SCIENTIFIC SECTION

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THE QUANTITATIVE DETERMINATION OF ALKALOIDS WITH BROMINE.*

BY ROBERT A. HATCHER AND ROBERT L. HATCHER.

Hart (1) reported that one molecule of quinine in solution absorbs four atoms of bromine, and that the former could be estimated quantitatively by means of $N/10$ bromine solution, 1 cc. of which is decolorized by 8.1 mg. of quinine.

Weiss and Hatcher (2) described a method for the quantitative estimation of small amounts of quinine and quinidine in pure solutions and in those which contain extracts of tissue, by means of an aqueous solution of bromine. The method depends on the absorption of the bromine, the end-point being the disappearance of the yellow color. Haag (3) reported that this method may be used for the quantitative determination of brucine, caffeine, cinchonidine, procaine, strychnine, theobromine and certain other alkaloids; but that it is not available for the estimation of atropine, cocaine, morphine, sparteine and certain other alkaloids.

In the course of studies involving the quantitative estimation of morphine in extracts of animal tissues, it was found that the presence of very small amounts of impurities sometimes interfered with the quantitative colorimetric determination of morphine by means of Marquis' reagent, and it was decided to investigate the availability of bromine solution for the estimation of morphine and various other substances.

TECHNIQUE.

The general method is as follows: Dilute 1 volume of the official (U. S. P. X) $N/10$ bromine solution with 9 volumes of distilled water. Dissolve the substance to be tested in distilled water; in the case of alkaloids in the proportion of $^1/_{400}$ of the molecular weight in grams in 1 liter. Place 1 cc. of $N/100$ bromine solution in each of a series of colorless or greenish (not yellowish) test-tubes of about 18 cc. capacity and of about 14-mm. diameter; add the solution of the substance to be tested in successively increasing volumes; then add 0.3 cc. of about 12 per cent HCl to each tube, and after the reaction is complete (or after thirty minutes), observe in which tubes the color of bromine persists. The tube is held at right angles to the light, preferably from a northern exposure—not in direct sunlight—and the solution is observed transversely. The end-point is the mean of the largest volume which fails to discharge the color of bromine, and the smallest volume which decolorizes it completely. If a greater degree of precision is necessary, the experiment is repeated with less difference in the successive volumes of the solution added to the bromine solution. Solutions of the substance of known concentrations, which in every case should approximate that of the one being examined, should be used in exactly the same way as a control. Moderately dilute solutions of all substances, the concentrations of which were not known to the observer, were used, the strength of the controls being known. Solutions of greater dilutions, differing in concentration by 10 per cent, and designated only by letters "A," "B" and "C," were classified correctly by the test. The combining powers of alkaloids vary; for example, one molecule of cinchonidine decolorizes two atoms of bromine, one molecule of procaine de-

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colorizes four, in moderate concentration, but the proportions are less exact with increasing dilution. That concentration which combines quantitatively with an equal volume of $N/100$ bromine solution is conveniently termed the "Standard Solution" of that particular substance. The concentrations of such "Standard Solutions" vary from 1–447 in the case of cinchonine, to 1–5850 in the case of antipyrine.

As little as 0.1 cc. of $N/1000$ bromine may be used for testing substances in very low concentrations. Where the concentration of bromine in the mixture of reagents is less than that of $N/5000$, 0.5 cc. of about 24 per cent HCl is used, and an interval of at least ten minutes is allowed for the release of the bromine and its combination with the substance to be tested.

A preliminary test for solutions of moderate concentration is made as follows: To 1 cc. of $N/100$ bromine solution in a test-tube, add 0.3 cc. of 12 per cent HCl, then the solution to be tested is added slowly from a pipette graduated in $^1/_{100}$ cc. until the color of bromine is discharged. In order to facilitate the detection of the least tinge of color due to bromine, comparison is made with a control in which there is a slight excess of the substance under examination. The average of several tests is taken. For some substances this test is about as accurate as either of the modified methods.

First Modification.—After the reaction is complete, 0.25 cc. of chloroform is added and the tube is shaken. Any free bromine is taken up by the chloroform, which assumes a yellowish tint. In some cases the end-point in the first method is obscured by the development of color. In such cases the use of chloroform is necessary, but even this fails in those cases where the color other than that of bromine appears in the chloroform.

