2816

A method for the quantitative determination of small amounts of quinin and quinidin with bromin water.

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We undertook to learn whether quinin could be determined quantitatively in extracts of animal tissues by means of the so-called thalleioquin color reaction, but it soon became apparent that the intensity of the color varies with too many factors to permit of its use for this purpose. It was observed, however, that the color of the bromin water disappeared sharply on the addition of a slight excess of quinin, and the present method depends upon the fact that when quinin is added to bromin they combine in definite proportions with the loss of the color of the bromin in reflected light.

Hart[1] attempted unsuccessfully to estimate quinin quantitatively by means of the thalleioquin reaction. He found, however, that four atoms of bromin are absorbed by the quinin, two of them loosely. He says, "It follows, then, that quinine may be determined from its bromine absorption for five minutes in the manner stated: 1 cc. N/10 Br. = 0.0081 g. quinine." This method has not been employed hitherto so far as we know.

Nine solutions of quinin, varying widely in concentration and containing from 0.5 to 2.0 milligrams of quinin base, were prepared by one of us (H) and examined by the other (W), who did not know the strength of the solution in any case. The determinations were made with an average error of about five per cent.

The necessary reagents are prepared in the following manner: Bromin water: Place a convenient volume of bromin in a bottle with a tightly fitting glass stopper, add water, and shake until saturation occurs with an excess of bromin in the bottom of the bottle. It keeps indefinitely.

Diluted bromin water: Dilute a convenient volume of bromin

[1] Hart, W. B., *Soc. Chem. Ind. J.*, 1921, lx, 72.

water with nine volumes of water immediately before using. This is placed in a bottle having a tightly fitting glass stopper and protected from light.

Standard quinin solution: Dissolve 120.1 milligrammes of quinin hydrochlorid (U. S. Ph.) in enough distilled water to make 100 cc. One cc. of this solution corresponds to 1 milligramme of anhydrous quinin base.

Standard quinidin solution: Dissolve 120.6 milligrammes of quinidin sulphate (U. S. Ph.) in enough distilled water to make 100 cc. One cc. of this solution corresponds to 1 milligramme of anhydrous quinidin base.

Method of procedure: Place 0.5 to 1.0 cc. of diluted bromin water, accurately measured, in a test tube of about 5.0 cc. capacity and of about 10 millimeters diameter; add the standard quinin solution until the color of the bromin is discharged. One milligramme of quinin (1. cc. of the standard solution) corresponds to about 0.85 cc. of the diluted bromin water, hence one must add about 1.2 cc. of the standard quinin solution to 1.0 cc. of diluted bromin water.

The disappearance of the color of bromin is observed best by reflected light with the tube held against a white background. A yellowish tint may still be observed by looking down through the depths of the solution. This must be disregarded. All glass used must be free from alkali and the test tubes must be free from a yellow color. The average of at least 3 readings is taken, and these must not vary more than 3 per cent.

Determination of quinin in the extract: The extract or substance to be tested should be made into a solution having as nearly as possible the same concentration of quinin as the standard, but in any event, not less than 1 milligramme of the base in 5 cc. of solution. When an extract from tissue is to be tested, it is convenient to add a few drops of alcohol to the extract with 0.25 cc. N/10 sulphuric acid for each milligramme of extract and warm. When solution is complete, it is diluted so that 1 cc. contains about 1 milligramme of the extract. Tests showed that traces of alcohol and a slight excess of sulphuric acid are without influence on the accuracy of the determination.

The solution of the extract is tested with the same amount of diluted bromin water and exactly as described for the standard quinin solution. When the preliminary test shows that 1 milli-

gramme of the extract contains less than 0.2 milligrammes of quinin, the extract must be repurified, if possible.

Calculations of quinin in the unknown: The number of cc. of the standard solution of quinin required to decolorize the diluted bromin water is divided by that required of the solution of the extract. The quotient is the amount of quinin in milligrammes (or fraction of a milligramme) in 1 cc. of the solution.

When numerous solutions are to be examined, the standard quinin solution should be tested after three or four solutions have been titrated. The reason for this precaution is that the bromin evaporates, and after a series of tests requiring an hour or more a slight loss in the concentration of the diluted bromin water may occur.

When diluted bromin water is allowed to stand at rest for several hours, the upper layer becomes colorless while the lower layer changes much less, or not perceptibly, when the container is tightly stoppered. The pipette used for measuring the diluted bromin water should be of such capacity that a residuum of bromin water remains in the pipette after the required amount is discharged. The balance in the pipette is thrown away. The loss of bromin can be avoided or minimized by the use of petrolatum on the stopper, but this is inconvenient owing to its adherence to the pipette.

Quinidin is estimated in exactly the same manner, but the standard is titrated against a known amount of quinidin when quinidin is to be determined quantitatively. In each of two tests of one specimen of quinidin, it required 8 per cent more diluted bromin water than an equal weight of the specimen of quinin used.