STUDIES IN SYNTHETIC DRUG ANALYSIS. IX. ESTIMATION OF ACETYLSALICYLIC ACID (ASPIRIN), PHENYLCINCHONINIC ACID (CINCHOPHEN) AND CAFFEINE IN ADMIXTURE.

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Among recent problems requiring the attention of this Bureau were two tablet preparations, containing as therapeutic agents aspirin and cinchophen on the one hand, and aspirin, cinchophen and caffeine on the other. While the current U. S. P. assays for both acetylsalicylic acid and phenylcinchoninic acid as such are apparently satisfactory, they make no provision for the isolation of these drugs separately, or as a group, from accompanying excipients and lubricants, nor for their evaluation when exhibited in simple admixture.

The only published attempt to determine cinchophen in the presence of aspirin (and salicylic acid resp.) was recently reported by Warren,[1] who made use of the difficult solubility of silver phenylcinchoninate in hydro-alcoholic media as a means of estimation. The present writer's experience with this method, however, has been less satisfactory, in that the values obtained for cinchophen were invariably high (one to four per cent) with correspondingly low ones for aspirin. It is believed, therefore, that such treatment cannot be relied upon to bring about more than an approximate separation in unknown mixtures.

In formulating the procedure presently to be described in detail, and designed to effect a quantitative group isolation of the active tablet constituents preliminary to their individual estimation, the following considerations were determinative. It was found, namely, that judicious treatment of the finely powdered tablets with aqueous sodium carbonate, and subsequent filtering, yielded a solution from which caffeine could, if present, be readily extracted with chloroform, and weighed. For the separation of aspirin and cinchophen, and their later estimation, the characteristic behavior of salicylates and phenol toward iodized potassium iodide solution (Wagner's reagent) in the presence of alkaline carbonates[2] immediately suggested itself, since a preliminary test had demonstrated the fact that cinchophen is wholly unaffected by this reagent under the experimental conditions observed, whereas aspirin yields the insoluble diiodophenylene oxide, $C_6H_2I_2O$, an obviously suitable compound for determining the salicylic acid nucleus. The clear filtrate obtained after elimination of the aspirin, and containing, in addition to iodides, iodates, and carbonates of sodium and potassium, the cinchophen (as a salt), yields on addition of dilute sulfuric acid in excess, a complex precipitate of iodine, both free and combined with cinchophen as periodide. On extracting this precipitate with an ether-chloroform mixture and treating with sulphurous acid, it is possible to isolate and weigh the cinchophen directly.

In the absence of caffeine, an alternative volumetric method for cinchophen suggests itself, based on the behavior of this substance, either alone or admixed with aspirin, toward iodized potassium iodide in the presence of glacial acetic acid. Thus, a solution of cinchophen in this acid yields with the iodine reagent in excess a black crystalline precipitate of the essential composition, $(C_{16}H_{11}NO_2)_2.HI.I_3$,

[1] JOUR. A. PH. A., 16, 32 (1927).

[2] Emery, *Ind. & Eng. Chem.*, 13, 538 (1921).

a periodide practically insoluble in the reacting menstruum and eminently suited to serve as a basis for volumetric estimation.[1]

EXPERIMENTAL.

The appended analytical findings are representative of many determinations carried out on both control and commercial mixtures. The individual components for these controls were selected from both domestic and foreign brands, and carefully checked as to purity. A specially purified cinchophen was obtained by means of the isatine-acetophenone synthesis of Pfitzinger,[2] and subsequent treatment with aqueous acetic acid. In general, the preliminary treatment of tablets consisted in exhausting the finely powdered sample with dilute sodium carbonate solution and filtering off the inert vehicular matter on a small suction plate.

METHOD I.