Cloudiness develops slowly in the chloroform in the presence of a trace of bromine, too slight to afford a tint, but since cloudiness is also induced under conditions that cannot be controlled, it was utilized in only a few tests. It may afford an extremely delicate test under conditions that avoid the disturbing factors.

Second Modification.—In some cases with moderate concentration, and in all cases with very low concentration, neither the general method nor the use of chloroform affords satisfactory results; in such cases a fraction of a milligram of apomorphine is added, and the test-tubes are shaken. A trace of free bromine (about 1–600,000) is indicated by a pink color appearing within thirty seconds. The mean of the results of several determinations is accepted as the end-point. Immediately after the addition of the apomorphine the observation is made obliquely downward through the solution. As previously stated, rather rigid control solutions are necessary, hence one must know roughly the concentration of the solution under examination.

The following factors concerned with the technique have received consideration:

1. *Time of Reaction:*

   (*a*) Substances which react slowly with bromine may require thirty minutes after the addition of HCl.

   (*b*) The chloroformic extraction of free bromine requires from a few seconds to one minute.

   (*c*) Apomorphine reacts rapidly with free bromine and since it displaces some substances slowly the mixture is observed within thirty seconds.

   (*d*) The rate of the reaction of codeine sulphate, heroine hydrochloride and morphine sulphate is accelerated by H_2SO_4, the acceleration increasing with the concentration of H_2SO_4.

2. *Concentrations:*

   (*a*) Bromine in very dilute solutions is extracted imperfectly by chloroform, and less than 1 in 600,000 is not detected even with apomorphine; hence the comparison of a very dilute solution of unknown concentration with a much more concentrated control would result in a large error.

   (*b*) The concentration of HCl required to liberate bromine rapidly varies with the concentration of the latter, but an excess of HCl should be used, the optimum being 0.3 cc. of 12 per cent HCl per cc. of $N/100$ or $N/1000$ Koppeschaar; very low concentrations of the solutions require as much as 0.5 cc. of 20 per cent HCl.

   (*c*) There is no exact limit to the dilution of a substance which may be estimated with a bromine solution, since the error increases with dilution. It is not usually feasible with chloroform to employ a solution of less than Standard/100 (1/100 the concentration of that which decolorizes an equal volume of $N/100$ bromine solution). When apomorphine is used without the addition of chloroform, the concentration in some cases may be Standard/400.

3. *Volatilization of Bromine:* (*a*) The loss of bromine through volatilization depends partly on the volume, more on the depth, of the solution. The loss is negligible with rapidly reacting substances but must be considered with those which react slowly. The loss of bromine is minimized by adding the HCl after having added the Solution to be tested. Heat accelerates the reaction but it may increase the loss of bromine. Brucine sulphate and codeine sulphate were estimated at 25° and 32° C. with an interval of thirty minutes for completing the reaction. In each case there was a difference of about 5 per cent due to the greater loss of bromine at the higher temperature. Slight differences of temperature are not important in estimating substances which react rapidly with bromine.

   (*b*) The loss of bromine is one of the chief objections to the use of the unofficial solution of bromine which Weiss and Hatcher employed. This may be minimized by using a micro-burette having a three-way stop-cock connected with an all-glass syringe. The tip of the syringe must be in contact with the intake of the burette in order to avoid the action of bromine on rubber tubing.

4. *Illumination:* Light and the background influence the precision of borderline observations. Daylight, and electric light with a blue lamp are satisfactory. White, unglazed paper affords the best background.

5. *Cloudiness in the Chloroform:* This appears eventually in the chloroform in solutions in which there is one part of free bromine in two millions; but it was of little value in our experiments because it occurred at times from unknown causes. It appears in chloroform underlying water when concentrated HCl is added, but not when chloroform is added to any concentration of HCl. Occasionally a tinge of color of unknown origin (having no relationship to the presence or absence of free bromine) appeared in the chloroform.

The simple description of the technique and statements of the approximate *average* error in the estimations of various substances would make the method appear more simple and accurate than it will be found in individual cases unless one has had practice, and will devote the necessary time to the estimation. The results presented here are based on a very large number of experiments involving many thousands of test-tube examinations.

Amidopyrine.—The simple decolorization method proved more satisfactory than the chloroform modification in the estimation of concentrations up to 1–30,000. For more dilute solutions this method is not practical. When an excess of amidopyrine is added, a purple color may appear, irregularly, it fades after a few minutes.

The chloroform modification is less satisfactory because of the pink color which appears in the chloroform and interferes with borderline observations; it also interferes with the apomorphine test when apomorphine is added after chloroform, but not if the chloroform is omitted.

