoated tablets are directly weighed and reduced to a fine powder. An aliquot part is taken—in either case representing about two grains of alkaloidal salt. The powder is transferred to a Florence flask of 300 cc. capacity, fitted with a tight stopper.

Ammonium hydroxide or sodium hydroxide T. S. is now added in quantity sufficient to liberate the alkaloid. A number of buckshot, depending upon the quantity of powder taken, are now added in amount necessary to subdivide the moist mass. Exactly 100 cc. of chloroform is added, the flask stoppered and rotated, thus coating the sides of flask with the moist powder.

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* Scientific Section, A. PH. A., Toronto meeting, 1932. No discussion.