of dichlorofluorescein indicator. Titrate the mixture with tenth-normal silver nitrate solution until the silver chloride flocculates and the mixture turns to a faint pink.

Assay of Ampuls of Sodium Iodide.—Transfer an accurately measured volume of the ampul solution, containing about 0.25 Gm. of sodium iodide, to a casserole, add 200 cc. of the distilled water and 10 drops of dichlorofluorescein indicator. Titrate the mixture with tenth-normal silver nitrate solution until the silver iodide flocculates and the mixture turns a faint pink.

Each cc. of tenth-normal silver nitrate is equivalent to 0.01499 Gm. of NaI.

Assay of Ampuls of Sodium Salicylate.—Transfer an accurately measured volume of the ampul solution, containing about 0.25 Gm. of sodium salicylate, to a 100-cc. glass-stoppered cylinder and add 75 cc. of ether and 5 drops of bromphenol blue T.S. as the indicator. Titrate the mixture with tenth-normal hydrochloric acid, mixing intimately the aqueous and ethereal layers by vigorous shaking, until a permanent pale green color is produced in the aqueous layer.

Each cc. of tenth-normal hydrochloric acid is equivalent to 0.01601 Gm. of $C_6H_4.OH.COONa$.

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Determination of Acetone

*By Melvin W. Green**

INTRODUCTION

Three methods are in general use for the determination of acetone. The official method depends upon the conversion of the acetone to iodoform with an excess of iodine and the subsequent determination of the iodine with thiosulfate. Another method makes use of the qualitative test for acetone in which the acetone is precipitated as a yellow mercury complex. The mercury in this complex is then determined mercurimetrically or gravimetrically. The third method takes advantage of the carbonyl group which reacts with hydroxylamine hydrochloride to form acetoxime, liberating hydrochloric acid which is titrated with alkali.

Since there has been some criticism of the present Pharmacopœial method, a comparison of the three methods was undertaken by this laboratory.

EXPERIMENTAL

Purification of Sample.—A commercial acetone, alleged to be of U. S. P. grade, was refluxed for two hours with calcium oxide and then fractionally distilled, collecting only that fraction which boiled at 56.3 to 56.4° C. (uncor.).

Mercurimetric Method.—In 1926, Jonescu-Matin (1) recommended the determination of acetone, as well as certain alkaloids, by forming the mercury complex and then determining the mercury contained therein.

The reagent was prepared by mixing 50 Gm. of mercuric oxide with 500 Gm. of concentrated sulfuric acid and then diluting to one liter with water. In conducting the test, 1 to 10 cc. of a 5% aqueous acetone were refluxed for twenty minutes in the presence of 10 cc. of 50% sulfuric acid, 10 cc. of the mercury reagent and enough water to make a total of 100 cc. The material in the flask was then cooled, filtered, washed with 100 cc. of water in divided portions, the residue washed into a flask by a stream of water from a wash bottle and digested with 25 cc. of a mixture of two parts sulfuric acid to one part of nitric acid until solution was effected. The mercury was then determined by the familiar thiocyanate method, using ferric alum as the indicator. Jonescu-Matin claims the complex to have the formula $Hg_2SO_4:3HgO.4(CH_3)_2CO$. This would correspond to an acetone content of nearly 17%. However, the author experienced a higher and variable result, so variable that the method was abandoned as too unpredictable and too time-consuming for practicality.

Oxime Method.—In the oxime method, advantage is taken of the reactivity of the carbonyl group with hydroxylamine hydrochloride according to the following equation:

$$(CH_3)_2CO + NH_2OH.HCl = (CH_3)_2C = NOH + HCl \quad (1)$$

The HCl thus liberated may be titrated with 0.1*N* NaOH using methyl orange as an indicator, although various other single and a few mixed indicators have been advocated. However, according to Morasco (2) the reaction is only 94.4% complete.

