(2) V. Reader, *Biochem. J.*, 23 (1929), 689.
(3) V. Reader, *Ibid.*, 24 (1930), 77.
(4) V. Reader, *Ibid.*, 24 (1930), 1827.
(5) Chick and Roscoe, *Ibid.*, 23 (1929), 498.
(6) Sherman and Spohn, *J. Am. Chem. Soc.*, 45 (1923), 2719.
(7) M. I. Smith, *U. S. Pub. Health Repts.*, 45 (1930), 116.
(8) Sebrell and Elvove, *Ibid.*, 46 (1931), 917.
(9) Chick, Copping and Roscoe, *Biochem. J.*, 24 (1930), 1748.
(10) Sherman and Sandels, *Proc. Soc. Exp. Biol. Med.*, 26 (1929), 536.
(11) Chick and Roscoe, *Biochem. J.*, 22 (1928), 790.
(12) Evans and Burr, *J. Biol. Chem.*, 77 (1928), 231.

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THE DETERMINATION OF HALOGENS IN PHARMACOPŒIAL ORGANIC COMPOUNDS.

BY U. O. OAKDALE* AND J. L. POWERS.

The United States Pharmacopœia, X, recognizes a large number of organic compounds containing halogens. For some of these a purity rubric based on halogen content is required; in the case of the others a similar requirement might be desirable.

Until comparatively recently the only accurate methods available for the determination of halogens were those of Carius[1] and the Lemp and Broderson[2] modification of the Parr[3] and Pringsheim[4] methods.

The Carius method is not desirable for general use because of several disadvantages which it presents. Among these are the use of a sealed tube, a bomb furnace in which to heat it, and the chance of fragments of glass falling into the tube upon opening it. This procedure requires an excessive amount of time, and it is not easy to determine when the material is completely oxidized.

When the Lemp and Broderson method is used there is always the possibility of incomplete fusion of the material, the danger of too rapid oxidation when the sample is mixed with sodium peroxide, and the difficulty in obtaining accurate results with volatile compounds.

With the exceptions of chloramine and dichloramine, the pharmacopœial assay methods for the determination of halogens are restricted to compounds containing iodine. In the determination of iodine in calcium iodobehenate, thymol iodide and thyroxin, the principle involved depends upon carbonizing the material which has previously been mixed with potassium carbonate, extracting the residue with water, and oxidizing the iodide thus formed to iodate by means of potassium permanganate. After filtering and collecting an aliquot portion of the filtrate, potassium iodide is added, the solution is acidified, and the iodine liberated is determined by titrating with sodium thiosulphate solution using starch as indicator.

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* Parke, Davis and Company Fellow, 1930–1931.

[1] Carius, *Ann.*, 136 (1865), 129.

[2] Lemp and Broderson, *J. Am. Chem. Soc.*, 39 (1917), 2069.

[3] Parr, *Ibid.*, 30 (1908), 764.

[4] Pringsheim, *Ibid.*, 41 (1904), 386.

This procedure may give low results due to loss of iodine from heating too rapidly and occlusion of potassium iodate by the manganese dioxide formed from the potassium permanganate. In the U. S. P. assay method for the determination of iodine in thyroid, a fusion mixture of potassium carbonate, sodium carbonate and potassium nitrate is used. The oxidation is carried out in acid solution using sodium hypochlorite solution. This method will be considered in the discussion of results.

Recently a very accurate and rapid general method for the determination of halogens in organic compounds was developed by Thompson and Oakdale[1] in this Laboratory. In this method the compound containing the halogen to be determined is oxidized with fuming sulphuric acid alone or in conjunction with potassium

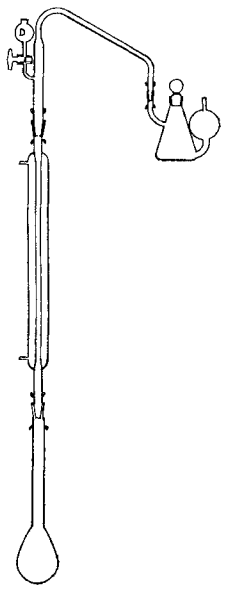

Fig. 1.

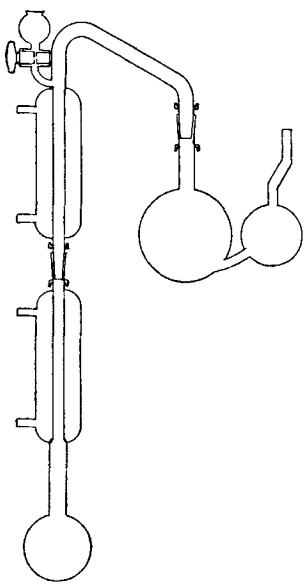

Fig. 2.

persulphate or chromic acid, using the apparatus shown in Fig. 1. Later, using the same principle but a much smaller apparatus (Fig. 2), thus making possible the use of very small samples for analysis, Willard and Thompson[2] developed a micro method for the determination of halogens.

