SCIENTIFIC SECTION

NOTES ON THE ASSAY METHODS OF THE U. S. PHARMACOPŒIA, X.*

BY T. F. PAPPE[1].

The articles of incorporation of the U. S. Pharmacopœial Convention state that the purpose of that organization is to encourage and promote the science and art of medicine and pharmacy by several means, among which is mentioned: "By establishing one uniform standard and guide for those engaged in the practice of medicine and pharmacy in the United States, whereby the identity, strength and purity of all such medicines and drugs may be accurately determined."

It is evident that the original purpose of the methods of assay outlined in the Pharmacopœia was to enable the physician and especially the pharmacist to establish the purity and strength of the crude drugs and raw materials used in compounding, and to enable them to determine the strength of the various galenicals after preparation. For this purpose it was necessary to write the assays as simply as possible, using the minimum of laboratory apparatus and reagents compatible with the purpose in view.

However, following the passage of the Federal Food and Drugs Act and the drug laws of the various states, which made the U. S. Pharmacopœia official for certain drug products, it was necessary to take a somewhat different viewpoint of the test. While the assay methods specified were usually satisfactory for the primary purpose, nevertheless some of them were of such a character that they were not entirely satisfactory for law enforcement.

In criticizing the assays, their dual nature at the present time must be kept fully in mind. These notes, which represent not only the experience of the writer and his immediate associates, but also that of members of other units of the Food, Drug and Insecticide Administration, were compiled, with few exceptions, from a regulatory standpoint, and should not be considered criticisms of methods outlined with only the needs of the physician and pharmacist in mind. The assays under consideration have been divided into the following classes for the purpose of this presentation: (1) methods which are inconsistent, or contain faulty directions or misstatements; (2) methods which are not specific for the material assayed; (3) methods which are indirect, where simple, direct methods are now available; (4) methods which, while direct, may well be replaced or supplemented by better or shorter methods that are the result of later research.

INCONSISTENT OR FAULTY METHODS.

Many assays in this group call for extraction with immiscible solvents where the obvious precaution of using a sufficient number of extractions to insure complete removal is not mentioned. Among such assays are those for caffeine sodiobenzoate, for quinine tannate, for ammonium benzoate and for ammonium salicylate.

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[1] Baltimore Station, Food, Drug and Insecticide Administration, U. S. Department of Agriculture.

The determination of mercury in mercuric salicylate and blue mass is extremely unsatisfactory, owing to the fact that the organic matter cannot be oxidized by nitric and sulphuric acids without some loss of mercury. Unfortunately, we have not been able to find a satisfactory substitute, although the electrolytic method may give satisfactory results in spite of its well-known difficulties in the case of mercury.

In the case of methenamine, concordant results can be obtained only if the formaldehyde formed in the decomposition is entirely removed. Boiling the solution will not usually remove all the formaldehyde, nor will one evaporation, but the solution should be evaporated to dryness and water added at least twice with subsequent evaporation to dryness.

The directions for the assay of potassium chlorate call for the addition of 50 cc. of acid ferrous sulphate T. S. to the analytical portion. It should be noted, however, that the strength of acid ferrous sulphate T. S. was changed in the 10th revision from 3 Gm. per 100 cc. to 7 Gm. per 100 cc. The addition of 50 cc. of this stronger solution gives an extremely large excess of ferrous sulphate, which makes the back titration unreliable on account of the danger of oxidation; 25 cc. of the T. S. will give an ample excess.

The determination of unsaponifiable matter in cod-liver oil[1] has been found to be unreliable, owing to the formation of acid soaps, which are not removed from the ether extract on washing with water. This difficulty has been obviated by two preliminary washings of the ethereal solution with dilute alkali, followed by the prescribed washings with water.

The determination of alcohol-soluble material in drugs containing volatile substances, such as benzoin,[1] asafœtida and gum myrrh, is open to criticism on account of the loss of these volatile materials under the conditions of the assay. The results of much work on benzoin done in the New York Station of the Food, Drug and Insecticide Administration have been reported to the Association of Official Agricultural Chemists, and will be published in the *Journal* of that Association. This report contains a method for the determination of alcohol-soluble extracts, which minimizes this loss and gives appreciably higher results.

The assay provided in the case of oil of cinnamon[1] for the determination of cinnamic aldehyde, and in the case of oil of caraway for the determination of carvone, includes alcohol in the results. As samples of oil of cinnamon have been found adulterated with alcohol, this method should be so modified as to correct for such an addition. Furthermore, the test for chlorinated products[1] in oil of cinnamon and benzaldehyde is lacking in the required delicacy.