Caffeine.—In a small tared 50-cc. beaker weigh out an amount of the finely powdered tablets containing not to exceed 0.3 Gm. of aspirin and cinchophen known or believed to be present, add 1 Gm. of anhydrous sodium carbonate and 10 cc. of water, stirring the mixture from time to time over a period of one-half hour. Pass through a small suction filter and plate into a 50-cc. Erlenmeyer, washing out the beaker and funnel with three 5-cc. portions of water. Transfer the aqueous liquid to a 150-cc. Squibb separatory funnel and extract by vigorous shaking with four 25-cc. portions of chloroform withdrawing when clear to a second separatory funnel and washing successively with 5 cc. of water. Pass each portion of chloroform through a dry 5.5-cm. filter into a 50-cc. tared beaker, evaporate the solvent by a gentle air blast, and weigh the residual caffeine.[3]

Aspirin.—Withdraw the aqueous alkaline liquids from both separatory funnels into a suitable evaporating dish and heat to dryness on a steam-bath, in order to expel all traces of alcohol taken up from the chloroform. If the sample under examination contains no caffeine, the above indicated treatment for its isolation becomes superfluous. Dissolve the alkaline residue containing the aspirin and cinchophen as salts in 25 cc. of warm water, transfer by filtration if necessary to a 400-cc. Erlenmeyer, add 1.5 Gm of anhydrous sodium carbonate (1 Gm. for each 100 mg. aspirin known or believed to be present), diluting with water to about 200 cc. Heat on the steam-bath and add from time to time 10-cc. portions of 0.2 *N* iodine,

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[1] A more detailed description of this compound, and of a lower copper-colored periodide, $(C_{16}H_{11}NO_2)_2.HI.I_2$, will appear in a later communication. Rabak, *J. Assocn. Official Agr. Chem.*, 1, 33 (1923), has already reported the formation of an iodine addition-product on adding a 0.1 *N* iodine solution to cinchophen in acetic acid, but was unable to develop any satisfactory method based on this observation.

[2] *J. prakt. Chem.*, (2) 38, 583 (1888).

[3] Except in specially prepared controls, it will be found that most, if not all, commercial brands of cinchophen carry an impurity which follows the caffeine on its extraction with chloroform. As a means of approximate purification, rub up the residual caffeine with 5 cc. of warm water, allow to stand one-half hour, then pass through a minute moistened filter into a small tared beaker, evaporate the filtrate by means of an air blast, and weigh the resulting crystals. Obvious precautions must of course be taken in this and similar operations to insure the quantitative transfer of dissolved substance by judicious washing of filter edges, funnel and separatory funnel tips, etc., with the respective solvent.

until after the final addition and protracted heating an excess of the reagent is apparent. Add 0.5 Gm of sodium carbonate and continue heating one hour, then filter hot through a tared Gooch, washing the reddish purple precipitate with about 200 cc. of hot water. Dry in an air-bath at 100° to constant weight. The weight of the precipitate multiplied by the factor 0.5238 yields the quantity of aspirin originally present in the sample taken.

Cinchophen.—Evaporate the filtrate on a steam-bath to about 10 cc., care being taken to avoid separation of iodine, then transfer the salt mixture by pouring and washing with a minimum of water to a 150-cc. separatory funnel, so that the final volume of liquid does not exceed 35 cc. Now add slowly 15 cc. of 10% sulfuric acid (sufficient at least for complete acidification) with constant agitation, whereupon the cinchophen is precipitated as periodide along with an excess of free iodine. Extract with four 50-cc. portions of a mixture of 40 cc. of chloroform and 10 cc. of ether, transferring the chloroform-ether mixture when reasonably clear to a second separatory funnel containing 5 cc. of a saturated aqueous solution of sulphurdioxide. Shake vigorously, then add, if necessary, a few more drops of sulphurous acid, sufficient to develop a bright yellow to straw color in the reacting media. Draw off the chloroform-ether mixture into a third separatory funnel containing 10 cc. of water. Shake moderately, whereupon the liquids should become colorless. Withdraw the lower layer through a small dry 5.5-cm. filter into a tared 100-cc. Erlenmeyer, and evaporate the solvents at a very gentle heat and with a moderate air blast nearly to dryness. Carry out the succeeding three extractions substantially as above indicated, except that in the third and fourth operations the addition of one to two drops of sulphurous acid to the contents of the first separatory funnel will be found advantageous in reducing superfluous free iodine. Familiarity with the method will soon enable one to determine the quantity of sulphurous acid conducive to smoothest operation. After the several chloroform-ether portions have been successively applied and united with the first, and the mixture finally evaporated to a few cubic centimeters, continue with a very gentle air blast but without the application of heat until the cinchophen separates as a crystallin mass, then heat at 100° in an air-bath to constant weight. Cinchophen thus isolated should be quite or nearly colorless.[1]

