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METHODS OF ANALYSIS OF ACETYLSALICYLIC ACID AND ADULTERANTS.

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In the spring of 1915, about ten months after the outbreak of the European war, the supply of acetylsalicylic acid was practically exhausted. The stocks still held by a few dealers were being offered at an advance of about two hundred per cent above the market price of the previous year. Owing to the scarcity of synthetic drugs of foreign origin, the "drug peddlers" and "drug brokers" who had been dealing in these products were unable to supply the demand and, not wishing to lose an opportunity to profit by the advanced prices, resorted to unscrupulous methods to obtain material for their trade.

Information that acetylsalicylic acid was being adulterated reached the Department in the early part of June 1915. An investigation was begun and samples collected and analyzed. These samples, mostly tablets, showed a marked variation in composition. Some were pure acetylsalicylic acid, others contained only small amounts, and a number did not contain any. As the combinations of substances found in the spurious samples differed widely, it was concluded that several manufacturers were adulterating acetylsalicylic acid, and steps to locate them were immediately taken. After some difficulty, consignments of spurious acetylsalicylic acid were found to have originated in Cleveland, Indianapolis, Memphis, St. Louis and Chicago. For the purpose of hiding their identity and to make it more difficult to trace shipments, some of these adulterators would use fictitious shippers' names and addresses on the packages sent out by them.

Previous to 1917, before the patent on acetylsalicylic acid expired, its sale was restricted in this country, and reputable wholesale druggists refused to handle it except under the trade name "Aspirin." As stated above, most of the acetylsalicylic acid was sold by drug brokers and peddlers who frequently carried their entire supply in a trunk or suitcase and disposed of it in such quantities as the druggist or dealer might wish to buy. A number of the shipments sent to brokers and peddlers by these adulterators were located and immediately confiscated by inspectors of this Department. The investigation showed that some of these manufacturers would deliver the unlabeled, spurious mixture to manufacturing pharma-

ceutical houses to have it compressed into tablets, while others would do their own compressing. The extent to which this fraud was perpetrated can be estimated when it is considered that one of these adulterators sold over twelve hundred pounds of this product in one month. This same manufacturer did not confine his activities to acetylsalicylic acid, but also made adulterated ichthyol, creosote, carbonate, potassium guaiacol, sulphonate, etc., samples of which were collected and analyzed. Most of these unscrupulous manufacturers have already been tried and convicted in the United States Courts, and several were sentenced to serve a term in prison.

During this investigation scores of samples of acetylsalicylic acid were collected and analyzed. While many of these were pure, the majority consisted of one of the following combinations:

1. Acetanilid or acetphenetidin, citric acid, salicylic acid, and milk sugar.
2. Acetanilid, salol, salicylic acid, and milk sugar.
3. Acetylsalicylic acid, milk sugar, and starch. (The tablets contained only 20 percent of the amount declared.)
4. Milk sugar, alum, and acetanilid.
5. Acetylsalicylic acid and salol.
6. Milk sugar, starch, and calcium acid phosphate.
7. Tartaric acid, milk sugar, and boric acid, both with and without acetanilid.
8. Acetanilid and tartaric acid.
9. Milk sugar, cream of tartar, and salicylic acid.
10. Milk sugar and cream of tartar.

Owing to the variation in composition of these samples, the usual methods for examining acetylsalicylic acid could not always be followed. Therefore special methods were compiled for the qualitative and quantitative analyses of the different combinations. These are given below.

Acetylsalicylic acid usually occurs in the form of colorless crystalline needles with an acidulous taste. It is soluble in 100 parts of water and freely soluble in alcohol, ether and chloroform. Its melting point is usually given as 135° C. A number of apparently pure samples were found to melt as low as 130° C.

QUALITATIVE TESTS.

Since many samples contain a very high percentage of chloroform-insoluble material, better results will be obtained by applying the qualitative tests to the chloroform-soluble substances and chloroform-insoluble substances after they have been separated. The taste of the insoluble residue will readily indicate sucrose, acids, or acid salts if present.

Free Salicylic Acid.—Shake 0.5 Gm. of the sample with 10 Cc. of cold water and filter the liquid. Add a drop of dilute ferric chloride solution to the filtrate. This should not produce more than a very faint violet color. If boiled with water for several minutes acetylsalicylic acid is hydrolyzed and the addition of ferric chloride to the filtrate will produce a deep violet color characteristic of salicylic acid.

