

TABLE I.—CHROMATOGRAPHIC DATA

Substance	R _f	Fluorescence
Acetyl digitoxin	0.82	Red
Digitoxin	0.72	Red
Digoxin	0.62	Blue
Lanatoside A	0.52	Red
Lanatoside B	0.41	Red
Lanatoside C	0.36	Blue
Desacetyl lanatoside C	0.27	Blue
Ouabain	0.09	Yellow-green

the plate, although for simple detection 1 mcg. is sufficient.

Visualization.—The plates are sprayed, then placed in a 100° oven for a few minutes. The perchloric acid produces a charring effect and a fluorescence which is visible under long-wave ultraviolet light (366 mμ.).

DISCUSSION

The sensitivity of detection by fluorescence is augmented by the visualization of the spots in ordinary

light. Good separation of the common cardiac glycosides can be obtained (Table I). The presence of certain contaminants is easily detected. The system has the advantages of simplicity, speed (approximately 0.5 hr.), efficiency of separation, and avoids the use of hazardous spray reagents, such as antimony trichloride.

Good results can also be obtained using a spray consisting of 0.5 ml. of *p*-anisaldehyde in 50 ml. of glacial acetic acid and 1 ml. of concentrated sulfuric acid (5). After heating the plate for a few minutes at 100°, the glycosides appear as blue spots with the exception of ouabain which appears as a yellow spot.

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Determination of Organically-Bound Iodine in Pharmaceuticals

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Eight official iodinated compounds with iodine contents from 50 to 66 per cent were assayed by the methods official in U.S.P. XVI and N.F. XI, by reduction with zinc in alkali, and by a standard procedure using oxygen flask combustion. An additional four compounds, formerly official, with iodine content as low as 23 per cent were also examined. The results indicate that the suggested standard procedure is equal or superior to the other methods in recovery of iodine and generally equivalent in reproducibility. The alkaline zinc reduction method is suggested for those compounds having an electronegative substituent *ortho* or *para* to the iodine atoms on the aromatic ring. The oxygen flask combustion is suggested for all other iodinated compounds and as a general method. The suggested standard procedure consists of combustion by the oxygen flask method, absorption of the combustion products in an alkaline sulfite solution, and titration of the acidified solution of iodide with standard silver nitrate using a silver-calomel electrode pair for potentiometric determination of the end point.

THE QUANTITATIVE determination of iodine in organic compounds has long proved a source of difficulty for the analytical chemist. This is reflected in the numerous proposals for the determination of iodine in organic substances. An assay procedure for the determination of iodine in an organic compound actually involves two problems. First, the organic compound must be decomposed to liberate the iodine, and then the iodine must be quantitatively determined. By far the more diffi-

cult of these problems is finding an efficient method for the decomposition of the organic compound which will not result in the loss of any of the iodine present.

EXPERIMENTAL

Reagents.—All reagents employed were reagent grade chemicals. All standard solutions employed were prepared and standardized according to the official compendia.

Methods.—*A. Parr Bomb Method (1-3).*—The organic matter is oxidized by fusion with sodium peroxide in a bomb and the halide present is converted to sodium halide.

B. Alkaline Permanganate Method (4, 5).—This assay is based on the conversion of organically-bound iodine to iodide by the action of permanganate in alkali and reduction with bisulfite.

C. Zinc-Sodium Hydroxide Method (6).—In this method the organic iodine is replaced with hydrogen generated in the nascent form by the reaction of zinc and sodium hydroxide.

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TABLE I.—IODINE CONTENT DETERMINED BY VARIOUS METHODS OF ANALYSIS

Compd. ^a	Theoretical % I	Official Methods ^b				Proposed Method ^c			
		A		B		C		D	
		Results % I	No. of Detn.	Results % I	No. of Detn.	Results % I	No. of Detn.	Results % I	No. of Detn.
Chinifon	26.5–29 ^d	23.6 ± .4	3	23.9 ± .3	3	23.8 ± .1	3
Diiodohydroxyquin U.S.P.	63.9	59.2 ± .8	6	63.6 ± .3	5
Iodipamide U.S.P.	66.8	65.4 ± .1	3	66.3 ± .5	4
Iodoalphonic acid N.F.	51.4	50.7 ± .5	3	49.8 ± .6	3	51.7 ± .2	4
Iodochlorhydroxyquin U.S.P.	41.5 ^f	40.6 ± .8	3	40.7 ± .2	4
Iodophthalein	61.8	60.3 ± .2	3	57.0 ± 1.0	4	60.6 ± .6	4
Iodopyracet N.F.	49.8	47.1 ± .1	3	46.9 ± .1	4	48.4 ± .4	4
Iopanoic acid U.S.P.	66.7	66.5 ± .5	3	64.5 ± .7	3	66.6 ± .6	4
Sodium methiodal N.F.	52.0	49.5 ± .1 ^g	3	50.4 ± .6 ^g	6	50.6 ± .3 ^g	5
Diatrizoic acid ^h	62.0	57.3 ± .4	6	58.4 ± .3	3
Sodium acetrizoate ⁱ	65.8	60.3 ± .7	3	60.7 ± .3	3	61.6 ± .3	4
Sodium diatrizoate ^j	59.9	53.7 ± .5	5	54.2 ± .5	4
Potassium iodide	76.5	76.5 ± .2	7