The assay for camphor in camphor liniment is somewhat inaccurate, owing to the oxidation of the cottonseed oil under the conditions outlined.[2] This may cause considerably lower results. It has been suggested that a control with 4 cc. of cottonseed oil be run along with the determinations, and the results corrected by adding the gain in weight of the control to the loss at 110° as a measure of the camphor actually present.

The U. S. P. X method, as well as the U. S. P. IX method, for the assay of podophyllum[2] have been criticised, the present official method, because it gives erroneously high results, and the 9th revision method on account of the time con-

[1] Noted by members of the New York Station, F. D. I. Administration.

[2] Noted by Members Drug Control, F. D. I. Administration.

sumed in the determination. Warren (*J. A. O. A. C.* (1927), 10, 272) has discussed this method and offered a substitute which has given good results on collaborative work. A further study of this method is suggested.

Concordant results have not been obtained in the determination of aldehydes as citral in lemon oil.[1] It seems probable that the substitution of an adaptation of the official method for citral in flavoring extracts would improve the assay of this product. This method determines the citral colorimetrically by means of meta phenylene diamine, and is detailed in the Book of Methods, of the Association of Official Agricultural Chemists. A refinement of this method has been suggested by Hiltner, one of the original authors, in the *Journal of Industrial and Engineering Chemistry*, 10 (1918), 608. This procedure obviates any off colors which may be formed because of oxidation in the case of certain oils, and renders the method more dependable.

Finally, it may be stated that provision may well be made in the assays of bismuth salts for solution of the bismuth oxide in acid, filtering and weighing of any acid-insoluble material to correct for the admixture of such substances if present; that the use of alcoholic potash in place of the aqueous solution prescribed assures saponification in the assay of acetylsalicylic acid; that the words "not less than" should be deleted from the statement "each cc. of tenth normal sulphuric acid corresponds to not less than 0.003705 Gm. of $Ca(OH)_2$" in the case of lime water; that the alcohol used to dissolve the alkaloids in the proximate assays for alkaloids should be evaporated on the steam-bath after addition of the measured acid and water, or else diluted to a concentration of 10 per cent or less, as the presence of much alcohol affects the end-point of the back titration materially in the case of some alkaloids; and that a loss of ferric chloride has been noted in the assay of tincture of ferric chloride[2] the volatility of ferric chloride when evaporated to dryness in the presence of strong hydrochloric acid being appreciable. Also that the wording of the test for galbanum[1] in asafœtida is misleading, and should be rewritten to show that the fleeting blue-green color is a test for asafœtida and not for the adulterant. Hence the non-appearance of the color indicates complete absence of asafœtida and the test can accordingly be only corroborative in the case of a product that does not show the characteristics of the drug.

METHODS NOT SPECIFIC FOR MATERIAL ASSAYED.

The assay for syrup of hydriodic acid and for the various inorganic bromides and iodides are typical instances of this class. The Volhard Method, which is specified for all halogen determinations, does not, of course, differentiate between chlorine, bromine and iodine. As there are methods which will satisfactorily determine bromine and iodine, such method should at least be studied with a view to their later adoption. Iodine in iodides may be determined by an adaptation of the method for organic iodine as outlined under thyroid, omitting the fusion. Iodine and bromine may be titrated directly in an actively boiling acid solution with potassium permanganate by the method of Winkler (*Z. angew. Chem.*, 28 (1915), 477, *Chem. Abstr.*, 10 (1916), 867), and the three halogens may be determined jointly by the official method. Combinations of these three methods will permit of the separate determination of the three elements. These refinements are necessary, as fre-

quently potassium bromide and potassium iodide which contained chlorides, sometimes in considerable amounts, have been found.

Another example of this class of assays is in the evaluation of Calx, quick lime. The method does not differentiate between calcium oxide, calcium hydroxide and calcium carbonate, and hence gives no measure of deterioration of the product.

INDIRECT METHODS.

Indirect methods are expedient in assaying certain products, such as the alkali metal salts of organic acids, as in most of these cases the determination of the acid is difficult and not particularly accurate. The salts of acetic acid, however, might well be evaluated by distillation and titration of the acid. The alkali benzoates and salicylates should be examined by the method outlined for ammonium benzoate and salicylate, as this direct determination is neither difficult nor inaccurate. Strontium salicylate is similar, and the assay should include a direct determination of the salicylic acid. Zinc chloride and solution of zinc chloride should also be assayed for zinc rather than for chlorine. Special emphasis should be laid on the assay for sodium cacodylate, as the official method gives no measure of its therapeutic activity. The arsenic can be readily determined by digestion of the material with sulphuric acid, potassium sulphate and starch, followed by titration with iodine.