METHOD II.

Cinchophen.—In the absence of caffeine, the following volumetric procedure may be used. Evaporate the solution of aspirin and cinchophen in aqueous sodium carbonate to dryness on the steam-bath and with air blast, then transfer the residual salts by solution in a minimum of glacial acetic acid to a 100-cc. volumetric flask, so that the resulting liquid does not greatly exceed 15 cc. Heat nearly to boiling and run in with constant rotation of the flask, from a burette or pipette, 25 cc. of a 0.1 *N* iodine solution. Stopper and allow to cool to room temperature, agitating the mixture from time to time. Fill to mark with water, mix thoroughly by repeated inversion and set aside for one-half hour. Withdraw a 50-cc. aliquot of

[1] Palkin has recently shown (JOUR. A. PH. A., 16, 634 (1927)) that cinchophen can be extracted from aqueous acid media quantitatively by immiscible solvents. Another method described by the same author depends upon the extraction of cinchophen as a bromine-addition-product by means of ether. Both methods were inapplicable to a solution of the problem in hand.

clear liquid, obtained by passing through a small (5.5-cm.), dry, closely-fitted filter into a graduated 50-cc. flask, rejecting, however, about 15 cc. of the first runnings. Transfer the aliquot by pouring and washing to a 200-cc. Erlenmeyer and titrate with 0.1 *N* sodium thiosulphate solution.

RESULTS BY METHOD I.

Series A.

Amounts taken.			Amounts found.		
Caffeine.	Aspirin.	Cinchophen.	Caffeine.	Aspirin.	Cinchophen.
....	0.1500	0.1500 grams	....	0.1509	0.1498 grams
....	0.1500	0.1000 grams	....	0.1511	0.1005 grams
....	0.1500	0.1300 grams	....	0.1503	0.1299 grams
....	0.1500	0.1500 grams	....	0.1493	0.1496 grams
....	0.1500	0.1300 grams	....	0.1495	0.1296 grams
....	0.1500	0.1500 grams	....	0.1499	0.1506 grams
0.0081	0.1620	0.1620 grams	0.0080	0.1628	0.1618 grams
0.0081	0.1296	0.1620 grams	0.0082	0.1287	0.1615 grams
0.0081	0.1620	0.1296 grams	0.0080	0.1625	0.1291 grams
		Series B.			
1/8	2.5	2.5 grains	1/8	2.35	2.30 grains
1/8	2.5	2.5 grains	1/8	2.45	2.43 grains
...	...	7.5 grains	...	...	7.41 grains
...	...	7.5 grains	...	...	7.46 grains
...	2.5?	2.5? grains	...	2.48	2.31 grains
...	2.5?	2.5? grains	...	2.31	2.37 grains

RESULTS BY METHOD II.

Series C.