Salicylic Acid and Salol.—Boil. 0.5 Gm. of the sample with 10 Cc. of normal sodium hydroxide for two minutes, cool, and add an excess of dilute sulphuric acid. Acetic acid will be liberated and delicately violet-colored crystals of salicylic acid will be precipitated. These crystals, when washed and dried, should melt at 157°. If phenyl salicylate (salol) is present, the odor of phenol will be recognized on acidifying. Salol may frequently be recognized by its characteristic odor.

Acetanilid.—Heat 0.2 Gm. of the sample with 5 Cc. of a ten percent solution of potassium hydroxide; then add 0.1 Cc. of chloroform and again heat. The odor of phenylisocyanide should not be evolved.

Acetphenetidin.—Heat 0.2 or 0.3 Gm. of the sample with 10 Cc. of dilute nitric acid. If an intense yellow color develops, acetphenetidin is indicated.

Sugars, Starch or Salts.—0.5 Gm. of the sample should be completely soluble in 15 Cc. of chloroform or alcohol.

Test any chloroform-insoluble residue for starch with iodine.

The presence of lactose is indicated (*a*) if a yellow color develops on boiling a 0.5 Gm. sample with potassium hydroxide solution, (*b*) if a water solution of the chloroform-insoluble residue reduces Fehling's solution.

Inorganic Impurities.—Shake two Gm. of the sample for several minutes with 25 Cc. of water, filter and test separate portions of the filtrate for metals, chlorides, sulphates, carbonates, borates and phosphates, according to the usual qualitative methods prescribed for these substances in the Pharmacopoeia, or other standard books.

QUANTITATIVE METHODS.

Substances Determined in the Original Sample.

Ash.—The residue of the ignited powder should not be over 0.2 percent. (On compressing acetylsalicylic acid into tablets some inert binding and lubricating materials are generally added which may increase the ash.)

Acetylsalicylic Acid.[1]—The following double titration is very satisfactory when examining pure acetylsalicylic acid or tablets containing the usual excipients sugar, starch and talc, since these substances do not interfere with the titration. In the presence of acids, acid salts, salol, etc., the method is not satisfactory.

Weigh 0.3 Gm. of the finely powdered material into an Erlenmeyer flask, dissolve as quickly as possible in about 10 Cc. of cold neutral alcohol (95 percent), add a drop of phenolphthalein solution, and titrate with $\frac{N}{10}$ potassium hydroxide. Note the volume of $\frac{N}{10}$ alkali required, then add a volume of the alkali equal to that required for the first titration plus 5 Cc., and place the solution on a steam bath for 15 minutes. Titrate the excess of alkali with $\frac{N}{10}$ acid.

If the product is pure the total amount of alkali required should be twice that of the first titration. Each cc. of $\frac{N}{10}$ alkali is equivalent to 0.009 Gm. acetylsalicylic acid.

If free acids are present, the amount of acetylsalicylic acid may be calculated by multiplying the number of Cc. of $\frac{N}{10}$ alkali required for the second or hot titration by 0.018.

The cold titration should be made with as little delay as possible since the acetylsalicylic acid hydrolyzes very rapidly liberating acetic and salicylic acid, which would require more alkali to neutralize than the original substance. If salol is present the odor of ethyl salicylate will be noticed after heating the alcoholic mixture.

Total Salicylates.[2]—Dissolve 1 Gm. of the material in a slight excess of dilute alkali, heat on a steam bath for 15 minutes, then carefully transfer to a separatory funnel, acidify with hydrochloric acid, and extract with from 3 to 5 25-Cc. portions of chloroform to remove all traces of salicylic acid from the mixture. Treat each portion of chloroform extract in succession in a second separator with 20 Cc. of water containing 1 Gm. of anhydrous sodium carbonate for every 100 mg. of salicylic acid to be neutralized. Shake the solutions vigorously, and after separating the layers wash each portion of chloroform in another separator with 5 Cc. of water. On completion of this operation, unite the water layer with the main aqueous soda solution. Dilute the alkaline solution to a known volume, transfer an aliquot representing about 100 mg. of salicylic acid, to a 200 Cc. Erlenmeyer flask, and dilute to about 100 Cc. Heat the solution nearly to boiling; then add from a burette 25 to 40 Cc. of strong (about $\frac{N}{5}$) iodine solution, sufficient to insure an excess of this reagent during digestion and heat the mixture for an hour on the steam bath. Discharge the free iodine with a few drops of thiosulphate solution, decant the clear liquid

through a tared Gooch filter, care being taken that most of the precipitate remains in the flask. To the latter add 50 Cc. of boiling water. Digest for 10 minutes on the steam bath; then filter, gradually washing all the reddish substance into the Gooch filter with 200 Cc. of hot water, dry at 100° and weigh. Multiply the weight of precipitate by the factor 0.4016 to obtain the quantity of salicylic acid present in the aliquot.