It is probable that concentrated solutions (up to about 1–5000) may be estimated with errors of 3 per cent by the decolorization method; the error being slightly greater with chloroform. Apomorphine without chloroform affords a more accurate and more delicate test, and it was used satisfactorily with concentrations as low as 1–700,000.

Antipyrine.—The reaction requires about two minutes. The error with chloroform may be as high as 2 or 3 per cent for concentrated solutions because of the development of a slight color. Less of antipyrine than of any other substance used is required to decolorize 1 cc. of bromine solution, and satisfactory estimations of it in concentration of 1–1,200,000 were made with apomorphine.

Apomorphine Hydrochloride.—The reaction with bromine begins at once but it requires some time for its completion when only traces are present. A pink or intense red color develops, dependent on the concentration; the chloroform takes up part of this color which interferes with the precision of the observations. One part of bromine in 600,000 parts of water may be detected with an excess of apomorphine. One part of apomorphine in 1,200,000 parts of water affords a pink tint with an excess of bromine. This affords a means of determining roughly the amount of apomorphine present in solution.

Brucine Sulphate.—The reaction requires about thirty minutes, and a slight color develops in the solution, hence the simple decolorization method is unsatisfactory. The chloroform modification affords a satisfactory result. Apomorphine is useful with concentrations of 1–40,000, or less, but not with much more concentrated solutions of brucine.

When brucine sulphate is added after the addition of HCl to bromine solution, a pink color develops immediately and changes rapidly to yellow, then to orange, and finally to pink with an excess of brucine sulphate. Hence it is possible to estimate the amount of brucine sulphate by this method. After the addition of chloroform the supernatant liquid in all tubes remains, or becomes, deep pink.

Caffeine.—The reaction requires about twenty minutes and the solution develops a slight color, hence the simple decolorization method is unsatisfactory, but the chloroform and apomorphine modifications afford satisfactory results. After the addition of chloroform a slight color appears in the chloroform. This must be disregarded, but it interferes only slightly with the observation of borderline cases. Dilutions up to 1–165,000 were estimated by both modifications with moderate errors.

Codeine Sulphate.—The reaction requires about thirty minutes. The chloroform and apomorphine modifications may be used for the estimation of concentrations of about 1–100,000. Apomorphine was not used with more dilute solutions. The decolorization method is unsatisfactory because of the development of a yellow color. In general, codeine behaves about like morphine.

Cinchonidine and Cinchonine.—The reaction is almost instantaneous. Solutions of 1–1000 may be estimated with an error of less than 1 per cent. In concentration of 1–100,000 the error is about 5 per cent. There was no appreciable difference in the results when these alkaloids were dissolved in 0.1 per cent, or 1.6 per cent sulphuric acid.

In a few experiments with cinchonine and some other substances a green color was observed in some of the test-tubes after the addition of apomorphine; this was not investigated further.

Dionine.—The reaction occurs slowly and there develops in chloroform a color which interferes with borderline observations. Only preliminary tests were made.

Emetine.—The reaction occurs slowly, and the solution becomes yellow. Chloroform-soluble emetine may be estimated by substituting ether for chloroform, but the reflection of the yellow color on the ether makes borderline observations uncertain.

Morphine Sulphate.—The reaction is practically complete in thirty minutes. The resulting solution develops a color resembling that of bromine, hence the simple decolorization method cannot be used, but this color does not pass into the chloroform; and the chloroform modification affords a satisfactory test. The error is less than 1 per cent for solutions up to 1–3000, and about 5 per cent for concentrations of about 1–200,000. The apomorphine test is applicable to very dilute solutions, and concentrations of about 1–420,000 were estimated with errors of about 5 per cent.

Procaine and Tutocaine.—The reactions are very rapid. Solutions of 1–2000 may be estimated with an error of less than 1 per cent; in concentrations of 1–300,000 the error is about 5 per cent.

Quinine and Quinidine.—These may be estimated by adding to the point of decolorization probably better than by the chloroform and apomorphine modifications, but one of us (R. L. H.) could not estimate them with errors of less than 10 per cent, owing to the development of a yellowish color, which, however, was invisible to the other (R. A. H.).

Strychnine Sulphate.—The reaction requires about ten minutes. Satisfactory estimations were made with dilutions up to 1–100,000. A few tests of solutions of about 1–150,000 gave errors of about 10 per cent.