The accuracy, rapidity and general applicability of the Thompson-Oakdale Method suggested its use in the determination of halogens in Pharmacopœial organic compounds, and a comparison with Pharmacopœial methods where possible. With the exception of thyroxin, the larger apparatus was used for halogen determinations described in this publication.

Determination of Iodine.—Depending upon the amount of halogen present, a 0.5–1 Gm. sample and 0.2 Gm. of copper sulphate are placed in the flask, A. A solution prepared from twenty Gm. of chlorine-free sodium hydroxide and 1 Gm. of arsenic trioxide dissolved in 75–100 cc. of water, is placed in the absorption flask, E. Twenty cc. of fuming sulphuric acid, containing 20–30% sulphur trioxide, are added through the dropping funnel, D. The mixture is boiled gently

[1] Thompson and Oakdale, *J. Am. Chem. Soc.*, 52 (1930), 1195.

[2] Willard and Thompson, *Ibid.*, 52 (1930), 1893.

for thirty minutes. Thirty-five cc. of 95% sulphuric acid are then added and the mixture boiled vigorously until it is clear. Fifty cc. distilled water are added cautiously to the boiling mixture. Water is removed from the condenser and 10 cc. of 30% hydrogen peroxide (superoxol) are added in portions, the boiling being continued to drive the liberated iodine through the condenser. Five cc. of a saturated aqueous solution of hydrazine sulphate are added to reduce any iodate which might have been formed, and the mixture is boiled for five minutes. Hydrogen peroxide is then added to expel any iodine. The contents of the absorption flask are now transferred to a 600-cc. beaker, and an excess of hydrogen peroxide is added and the mixture heated to boiling to oxidize any sulphite to sulphate. The solution is cooled and acidified with dilute nitric acid, phenolphthalein being used as indicator. The iodide is then precipitated with an excess of silver nitrate solution, and 5–10 cc. of concentrated nitric acid is added to dissolve any silver salts other than halides. The solution is boiled until the silver iodide is coagulated. The precipitate is collected on a weighed filtering crucible, washed with a 2% solution of nitric acid, then with distilled water and finally with acetone. The crucible is then dried at 130°.

Determination of Chlorine and Bromine.—The procedure is the same as for the determination of iodine except that after the sample has been heated with the fuming sulphuric acid for thirty minutes, 10 Gm. of finely powdered chlorine-free potassium persulphate, suspended in 20 cc. of 95% sulphuric acid, are added through the dropping funnel. The mixture is boiled vigorously until it is clear. An excess of a saturated solution of potassium permanganate is then added, the water is drained from the condenser, and the boiling is continued for five minutes to expel any chlorine or bromine which may remain in the flask or condenser. The above variations are necessary because iodinated compounds are more easily decomposed than those containing chlorine or bromine. In both determinations an analysis may be completed in from one to one and one-half hours. The results reported in the following table were obtained from analyses of U. S. P. X products procured from reliable sources and were not purified or altered in any way, except to be dried in a desiccator over calcium chloride or sulphuric acid to constant weight. The results, with the exception of thyroid, are of two or more determinations checking within 0.05% of each other by the Thompson and Oakdale method, and within 0.2% when the U. S. P. procedures were used.

TABLE OF RESULTS.

Compound.	U. S. P. requirements.	Calculated.	% Halogen found, U. S. P. Method.	% Halogen found, Thompson-Oakdale Method.
Calcium iodobehenate	23.5% I	26.56% I	24.67% I	25.14% I
Carbromal		33.71% Br	...	33.63% Br
Chloral hydrate	99.5% pure	64.31% Cl	...	63.97% Cl
Chloramine	11.5–13.5% Cl	12.58% Cl	12.70% Cl	12.92% Cl
Dichloramine	28–30% Cl	29.54% Cl	29.65% Cl	29.50% Cl
Iodoform		96.67% I	...	96.70% I
Thymol iodide	43% I	46.14% I	45.91% I	45.93% I
Thyroid	0.17–0.23% I		0.23% I	0.122% I
Thyroxin	63% I	65.36% I	63.00% I	64.17% I
Trichloracetic acid	99% pure	65.09% Cl	...	64.97% Cl

DISCUSSION OF RESULTS.

Calcium Iodobehenate.—The results show that the U. S. P. assay method for iodine determination gives lower results than the Thompson-Oakdale Method. The first method required several trials in which varying results were obtained before two determinations checking within 0.1% were possible. The latter method possesses the advantage of requiring only ordinary precautions in carrying out a determination, and is more accurate and more rapid.

The determination of halogens in carbromal, chloral hydrate, iodoform and trichloracetic acid illustrates the accuracy of the Thompson and Oakdale Method and suggests the possible desirability of a purity rubric based on halogen content. The purity of chloral hydrate was determined by the U. S. P. Method and found

to be 99.54%. This is in close agreement with 99.48% purity based on halogen content. The purity of the sample of trichloracetic acid was found by the U. S. P. assay method to be 99.79% which is also in close agreement with that calculated from the halogen determination and found to be 99.48%.

