TABLE I.—ABSORBANCE VALUES AT 300 mμ FOR SALICYLIC ACID IN THE ABSENCE OF ASPIRIN (FOR CALIBRATION) AND IN THE PRESENCE OF ASPIRIN OR ASPIRIN CONTAINING MIXTURES

Concn. of Salicylic Acid, mg./1000 ml.	Absorbance in Presence of—				
	No Added Aspirin	Aspirin		APC[a]	
		3.5 gr.	5 gr.	Capsule Contains 3.5 gr. Aspirin	Tablet Contains 3.5 gr. Aspirin
0	0	0	0	0	0
5	.125	...	...	...	...
10	.255	.257	.260	.255	.255
20	.505	...	...	...	...
40	1.05	1.02	1.01	1.05	1.05
80	2.06	2.06	2.06	2.05	2.04
100	2.55	2.57	2.57	2.55	2.59
200	5.05	5.05	5.06	5.04	5.04

[a] Marketed as A. S. A. Compound by Eli Lilly and Co., Indianapolis, Ind.

obtained from the standard curve for salicylic acid. Ten such values were averaged giving an average discrepancy of 0.4%, with no individual discrepancy greater than 1.2%. These values include the errors of weighing salicylic acid and all volumetric and spectrophotometric errors.

DISCUSSION

This method is presented as a procedure applicable exclusively to pharmaceutical forms, offering a convenient, economical, and accurate method for following the hydrolysis of aspirin under research or market conditions. It is expected that the capsule itself may contain products of hydrolysis of acetylsalicylic acid and should not be excluded from the analysis if, for instance, the stability of aspirin in a packaged formulation is under study. The same can be said of any special tablet coating which might be employed.

Since this method is presented solely for the study of the hydrolysis product, salicylic acid, in acetylsalicylic acid medicinal forms, the unit, tablet or capsule, is assumed to have substantially constant weight, and results are expressed as weight of salicylic acid per unit (capsule or tablet).

It is obvious that the test procedure presented here can be so organized that a series of samples (capsules or tablets) can be set up for simultaneous assay limited only by the time required for each successive operation. It has been feasible in our experience to carry out such a test with a series of 24 units. Centrifuging for a series of 24 tubes was done in two runs on a 12-place head. The entire operation for such a series requires approximately 2 hr. when run by one operator.

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Quality of Reagents in Micro Iodine Methods

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Iodine, present as an impurity, has been found in widely varying amounts in different batches of certain reagent chemicals used in iodine assay methods. Reagents containing iodine impurities cannot be used in sensitive analytical procedures for the determination of trace levels of iodine in biological materials. Methods are described for the estimation of the iodine (or iodine-like) content of reagent grade chemicals. Maximum permissible levels of iodine impurities in chemicals used to prepare reagents for iodine assay methods are specified.

A MODIFICATION (1) of the serum protein-bound iodine method of Bodansky *et al.* (2) has been described for assay of iodine in desiccated thyroid and other biological materials. The procedure consisted of a chloric acid oxidation of organic material prior to measurement of the iodine content in the digested samples by means of the Sandell-Kolthoff reaction (3) in which the reduction of Ce (IV) in the presence of arsenious acid was catalyzed by traces of iodine. This wet-ash digestion technique permitted the rapid and convenient processing of most biological materials.

Chloric acid was prepared by adding perchloric acid to a boiling, saturated, aqueous solution of potassium chlorate (2). Many batches of reagent grade potassium chlorate have been found to contain iodine or other impurities functioning like iodine in the Sandell-Kolthoff reaction. Chloric acid prepared from potassium chlorate which contained excess iodine was unsatisfactory for wet-ashing samples in sensitive iodine assay methods in

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TABLE I.—IODINE CONTENT OF POTASSIUM CHLORATE SAMPLES

Brand and Lot No.	mcg. of Iodine/Gm. of Sample[a]	Observed Quality of Chloric Acid Reagent[b]
Brand *A*, lot 1	0.004 ± 0.0005 (*4*)	Poor
2	0.006 ... (*2*)	Not tested
3	0.036 ± 0.006 (*3*)	Not usable
4	0.061 ± 0.004 (*8*)	Not usable
5	0.886 ± 0.031 (*8*)	Not usable
6	3.403 ± 0.026 (*3*)	Not usable
7	0.040 ... (*2*)	Not usable
8	Less than 0.001 ... (*3*)	Excellent
9	0.026 ... (*2*)	Not usable
10	0.088 ... (*2*)	Not usable
Brand *B*, lot 1	0.001 ± 0.0003 (*4*)	Good
Brand *C*, lot 1	Less than 0.001 ... (*4*)	Excellent
2	Less than 0.001 ... (*3*)	Not tested
3	Less than 0.001 ... (*4*)	Excellent
4	0.001 ± 0.0002 (*6*)	Excellent
Brand *D*, lot 1	0.005 ± 0.0006 (*4*)	Poor
2	Less than 0.001 ... (*4*)	Excellent
3	Less than 0.001 ... (*5*)	Excellent
4	0.007 ± 0.0004 (*6*)	Not usable
5	0.001 ... (*3*)	Good
6	Less than 0.001 ... (*3*)	Excellent
7	0.008 ... (*2*)	Not usable
Brand *E*, lot 1	Less than 0.001 ... (*3*)	Excellent