Substances Soluble in Chloroform.—Separation of constituents (in mixtures containing sugar, inorganic salts, salicylic acid, acetphenetidin, and acetanilid, with little or no acetylsalicylic acid).

Accurately weigh 2 or 3 Gm. of the finely powdered sample into a counterpoised filter, and wash it with successive small portions of chloroform to extract all the soluble substances. The last portion of the chloroform should be dropped along the upper edge of the filter to remove any crystals which may have formed. Collect the solvent in a tared dish, evaporate at 60°, dry and weigh the residue, redissolve in 10 Cc. of cold 95% alcohol, add a drop of phenolphthalein solution, and titrate with $\frac{N}{10}$ alkali. Calculate the acidity as salicylic acid. Add an excess of 0.5 Cc. of $\frac{N}{10}$ alkali, and heat on a steam bath for 2 or 3 minutes. If the solution is partly or completely decolorized, acetylsalicylic acid or salol is indicated. (Salol will produce ethyl salicylate, which is readily detected by its wintergreen-like odor.)

Transfer the titrated solution to a separatory funnel, using 20 Cc. water and 25 Cc. of chloroform to wash the dish. Then add about 10 Cc. of 1% potassium hydroxide solution, shake, and when the two liquids have separated, carefully transfer the chloroform to another separator. Repeat the extraction twice, using 15 and 10 Cc. chloroform. Filter the chloroform into a tared dish, evaporate, dry and weigh. This residue contains the acetphenetidin, acetanilid, and salol.

Combine the aqueous washings and reserve for the determination of total salicylates. The melting point of the chloroform residue should be determined and other tests that may be necessary to identify the residue should be made. Acetanilid melts at 115°, acetphenetidin at 135°, and salol at 42°.

Acetanilid and acetphenetidin may also be determined by the following methods:

Acetanilid.[3]—Transfer 0.30 Gm. of the chloroform-soluble residue redissolved in chloroform to a 200 Cc. flask, add 10 Cc. of dilute sulphuric acid (1 to 10 by volume), and heat on a steam bath until the volume is reduced to 5 Cc. If the odor of acetic acid is present, add 10 Cc. of water and continue the digestion until the liquid is again reduced to 5 Cc.; then cool, transfer to a separatory funnel with water, so that the final volume does not exceed 20 Cc. Add 30 Cc. of chloroform, shake the mixture gently for 1 minute and, after the layers have separated, withdraw the lower layer through a small dry filter. Repeat the extraction with 20 and 15 Cc. chloroform. Discard the chloroform and carefully draw off the aqueous acid solution remaining in the separator into an Erlenmeyer flask. Rinse the filter and funnel with several 5 Cc. portions of water to remove all traces of the former contents. Heat the liquid on a steam bath for 10 minutes to expel the chloroform. After cooling, add 10 Cc. of concentrated hydrochloric acid. Run in gradually, and with frequent shaking, a standard solution of potassium bromide-bromate until a faint yellow color persists. From the amount of standard solution required calculate the percentage of acetanilid in the chloroform-soluble residue, as well as in the original mixture.

The standard bromide-bromate solution is prepared as follows: Dissolve 50 Gm. of potassium hydroxide in a small quantity of water. Add a slight excess of bromine, dilute with water to dissolve any separated salts, boil to expel excess of bromine, and dilute to 1 liter. Standardize the solution against well-dried, recrystallized acetanilid, adjusting the solution so that each Cc. is equivalent either to 5 or to 10 mg. of acetanilid.

Acetphenetidin.[4]—Proceed as directed under "Acetanilid" up to and including the extraction of the hydrolyzed acid mixture with chloroform. Treat the aqueous acid solution of phenetidin sulphate remaining in the funnel with successive small portions of solid sodium bicarbonate until an excess of the former persists at the bottom of the mixture. Add 50 Cc. of chloroform

and, for every 100 mg. of acetphenetidin present, 5 drops of acetic anhydride. Shake the mixture vigorously for some time, allow the layers to separate, and withdraw the chloroform into a second separator containing 5 Cc. of water. Shake this mixture, and after separation pass the lower layer through a small dry filter into a 200 Cc. Erlenmeyer flask. Distil over about 40 Cc. of the chloroform, using the distillate for further extraction. Make a second and third extraction of the original aqueous solution and wash and distil as before. Completely transfer the chloroform residue to a tared 50 Cc. crystallizing dish or beaker, evaporate on the steam bath to apparent dryness, and finally remove any considerable excess of acetic anhydride by repeated additions of 1 Cc. of fresh chloroform to which has been added a drop of alcohol.