...	...	0.0500 grams	...	...	0.0492 grams
...	...	0.1000 grams	...	...	0.1005 grams
...	...	0.1500 grams	...	...	0.1495 grams
...	...	0.2000 grams	...	...	0.2008 grams
		Series D.			
...	...	0.0500 grams	...	...	0.0495 grams
...	...	0.1000 grams	...	...	0.0996 grams
...	...	0.1500 grams	...	...	0.1504 grams
...	...	0.2000 grams	...	...	0.2002 grams
		Series E.			
...	...	0.0500 grams	...	...	0.0496 grams
...	...	0.1000 grams	...	...	0.0999 grams
...	...	0.1500 grams	...	...	0.1500 grams
...	...	0.2000 grams	...	...	0.2003 grams
...	0.1500	0.1500 grams	...	...	0.1507 grams
...	...	0.1296 grams	...	...	0.1298 grams
...	0.1620	0.1620 grams	...	...	0.1626 grams

Since the composition of the black insoluble iodine addition-product constituting the basis for the foregoing operation was found on analysis to be $(C_{16}H_{11}NO_2)_2.HI.I_3$, it will be noted that for every molecule of cinchophen the equivalent of 1.5 atoms of iodine is required, hence from a titrimetric standpoint one atom of iodine is equivalent to a two-thirds molecule of cinchophen. If, therefore, the quantity of iodine expended in the formation of insoluble periodide is ascertained as the result of such titration, the quantity of cinchophen is readily calculated from the expression:

$$\text{cinchophen} = I(0.0016609 \times N),$$

in which 0.0016609 represents the quantity of cinchophen in 1 cc. of a 0.1 *N* solution of this substance; N, the normality of standard sodium thiosulfate employed; and I, the number of cubic centimeters of such thiosulphate corresponding to the iodine entering into combination with cinchophen isolated as periodide.

The above procedure was developed essentially for application to mixtures of aspirin and cinchophen, but there is every reason to believe it might serve as a works' method in the evaluation of cinchophen tablets, the powdered sample could be directly extracted with hot acetic acid, and the solution filtered into the volumetric flask for treatment with iodine, thus eliminating the preliminary extraction with aqueous sodium carbonate.

The samples examined under Series B represent various commercial tablets, and their therapeutic content in grains, indicated under amounts taken, is that claimed by the manufacturer. All the other series involve controls. In Series C the iodine was added slowly to the acetic acid solution of cinchophen, whereas in Series D the cinchophen solution was added rapidly to the iodine. In Series E the order of treatment was like C, except that the addition of iodine was rapid. So far as final results are concerned it would appear immaterial whether the addition of iodine to cinchophen is slow or rapid.

SUMMARY.

Two methods are described, the one, gravimetric for the evaluation of aspirin and cinchophen, or of aspirin, cinchophen and caffeine in admixture, the other volumetric for the estimation of cinchophen with or without the presence of aspirin. By the first, caffeine is isolated by extraction with chloroform from a solution of the mixture in aqueous sodium carbonate. Separation of aspirin from cinchophen is effected by conversion of the former to the characteristic diiodophenylene oxide, $C_6H_2I_2O$, which is filtered off and weighed. The cinchophen is thereupon precipitated from the acidified filtrate as the periodide, $(C_{16}H_{11}NO_2)_2.HI.I_3$. The latter is extracted with an ether-chloroform mixture, then deiodized by aqueous sulphurous acid. The liberated cinchophen is recovered and weighed as such. By the second method, the cinchophen, either alone or admixed with aspirin, is converted to the above periodide in aqueous acetic acid, the resulting menstruum is diluted to a measured volume and the unexpended iodine determined in an aliquot by titration. The data thereby obtained serve as a basis for volumetric estimation.

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PROGRESS OF MEDICINE.

The *Washington Post* of January 8th devotes two pages of its "Magazine Section" to an interesting and informative article by Surgeon-General Hugh S. Cumming on "The Progress of Medicine." The Surgeon-General closes with the following comment:

"Great as have been the triumphs of modern preventive medicine, the problems remaining for solution are still greater. The hope of the future lies in the continued and increasing growth of scientific knowledge which can be applied to the protection against disease, and the promotion of the public health."