[a] Mean and standard error of the mean. Number of separate assays of each individual lot number is indicated in parentheses. [b] Chloric acid was prepared from the indicated potassium chlorate as described by Bodanksy *et al.* (*2*) for use in the iodine assay procedure.

which traces of iodine (0.01 to 0.06 mcg.) were measured. When iodine, as a contaminant from chloric acid, was present in excessive amounts in the final reaction mixture, reduction of Ce (IV) occurred too rapidly and satisfactory absorbance measurements of samples, standards, and reagent blanks could not be made.

Acetic acid–acetate buffers have been used in an ion-exchange column procedure for separation of iodinated amino acids (4). After chromatographic separation, the iodinated amino acids were quantitated by measuring the iodine content in aliquots of the column effluents (5). Buffers prepared from certain lots of reagent grade glacial acetic acid and sodium acetate contained amounts of iodine which interfered with iodine analysis of the iodinated amino acids.

The iodine (or iodine-like) content in several lots of reagent grade potassium chlorate, glacial acetic acid, and sodium acetate was determined. An estimate of the very low allowable iodine levels in potassium chlorate permitting preparation of good quality chloric acid can be made from these results.

PROCEDURE

Iodine Assay.—Reagent preparation and details of the iodine assay method have been described previously (1, 2, 5).

Preparation of Samples for Iodine Analysis.—Solutions of glacial acetic acid and sodium acetate were prepared in glass-distilled water so that 1.0-ml. samples contained 0.01 to 0.06 mcg. of iodine, the limits of the standard iodine calibration curve. These samples were analyzed in duplicate for iodine.

The iodine content of potassium chlorate (which has limited solubility) was estimated by suspending 5 to 20-Gm. samples in 10.0 ml. of glass-distilled water. After heating to a gentle boil with stirring to extract the iodine from the potassium chlorate crystals, the suspensions were cooled in an ice bath and then centrifuged. Duplicate 1.0-ml. samples of the clear supernatant solutions were analyzed for iodine.

The results were calculated as micrograms of iodine per gram of sample. The iodine content of some samples was below the detectable limits of the method. Such samples are reported as containing less than 0.001 mcg. of iodine per gram.

RESULTS AND DISCUSSION

During the past 4 years, potassium chlorate from five different well known manufacturers of reagent chemicals has been used to prepare chloric acid for the wet-ashing step in the iodine assay procedure. Twenty-three different batches of potassium chlorate have been analyzed for iodine, and in most cases the analyses were done on samples removed from freshly opened bottles. The results are given in Table I. Since impurities interfering with the iodine analysis have never been found in perchloric acid[1] used in the preparation of the chloric acid, the quality of the chloric acid depends on the potassium chlorate used. The quality of chloric acid prepared from each lot of potassium chlorate in Table I was rated not usable, poor, good, or excellent based on the reagent blank absorbance in the iodine assay procedure.

Eleven different lots of potassium chlorate produced chloric acid which was poor or not usable, while chloric acid made from ten other lots of potassium chlorate was of good or excellent quality. The iodine content of two additional lots of potassium chlorate, not available in sufficient quantity for chloric acid preparation, is reported in Table I. Brand *A*, lot 2, would be expected to produce unsuitable chloric acid, while brand *C*, lot 2, should make good chloric acid.

The range of the iodine content in ten different lots of brand *A* potassium chlorate is remarkable.

[1] G. Frederick Smith Chemical Co., Columbus, Ohio.

TABLE II.—IODINE CONTENT OF GLACIAL ACETIC ACID SAMPLES

Brand and Lot No.	mcg. of Iodine/Gm. of Sample[a]
Brand *B*, lot 1	Less than 0.001 (*4*)
Brand *C*, lot 1	0.153 ± 0.005 (*7*)
2	Less than 0.001 (*9*)
3	Less than 0.001 (*2*)
Brand *E*, lot 1	Less than 0.001 (*4*)

[a] Mean and standard error of the mean. Number of separate assays of each individual lot number is indicated in parentheses.