^a All assays done on samples as received. ^b The standard deviation was calculated by the Dean and Dixon method (10). ^c Sample weight chosen to yield about 10 mg. of iodine. ^d N.F. XI specifications are given since chinifon is a mixture. ^e Method will not produce quantitative dehalogenation. ^f U.S.P. specifications are 40–42% I. ^g Loss on drying was 3.25%. ^h Used to prepare meglumine diatrizoate injection U.S.P. ⁱ Used to prepare sodium acetrizoate injection U.S.P. XVI. ^j Used to prepare sodium diatrizoate injection U.S.P.

D. Oxygen Flask Combustion Method (7–9).—This is an extremely rapid, simple, and inexpensive technique for combustion analysis of organic materials in oxygen. The procedure converts organic materials into soluble combustion products which are then analyzed.

Methods *A*, *B*, and *C* were official in U.S.P. XVI and N.F. XI, and the complete procedures may be found in the compendia in the monographs for the various compounds used in this study.

Preliminary testing indicated that, of the official methods, method *C* offered the best possibility of a general assay method. All compounds used in this study were assayed by the official method, by method *C*, and by the proposed general method, method *D*, which is given in detail under *Proposed General Method*. Table I shows a comparison of the four methods.

Proposed General Method

Apparatus.—Five-hundred-milliliter oxygen combustion flask (Arthur H. Thomas Co., Philadelphia, Pa.), 10-ml. buret, pH meter, silver and calomel electrodes, magnetic stirrer.

Reagents.—Sodium hydroxide solution (1 in 100), sodium bisulfite solution (1 in 100), approximately 1 *N* nitric acid solution, oxygen, 0.01 *M* AgNO₃ solution prepared by weighing as primary standard, low-ash filter paper (Whatman 42).

Combustion Method.—Weigh the sample accurately on a piece of the filter paper and fold the paper to enclose the sample. Place the sample, together with a filter paper fuse-strip, in the platinum wire loop fused into the flask stopper. Place 5 ml. of the sodium hydroxide solution and 2 ml. of the sodium bisulfite solution in the flask. Flush the air from the flask with a stream of rapidly flowing oxygen, moisten the stopper joint with water, and ignite the fuse strip. Immediately plunge the sample holder into the flask, and hold the stopper firmly in place. After combustion is complete, shake the flask vigorously, inverting and rotating the flask to absorb all of the iodine vapor, and allow the flask to stand for 3 min.

Completion Method.—Place a few drops of water around the stopper joint and twist the stopper to loosen it. Rinse the stopper and sample holder with water and transfer the flask contents to a 250-ml.

beaker, washing the flask several times with a total of about 70 ml. of water. Add 2 ml. of the nitric acid solution, and if the solution turns a pale yellow, add 1 or 2 drops of the bisulfite solution to decolorize. Titrate potentiometrically with 0.01 *M* silver nitrate using a silver indicator electrode and a calomel reference, adding titrant in 1-ml. increments until the potential is about –100 mv. Now add titrant dropwise, taking a reading after each addition until the meter shows a change of 150–200 mv. This maximum potential change occurs at about 0 mv. and is evidenced by a slow drift in the meter pointer. The volume of titrant is recorded. The meter pointer will stabilize in a few minutes and the addition of 1 drop of excess titrant will result in a potential change of about 40 mv. The end point is further shown by the coagulation of the silver iodide particles. The silver electrode may be cleaned by immersing it in a 50% nitric acid solution until the electrode gases freely.

DISCUSSION

Some of the assay methods for iodinated compounds in the U.S.P. XVI and N.F. XI can produce low results and be quite time-consuming. Because the zinc-sodium hydroxide method requires no elaborate equipment and utilizes a simple procedure, it was hoped at the onset of this work that this method might be applicable to all of the compounds studied. In proving this, all of the compounds used in this study were analyzed by this procedure. The data reveal that the method is acceptable for those compounds which have a carboxyl or sulfonic acid group attached directly to the ring structure with the iodine atoms being *ortho* or *para* to the acid groups. In those compounds in which the acid function is separated from the ring structure by one or more carbons, the results with this method are a few per cent lower than those obtained with other methods. In compounds without an acid group present, the zinc-sodium hydroxide method will not work.