The reaction was conducted as follows: Approximately 2 cc. of the purified acetone were added by

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pipette to a weighed 200-cc. volumetric flask containing about 50 cc. of distilled water. The flask was shaken and then made up to the mark with water. In each of two beakers, 400 cc. of a 0.2% hydroxylamine hydrochloride solution were placed and the contents of one beaker was neutralized to the indicator and to the other beaker were added 25 cc. of the aqueous acetone solution previously prepared. After standing fifteen minutes, the acid liberated by the reaction was titrated to match the control fluid. One cc. of 0.1*N* NaOH = 0.00614 Gm. of acetone.

Using this technique, values from 100.5% to 101.8% of theory were obtained. It is worthy of note that the Morasco factor is based upon the assumption that the iodoform reaction is exactly correct (*i. e.*, 1 cc. of 0.1*N* I_2 = 0.009675 Gm. of acetone). However, Haughton (3) believes the reaction to go to 97.1% completion rather than 94.4% and that, therefore, the factor should be 0.00598. The work in this laboratory seems to substantiate a need for a lower factor. However, here it was found that the factor varied with the sample of hydroxylamine hydrochloride and with the concentration of acetone employed.

Any attempt to change the time, temperature or concentration factors failed to yield a constant result. Consequently, it was concluded that such a method would be of little value officially unless some method of control could be found that would yield at least a constantly dependable and reproducible factor.

Messinger's Iodoform Method.—The technique used was that described in the U. S. P. XI. The results obtained by following directions closely are presented in Table I.

TABLE I.—IODIMETRIC DETERMINATION OF ACETONE

No.	Acetone Added, Gm.	Acetone Recovered, Gm.	Per Cent of Theory
1	0.01968	0.01973	100.25
	0.01968	0.01972	100.20
2	0.01982	0.01986	100.18
	0.01982	0.01987	100.25
3	0.01990	0.02001	100.55
	0.01990	0.01996	100.31

It was thought that perhaps the amount of alkali was a factor. To determine the effect of the alkali concentration, the assay was conducted as directed in the U. S. P., excepting that the alkali (and acid) concentrations were varied from 0.1*N* to 2.5*N*. Using 0.1*N* alkali, only about 40% of theory was obtained. The conditions under which exactly six atoms of iodine react with one molecule of acetone, were considered to give 100% of theory. Constancy of values arises at about 100.8% of theory in alkali concentrations from 0.85*N* to 2.5*N* NaOH. The alkali value corresponding to exactly 100% varied from about 0.6*N* to 0.85*N* and was too variable to be dependable. Consequently, it would seem that 1*N* alkali is necessary to be assured of constancy.

Changes in the time which the iodine was in contact with the alkaline acetone and the speed of the addition of the iodine produced no material deviation from the foregoing results.

The results of this reaction would, of course, be high in the presence of alcohol. Rakshit (4) has modified the U. S. P. method by replacing the sodium hydroxide with lime water, claiming that the presence of alcohol does not then interfere. However, it was the experience of this laboratory that lime water gave an impossibly low result.

For estimation of small quantities of alcohol in the presence of acetone, Craven (5) observed that 1 cc. of pure acetone with 3 cc. of a reagent containing 0.5 Gm. of potassium dichromate in 100 cc. of pure colorless nitric acid (sp. gr. 1.310) developed a green color only after three hours whereas with 0.5% alcohol or formaldehyde, a blue color developed almost immediately. The author applied the reaction to both commercial acetone U. S. P. and the purified acetone. No green color developed with the acetone as long as the nitric acid was free from colored nitrogen oxide fumes. It was found that the threshold concentration of alcohol, 4:1000, developed a blue color within five minutes. Since the commercial acetone never developed the color within the time limit, it was concluded that the alcohol concentration was never over 4:1000 and that, therefore, any high results could not be due to the high alcohol content.

Haughton (3) has suggested that in addition to the main reaction a side reaction takes place as follows:

$$CH_3COCH_3 + 5NaOH + 5I_2 = 2CHI_3 + HCOONa + 4NaI + 4H_2O \quad (2)$$

This side reaction consumes ten atoms of iodine instead of the six of the normal reaction and hence would possibly account for the high results.