TABLE III.—IODINE CONTENT OF SODIUM ACETATE SAMPLES

Brand and Lot No.	mcg. of Iodine/Gm. of Sample[a]
Brand *A*, lot 1	1.051 ± 0.080 (*6*)
2	0.522 ± 0.023 (*6*)
Brand *B*, lot 1	0.005 ± 0.003 (*4*)
Brand *C*, lot 1	0.344 ± 0.024 (*4*)
2	0.049 ± 0.009 (*4*)
3	0.046 ± 0.007 (*3*)

[a] Mean and standard error of the mean. Number of separate assays of each individual lot number is indicated in parentheses.

Usable chloric acid was obtained from only one of ten lots of brand *A* tested (lot 8). All lots of potassium chlorate tested were analytical reagent grade except brand *B*, lot 1 (Table I), which was N.F. grade and, unexpectedly, had a low iodine content and produced good quality chloric acid. A good correlation exists between the quality of the chloric acid and the iodine content of the potassium chlorate from which it was prepared (Table I).

It appears from Table I that the most satisfactory chloric acid can be prepared only with potassium chlorate having 0.001 mcg. of iodine or less/Gm., although potassium chlorate containing as much as 0.002 mcg. of iodine per gram probably could be used to prepare fairly good quality chloric acid. However, chloric acid prepared from potassium chlorate containing 0.004 to 0.005 mcg. of iodine/Gm. (brand *A*, lot 1, and brand *D*, lot 1, Table I) was of poor quality.

Levels of iodine ranging from 0.005 to 0.09 mcg./Gm. of potassium chlorate would not be expected to interfere in most analytical procedures. However, it can be estimated that in the iodine assay method of Bodansky *et al.* (2) the volume of chloric acid used for wet-ashing each sample contains acid-soluble material from an equivalent of more than 2 Gm. of potassium chlorate. Thus, in this procedure in which iodine varying from 0.01 to 0.06 mcg. per unknown sample (*e.g.*, thyroid powder, iodinated compounds, or serum) is being determined, chloric acid prepared from potassium chlorate having 0.005 to 0.09 mcg. of iodine/Gm. could contribute from 0.01 to 0.18 mcg. of iodine to each sample as an impurity.

As far as can be determined, no manufacturer of potassium chlorate attempts to control the iodine limits of the product, and certainly no label value for iodine content of reagent grade potassium chlorate is given. Batches of potassium chlorate have sometimes been found having low enough iodine levels for the preparation of satisfactory chloric acid. However, depending upon chance to obtain a satisfactory reagent chemical is expensive in time and effort since each new batch purchased must be evaluated before use.

For a period of several months no satisfactory reagent grade potassium chlorate could be obtained from any manufacturer. However, a plentiful supply of an unusable batch of potassium chlorate was available. In order to maintain the schedule of iodine assays in this laboratory, purification of potassium chlorate (brand *A*, lot 6, Table I) by recrystallization was attempted. This was done by filtering a boiling saturated solution of the potassium chlorate. After chilling the filtrate at 5°, the crop of crystals was harvested by filtration and dried at 100°. It was necessary to repeat the recrystallization four times in order to obtain potassium chlorate which could be used to prepare fairly satisfactory chloric acid. After four recrystallizations, brand *A*, lot 6, potassium chlorate yielded a product estimated to contain about 0.002 mcg. of iodine/Gm.

Samples of reagent grade chemicals used in ion-exchange column chromatographic separation of iodinated amino acid (4, 5) were analyzed for their iodine content. Four lots of reagent grade glacial acetic acid from three manufacturers contained no detectable iodine (Table II), but samples from freshly opened bottles of brand *C*, lot 1, contained 0.15 mcg. of iodine/Gm.

Six lots of reagent grade sodium acetate from three manufacturers were found to contain 0.005 to 1.05 mcg. of iodine/Gm. of sample (Table III). Only sodium acetate, brand *B*, and lots 2 and 3, brand *C* (Table III), could be used (with iodine-free acetic acid) to prepare satisfactory buffers for ion-exchange column chromatographic separation of iodinated amino acids.

CONCLUSIONS

Reagents used in several methods for iodine analysis may be a source of iodine contamination since it appears that, in general, most reagent grade chemicals used in iodine analysis procedures are not limited by ACS specifications with reference to iodine content. Wide variation of iodine content was found in different batches of reagent grade potassium chlorate prepared by one manufacturer. Analysis of iodine in biological materials in the range of 0.01 to 0.06 mcg. per sample by the chloric acid wet-ashing digestion method would be considerably less difficult if producers could be persuaded to introduce low limits of iodine impurities in reagent grade potassium chlorate. At least, label assays of the iodine content of reagent grade potassium chlorate, glacial acetic acid, and sodium acetate should be available.

The results reported here indicate that analytical chemists should be aware that reagent chemicals may contain interfering impurities and should be concerned with the quality of analytical reagents used.

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