The regenerated acetphenetidin should finally appear as a white, crystalline mass, with a faint acetous odor. The latter will completely disappear on standing some hours in the open or in a vacuum desiccator over lime. Weigh the residue from time to time until the final weight differs from the preceding not more than 0.05 mg.

Salol.[5]—If salol is substituted for acetylsalicylic acid, its presence is readily detected in the chloroform-soluble residue by qualitative tests, odor, and by the melting point (42° C.). In mixtures containing salol, acetanilid, or acetphenetidin, the salol may be determined by difference or by titration with standard bromide-bromate solution.

1. *Salol by Difference.*—In 0.3 Gm. of the chloroform residue determine the amount of acetanilid or acetphenetidin by acid hydrolysis as directed in the methods for acetanilid. To determine the amount of salol, subtract the weight of acetanilid or acetphenetidin found from the weight of chloroform residue taken.

2. *Salol by Alkaline Hydrolysis.*—Heat the chloroform-soluble residue from 1 Gm. sample with 20 Cc. of 2.5 percent caustic alkali on a steam bath for 10 minutes, cool and transfer quantitatively to a separatory funnel. Extract the solution with four 30 Cc. portions of chloroform. Discard the chloroform and transfer the alkaline solution from the separatory funnel into a 200 Cc. graduated flask. Wash the funnel, add the washings to the flask, and make up to the mark with water. Take an aliquot representing not more than 75 mg. salol, add an excess (about 45 to 50 Cc.) of standard bromide-bromate solution, and follow this with 10 Cc. of concentrated hydrochloric acid. Close the flask and shake 1 minute; then shake at intervals over a period of $^1/_2$ hour. At the end of this time add 10 Cc. of 15 percent potassium iodide solution, agitating the closed flask at intervals for 15 minutes. Titrate the free iodine with standard thiosulphate previously adjusted to the standard bromine solution, 1 Cc. of which is equivalent to 0.001784 Gm. salol. From the number of Cc. of standard bromine solution required, calculate the salol on the basis of 12 atoms of bromine to 1 molecule of salol.

If salicylic acid also is present the amount should be determined on a separate portion of the chloroform-soluble residue by dissolving a known weight of the residue in 10 Cc. of alcohol, adding 10 Cc. of water and 2 drops of methyl red indicator, and titrating with $\frac{N}{10}$ alkali. One Cc. of $\frac{N}{10}$ alkali represents 0.0138 Gm. salicylic acid. The amount of salicylic acid found, as well as the amounts of acetphenetidin or acetanilid found, should be subtracted from the weight of the residue taken, if salol is determined by difference.

If Method 2 is used in the presence of salicylic acid the quantity of standard bromide-bromate solution required for the salicylic acid must be deducted before the amount of salol is calculated.

SUBSTANCES INSOLUBLE IN CHLOROFORM.

The chloroform-insoluble residue remaining on the filter paper after the original extraction with chloroform is allowed to dry in the air. It is weighed and is

then washed with water until all water-soluble matter has been removed. The residue on the filter consists of starch, talc, etc., while in the aqueous solution the following adulterants, if present, will be found:

Alum, boric acid and salts, citric acid and salts, tartaric acid and salts, phosphates, and milk sugar.

Make the solution to a known volume and in aliquots determine:

Acidity	Alum
Reducing sugars	Citric acid
Phosphoric acid	Tartaric acid

according to methods outlined in Leach "Food Inspection and Analysis," John Wiley & Sons, or *Journal of the Association of Official Agricultural Chemists* (1916), "Methods of Analysis," Williams & Wilkins Co., Baltimore.

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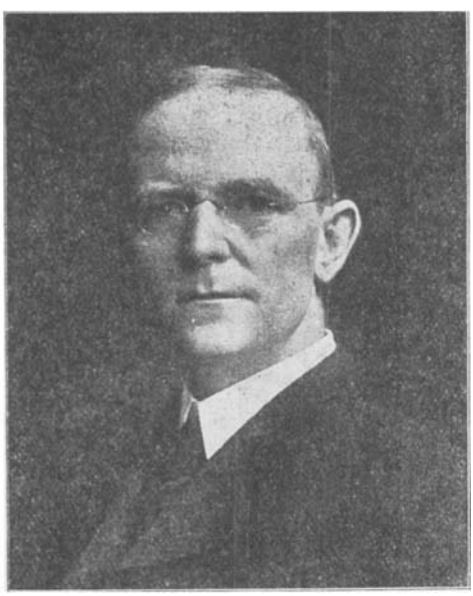

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