METHODS WHICH MAY BE REPLACED OR SUPPLEMENTED.

The assays for ipecac and henbane and their preparations, and occasionally for the preparations of belladonna, fall into this group. Complete extraction of the active ingredient is almost impossible by the official method, and it is strongly urged that the use of the automatic extractor described by Murray, Watkins and Palkin (*Ind. Eng. Chem.*, 17 (1925), 612) be made official. A more satisfactory assay for opium preparations has been described by Buchbinder in an Information Sheet issued by the Food, Drug and Insecticide Administration. The method for spirits of ethyl nitrite described by Kebler *et al.* in JOUR. A. PH. A., Vol. 4, No. 8, while not so specific as the official method, is much simpler and requires no special apparatus. This method depends upon the reduction of potassium chlorate by the ethyl nitrite and subsequent titration of the potassium chloride formed. The substitution of the reduction method for spirit of nitroglycerin is also urged. This method makes use of DeVarda's alloy to reduce the nitroglycerin in alkaline solution, followed by distillation and titration of the ammonia formed. The substitution of solid potassium hydroxide in the test for aldehydes in ether also is advantageous, the ether being allowed to stand over night in the refrigerator in contact with a small piece of the alkali.

In general, it may be stated that many assays can be more satisfactorily performed using the automatic extractor mentioned above, and it is suggested that this apparatus be made official as an alternative to the ordinary separator. Also the xylol distillation method is of particular value in determining moisture in drugs containing other volatile ingredients, and its inclusion as an alternative method is suggested. It has been found useful in assaying aloes where the drying method does not give reliable results.

There are two points which are not exactly germane to the purpose of this paper

which I desire to mention. The first is the addition of directions for assay of various alkaloidal salts, the purity of which is sometimes questionable. Most of these can be assayed by extraction from ammoniacal solution with subsequent titration. An alternative method (*J. A. O. A. C.*, 11, No. 1, 49) for cocaine provides for saponification and determination of the benzoic acid formed.

The second point has reference to the standards for acid insoluble ash as a measure of foreign inorganic matter in crude drugs. As you know, the U. S. P. IX relied on total ash as an index of foreign material of this type. On account of the limitations of this figure, a change was made in the present revision to the acid-insoluble ash figure. Limestone and similar acid-soluble extraneous matter will not be detected by this determination, and it, therefore, seems advisable to recommend a combination of total ash and acid-insoluble ash as standards. This combination is used largely in judging spices and similar food products under the Federal Food and Drugs Act, and has given reasonable satisfaction.

STABILITY OF SOLUTION OF POTASSIUM ARSENITE.*

BY ELIZABETH PICKERING.

The U. S. P. X assay for solution of potassium arsenite detects only its content of trivalent arsenic. Since during the aging of this solution it undergoes gradual oxidation to potassium arsenate or pentavalent arsenic, there results an apparent loss in strength although the total arsenic remains the same. Trivalent arsenic is much more active therapeutically and of higher toxicity than pentavalent arsenic. Oxidation of potassium arsenite solution therefore reduces both its toxicity and therapeutic effect. For this reason, both the therapeutic activity and assay, as determined by the U. S. P. X method decrease on aging, although not in the same proportion. It has been suggested that the U. S. P. assay should be so changed as to include all forms of arsenic, if solution of potassium arsenite undergoes oxidation to a noticeable extent.

Arsenious acid is oxidized by air when the solution is warmed (1), and this reaction is accelerated in the presence of ether (2) or copper salts (3). A solution 0.5 Gm. arsenious oxide is completely oxidized by boiling for 26.5 hours in 5 cc. of water and 3 cc. of 95% ethyl alcohol (4).

Literature published on alkaline arsenites is not in agreement as to their stability to the oxidizing effect of air but the majority of authors believe such oxidation to occur slowly. Solutions of potassium arsenite are said to be more stable than those of sodium arsenite, remaining "practically" unchanged for six or eight months when made from carbonate and bicarbonate, respectively (5). Cooper and Freak have reported (6) the partial oxidation of the alkaline sodium arsenite dipping solution to the arsenate and its increased rapidity in the presence of wood tar and cresylic acid, especially on exposure to sunlight. In agreement with the earlier statement that the oxidation of arsenious acid is accelerated by ether or copper salts, Reinders and Vles, found that alkaline arsenites are not oxidized by oxygen in the absence of catalysts, such as copper or carbon. Even in the presence of such catalysts, oxidation is immeasurably small in acid and neutral solution, but

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