If this side reaction occurs, one should be able to detect formic acid in the reaction mixture. To test for formate, 2 cc. of acetone were placed in excess alkali and an excess of 0.1 *N* iodine added. After about thirty minutes, the iodoform was filtered off. After acidifying the filtrate with sulfuric acid, mercury was added to combine with any free iodine and the mixture filtered and steam-distilled. The distillate was made alkaline to litmus with caustic, a slight excess added and evaporated down to about 200 cc. This liquid was refluxed for one hour in the presence of 5% mercuric chloride after first neutralizing with hydrochloric acid. By this method, 1.55 Gm. of acetone yielded 0.068 Gm. of mercurous chloride. A blank made by distilling acetic acid, in the approximate concentration obtained from the normal iodination of acetone, yielded only the slightest trace of turbidity equivalent to less than 0.1 mg. of calomel.

From these results, it would seem that the side reaction probably does occur to a limited extent. It is interesting to note that Cassar (6) had a similar experience in determining methyl ethyl ketone in the presence of secondary butanol. In this case the main reaction is:

$$CH_3COCH_2CH_3 + 3I_2 + 3NaOH = CHI_3 + CH_3CH_2COOH + 3NaI + 2H_2O \quad (3)$$

and the secondary reaction is:

$$CH_3COCH_2CH_3 + 5I_2 + 4NaOH = 2CHI_3 + CH_3COOH + 4NaI + 3H_2O \quad (4)$$

Cassar found that the iodoform method yielded about 110.6% of theory and he believes that the high results may be attributed to the secondary reaction and its subsequent consumption of more iodine.

SUMMARY

1. There are three general methods for assaying acetone: the mercury complex method, the oxime method and the iodoform method.

2. The mercury method gives the lowest results, the oxime method high results and both give variable results.

3. The iodoform method gave the most uniform results, but the results are high. It is believed that these high results are due to a secondary reaction involving formate and consuming ten rather than six atoms of iodine.

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Decomposition Rate of Ethyl Nitrite in Brown Mixture, U. S. P.

By Edward Greenfield and H. Walter Kuhl**

Several samples of Brown Mixture, U. S. P. XI, known to be approximately six months old, were tested for their ethyl nitrite content. Analysis showed none of these samples to contain any ethyl nitrite. In view of this, experiments were made to determine the rate of decomposition of this ingredient in the mixture.

EXPERIMENTAL

A modified Peter Griess (1) method for the determination of nitrous acid was employed in these experiments. This colorimetric method is extremely sensitive, depending upon the formation of intensively colored azo dyes (2) and detecting as little as 0.001 mg. HONO in a liter. It was assumed that the ethyl nitrite was hydrolyzed to nitrous acid by the action of the acetic acid in the reagent. A standard solution of sodium nitrite was used for quantitative colorimetric comparison.

Procedure.—A well-shaken sample of Brown Mixture was diluted to 4000 volumes to yield a theoretical concentration of 0.3 mg. C_2H_5ONO per liter in a practically colorless solution. Fifty cc. of this latter solution, theoretically containing 0.015 mg. C_2H_5ONO, was used for the actual test. After negative results were obtained with the original samples of Brown Mixture, the theoretical amount of Spirit of Nitre, as per U. S. P., was added to a composite of these samples. At the same time a fresh batch of Brown Mixture was prepared by the official U. S. P. method. These two samples were individually assayed each day, after preparation, up to a month. The composite results follow:

RATE OF DECOMPOSITION OF ETHYL NITRITE IN BROWN MIXTURE

Time after Manufacture of Mixture	Ethyl Nitrite Remaining, in Per Cent
0	100.0
4 Hours	66.5
28 Hours	39.5
2 Days	33.3
5 Days	13.3
6 Days	6.7
1 Month	Negligible

From the above results, it is evident that the ethyl nitrite begins to decompose as soon as it is added to the preparation. It is doubtful whether it has any therapeutic (3), (4) or other value in the preparation unless it is used immediately after manufacture. It has been shown (5) that on deterioration ethyl nitrite develops no new kind of pharmacologic activity.

CONCLUSION

The decomposition rate of ethyl nitrite in Brown Mixture U. S. P. is so rapid and extensive as to render it valueless in the latter preparation.

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