These observations can be explained on the basis of electron densities in the ring systems. The carboxyl group, a *meta* director, decreases the electron densities in the *ortho* and *para* positions, thus decreasing the strength of the carbon-iodine bonds in these positions and allowing dehalogenation to take place readily. When the carboxyl function is sep-

arated from the ring, a decrease in electron density is effected to a lesser extent, possibly explaining the lower results obtained with compounds of this type. In the hydroxyquinoline compounds, the hydroxyl group, an *ortho-para* director, increases the electron density at the *ortho* and *para* positions, strengthening the C-I bond, thus resulting in the failure of the zinc-sodium hydroxide method.

Tetrabromophenolphthalein ethyl ester (0.1% in glacial acetic acid) is the adsorption indicator used in the official compendia for the titration procedure following decomposition by zinc-sodium hydroxide. In the course of this work, it was noticed that the indicator was stable only for a period of 3-4 weeks. This is believed due to hydrolysis of the ester linkage, since refrigeration of the indicator prolonged the stability by several weeks. The freshly prepared indicator produces a sharp color change at the end point, but the sharpness of this change decreases as the indicator solution ages. It is important, therefore, to follow the U.S.P. XVII requirement that the indicator be freshly prepared.

The oxygen flask procedure produced values closer to the theoretical values than were obtained with any of the other methods used except the longer, more involved Parr bomb procedure. No difficulty was encountered from loss due to the transfer of the absorbing medium to another container prior to the titration procedure. Many of the compounds used in the study contained over 60% I and not a single case of incomplete combustion was encountered, probably because of the small sample size.

The potentiometric titration technique was employed because of the difficulties mentioned with tetrabromophenolphthalein ethyl ester and because of the difficulty in visual detection of the equivalence point at low iodide concentration. The validity of the titration method was shown by the recovery of 100.0% of potassium iodide from seven different samples having iodide concentrations equivalent to that obtained from the combustion procedure.

The suggested technique differs from that official in U.S.P. XVII and N.F. XII in that the iodine is converted into iodide and titrated with silver nitrate as is required for iodochlorhydroxyquin U.S.P. The official method converts iodine to iodide, then to iodate by oxidation with bromine in an acetate-buffered medium, removal of excess bromine by formic acid, removal of oxygen by displacement with nitrogen, conversion of iodate to iodine by treatment with iodide solution, and titration of the liberated iodine with standard sodium thiosulfate.

The official procedure has the advantage of producing six equivalents of iodine for each atom of iodine originally present, and is not subject to interference by chloride. The sample size allows titration volumes of about 30 ml. of 0.02 *N* thiosulfate.

The suggested procedure eliminates the need for further chemical treatment after combustion, but produces only one equivalent of iodide for each atom of iodine originally present. The sample size re-

quires about 9 ml. of 0.01 *N* silver nitrate. The potentiometric end point prevents interference by chloride.

The choice between the two methods is certainly open to debate. The official method requires more handling, more reagents, a cylinder of nitrogen, use of a hazardous bromine solution, storage of standard sodium thiosulfate whose normality must be frequently checked and which is a secondary standard, and use of starch T.S. indicator which must be freshly prepared. In contrast, the suggested method uses the solution from the combustion and a stable solution of primary standard for titration, but does require the less common 10-ml. buret and silver electrode as well as a pH meter capable of use as a millivoltmeter. The official method might be best for a laboratory which routinely uses 0.02 *N* thiosulfate for iodine determinations, whereas the suggested method might be better for laboratories with a low volume of iodine titrations but with adequate instrumentation.

In several instances the data obtained by all methods is at least 4% lower than the theoretical values for the compound. In all cases where this is true, the compound is not official as the powdered material supplied to the authors for this investigation, but as the injection. The data obtained do not reflect on the validity of the assay methods; in many cases it is the manufacturers' policy to assay the powdered material for iodine content and then prepare the injection to meet the standards of the official compendia.

CONCLUSIONS

The proposed method utilizing the oxygen flask combustion is in all cases superior to or equal to other methods in recovery of iodine and generally equivalent in reproducibility. With the procedure suggested, an analyst with a minimum of practice can complete an analysis in 20 min., far faster than any of the presently official methods. The method is so rapid and simple that a technician could obtain excellent results in routine laboratory work.

Despite the general applicability of the suggested method, it is recommended that the alkaline zinc method be retained for appropriate compounds because of its ease, absence of hazard, use of common equipment, and an indicator end point.

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