The Thompson-Oakdale Method applied to chloramine and dichloramine determines total chlorine present, while the U. S. P. Method determines active chlorine.

Iodine was determined in thyroxin,[1] using the micro method. Samples of 15–25 mgms. weighed on a Bunge air-damped balance, type 4 DM, with a capacity of 200 Gm., were used. Using chlorine-free chemicals, no blank determination was found to be necessary. This method is unquestionably superior to any fusion method as far as accuracy is concerned, especially in cases which demand the use of extremely small samples for analysis.

Wide variation between the results obtained for iodine content of thyroid[2] determined by the U. S. P. Method and the Thompson-Oakdale Method will be noted. The only probable error using the latter method is the possibility of volatile iodine compounds being formed in the oxidation of the thyroid which would not be absorbed by the alkaline sodium arsenite solution. This possible source of error was eliminated by placing a narrow bore quartz tube about 25 cm. long between the condenser and the absorption flask, connection being made by black rubber tubing. Heating the central portion of the tube to redness by means of two Meker burners decomposes any volatile organic iodide and permits complete absorption of the iodine.

It seemed that the discrepancies obtained by the U. S. P. assay methods might be due to, (*a*) the presence of iodides in the reagents used, or (*b*) to the production of compounds, during the oxidation, which might liberate iodine from potassium iodide. The results obtained from several blanks were constant and accounted for an error of 0.04%, thus reducing the percentage of iodine from 0.23% to 0.19%. This, obviously, does not account for the variation of results between the two methods. Iodine in several samples of thyroid was determined by fusing a sample with the mixture directed to be used in the U. S. P. Method, dissolving the fused mixture in water, acidifying with nitric acid, and precipitating the iodide as the silver salt. Fairly consistent results were obtained averaging 0.081% iodine. This would indicate that some iodine is actually lost by the fusion method. If, in the oxidation of iodides formed by the fusion of thyroid, periodates were formed instead of iodates, a greater amount of iodine would be liberated by the action of potassium iodide. To determine the possibility of this, samples of pure potassium iodate were dissolved in water and acidified with sulphuric acid. The iodate was reduced to iodide with sulphur dioxide, the solution was then made alkaline with sodium hydroxide and the excess sulphur dioxide was oxidized to sulphate with hydrogen peroxide, the excess of which in turn was destroyed by boiling. The amount of fusion mixture used in the assay of thyroid was then added to each sample and the iodide was oxidized exactly as directed by the U. S. P.

[1] We are indebted to Dr. John F. Anderson of E. R. Squibb and Company's Biological Laboratories who furnished the thyroxin for this analysis.

[2] We wish to express our appreciation to Dr. Frederic Fenger of the Armour Laboratories who furnished the thyroid used in these analyses.

assay method. Upon acidifying, adding potassium iodide and titrating with tenth normal sodium thiosulphate solution, the calculated amount of iodine was found to be liberated. Because of the extreme accuracy of the Thompson and Oakdale Method, the difference in results is thought to be due to some error in the U. S. P. assay method as yet unexplained.

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A COMPARATIVE STUDY OF THREE ASSAY PROCEDURES FOR CAMPHORATED TINCTURE OF OPIUM, U. S. P.*

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INTRODUCTION.

Camphorated Tincture of Opium, commonly called "*Paregoric*" (meaning "*soothing*") and originally known as "*Elixir Paregoricum*," is found in the U. S. P. X, but no assay procedure is provided in the Tenth Revision or in any previous revision. The small amount of Opium in this tincture and the presence of the other ingredients make an assay procedure for this preparation more or less involved.

Eaton (1) proposed an assay method for Camphorated Tincture of Opium which, in the hands of some workers, has given satisfactory results. This method was included in this study as "Method III."

Kippenberger (2), Warthle (3), and Puckner (4) each suggested methods of assay for this preparation which appeared to yield more or less satisfactory results as carried out by these investigators. Buchbinder (5) proposed a method in 1917 which was based on the work of the four investigators whose names have been mentioned above. This method was included also in this collaborative study as "Method I." St. John (6) elaborated a method which is adapted for small samples of this tincture. This method was studied too as "Method II."

Caines (7) suggested a colorimetric method for the determination of small amounts of morphine. Warren and McClosky (8) in commenting on this method state that, as applied to Camphorated Tincture of Opium, the morphine is obtained in comparative purity by suitable treatment, and the color produced with sulphuric acid and a saturated solution of potassium iodate is compared with a known standard under similar treatment.

The American Drug Manufacturers' Association, Subcommittee on Alkaloids and Drug Standards (9), studied the Buchbinder Method and an unpublished method devised by one of its members. In 1929 this sub-committee felt that the differences in the results obtained by four workers warranted further study. In 1930 this sub-committee reported that no further work is required on this product at this time. In view of the fact that the maximum and the minimum findings of this group were 0.055 and 0.027, one fails to understand the recommendation made by this subcommittee.

The three methods which follow were applied to portions of a very carefully prepared Camphorated Tincture of Opium, U. S. P. X.

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* Scientific Section, A. PH. A., Miami meeting, 1931.