Theobromine.—The chloroform and apomorphine modifications afford satisfactory results. The reaction is complete in about thirty minutes. The method of simple decolorization affords at least a rough test. Dilutions up to 1–350,000 were estimated with errors of about 5 per cent.

Picrotoxin.—An aqueous solution of picrotoxin, 1–1000, may be estimated with an error of about 1 per cent, and solutions of much lower concentration may be estimated with a slightly greater error. This is the only simple quantitative chemical test for picrotoxin with which we are acquainted.

Salicin.—The reaction requires about thirty minutes; the chloroform method is satisfactory. Apomorphine was not tried.

Salicylic Acid.—The complete reaction requires about 30 minutes, but it is nearly complete within 3 minutes. The chloroform modification permits of an accurate test within 5 or 10 minutes after the addition of HCl; the general method (decolorization) is less satisfactory, but it may be used with a control. Salicylic acid decolorizes $N/10$ bromine solution almost as actively as antipyrine, and with the apomorphine modification it was estimated in a concentration of about 1–900,000. Salicylic acid in concentrated solution forms with bromine a precipitate, which is soluble in chloroform; it does not interfere with the test.

Protocol showing the volume of solution of procaine HCl $N/200$ (1.364 mg. per cc.) which absorbs the bromine in 1 cc. of $N/100$ bromine solution.

Preliminary Test.—In each of two tests the gradual addition of 0.53 cc. $M/200$ Procaine HCl-solution to 1 cc. of $N/100$ bromine and 0.3 cc. 12 per cent HCl caused complete decoloration.

Final Test.—To each of 16 test-tubes there was added 1 cc. of bromine solution $N/100$ and 0.3 cc. of 12 per cent HCl. Successively increasing amounts of Procaine solution $M/200$ were added. Observations were made immediately; after 1 minute; after 10 minutes; at the end of which time chloroform was added; and 5 minutes later a trace of amomorphine was added. The intensity of color in the aqueous layer; in the chloroform; and that induced by apomorphine affords a clew to the amount of unabsorbed bromine. The degree of color is expressed as "xxx" (deep color); "xx;" "x" (barely perceptible color); "—" (no color perceptible).

Procaine	Immed.	1 Min.	10 Min.	Chlor. 10 Min.	Apomorph. 15 Min.
0.3 cc.	xxx	xxx	xxx	xxx	xxx
0.35	xxx	xxx	xxx	xxx	xxx
0.4	xxx	xxx	xxx	xxx	xxx
0.42	xxx	xxx	xxx	xxx	xxx
0.44	xxx	xxx	xxx	xxx	xxx
0.46	—	xx	xx	xx	xxx
0.47				x	xx
0.48	—	x	x	x	xx
0.48				x	xx
0.49				x	xx
0.5	—	—	—	?	x
0.5				?	x
0.51				—	—?
0.52				—	—
0.52	—	—	—	—	—
0.54	—	—	—	—	—

The results indicate that 1 cc. of $N/100$ bromine solution is decolorized by 0.5 cc. Procaine HCl $M/200$, as shown by the chloroform test, and by 0.505 cc. shown by the apomorphine test. Hence 0.682 mg. Procaine HCl absorbs 0.7992 mg. bromine.

SUMMARY.

1. Tenth normal bromine solution (Koppeschaar's solution) was used for the quantitative estimation of a number of alkaloids. Two general modifications of the method are described.

2. By this means quantitative estimations of a number of alkaloids may be made with an error of about 0.5 per cent in concentrations of about 1–1000.

3. The error in the quantitative estimation of substances in solutions of unknown concentration increases with the dilution, but antipyrine, amidopyrine and salicylic acid in concentrations of 1–1,000,000 may be determined with errors of from 5 to 10 per cent.

4. The estimations are made with controls in which attention must be paid to concentration, temperature, rate of reaction and other factors discussed in the paper.

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THE AIR-LIFT EXTRACTOR APPLIED TO THE ANALYSIS OF ALKALOIDAL DRUG EXTRACTS.

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The extraction of alkaloids from pharmaceutical preparations has always been time-consuming and many attempts have been made to accomplish the extraction by means of automatic devices. Most of these have been regarded as unsuitable because they depend upon refluxing of the solvent by heat (1) with a resultant possible decomposition of the alkaloid and because of the difficulty of determining when the extraction is complete (2). The air-lift extractor (3) ac-