Assay for Free Salicylic Acid in Individual Capsules and Tablets of Aspirin or Aspirin Containing Medicinals

By ROBERT C. REED and WILLIAM W. DAVIS

A procedure is described by which individual capsules or tablets of aspirin containing medicinals can be conveniently and accurately assayed for salicylic acid. Each unit, including the capsule or any tablet coating, is extracted with alcohol and subsequently diluted with water. The salicylic acid absorbance at 300 mμ is measured employing an appropriate aspirin containing reference solution. Results obtained with known amounts of added salicylic acid give satisfactory reproducibility; average discrepancy from known content of salicylic acid is 0.4 per cent. While the procedure is designed principally for following appearance of free salicylic acid, as aspirin containing medicinals age or deteriorate, it is applicable down to allowable limits in marketed aspirin and APC products.

A SEARCH of the literature for tests applicable to following the hydrolysis of aspirin showed numerous tests for salicylic acid, but none with the features of accuracy and convenience desired. Several colorimetric (1–3) and chromatographic (4–7) tests are available. The work of Edwards (8) and Tinker and McBay (9) describe ultraviolet tests in which it is clearly feasible to determine salicylic acid in the presence of aspirin by ultraviolet spectrophotometry.

The present paper describes a simple direct spectrophotometric determination of salicylic acid in either one capsule or one tablet of an aspirin containing medicinal, providing no interference of the other components with the salicylic acid absorption at 300 mμ is encountered. The ultraviolet spectra of salicylic acid, aspirin, and a marketed combination of aspirin, phenacetin, and caffeine with and without added salicylic acid indicated no interferences from the other components. Also, no interfering ultraviolet absorbers were extracted from the capsules in the alcohol–water solvent used in the assay. Only clear gelatin capsules were used. If colors employed in capsules or tablet coatings are soluble in alcohol, these should be checked for interference with the spectrophotometric determination at 300 mμ.

EXPERIMENTAL PROCEDURE

The contents of one capsule are emptied into a 20-ml. centrifuge tube, and both ends of the empty capsule are punctured with pin pricks and the parts dropped into the tube with the contents. If a tablet is being tested, one whole tablet is dropped into the centrifuge tube. Ten milliliters of alcohol is added to the tube with a delivery pipet. The tube is covered with parafilm and shaken vigorously for 1 min. The extraction should continue for 1 hr., shaking the tube at regular intervals. Alternatively, of course, a reciprocal or rotary shaker could be used. A fresh tablet or capsule of the aspirin containing medicinal is run as a blank. If none are available for which complete confidence exists, an equivalent weight of fresh aspirin can be weighed and used. After the 1-hr. extraction, the tube is centrifuged for 5 min. to produce a clear supernatant with the powder and capsule on the bottom. A 1-ml. aliquot of supernatant is put into a 100-ml. volumetric flask and made up to 100 ml. with distilled water. If the absorbance is above 0.5, a further dilution of the solution is made both in sample and blank solutions. Likewise, if absorbance is below 0.1, then lower dilution should be used, such as 1 ml. to 25 ml.

The sample is read against the blank prepared in the same way employing an ultraviolet spectrophotometer at 300 mμ. Care is taken with the wavelength setting since at this wavelength the absorbance of salicylic acid is wavelength dependent. From the observed absorbance, the concentration of salicylic acid is read off of standard salicylic acid concentration *versus* absorbance curves. Appropriate dilution factors are applied. The result should be expressed as mg. of salicylic acid present per capsule or tablet.

It might be noted that in setting up the experiment, the appropriate dilutions were made to keep the absorbance readings between 0.10 and 0.50. Our readings were made on the Beckman model DB spectrophotometer. The standard curve of salicylic acid was run by the same procedure, except the 1-hr. extraction was eliminated. The values represented on the calibration curve for salicylic acid are from separately weighed samples. The salicylic acid used was U.S.P. grade, and the aspirin used meets the U.S.P. standards on salicylic acid content (less than 0.1%).

RESULTS

The absorbance–concentration curve for salicylic acid shows conformity to Beer's law as do the curves for added salicylic acid in the presence of aspirin or the aspirin containing medicinal. Table I presents the data for absorbance at 300 mμ of varying amounts of salicylic acid added to aspirin compared with the data presented in the standard curve. The absorbance values in the table are those which result from dilution of the specified weight of salicylic acid by the standard procedure, *i.e.*, mg./1000 ml.

Whether 3.5 or 5 gr. of aspirin is added, the results are indistinguishable from results in the absence of aspirin. Table I also presents the data for absorbance of salicylic acid in the presence of the aspirin containing medicinal (3.5 gr. aspirin, 2.5 gr. acetophenetidin, and 0.5 gr. caffeine plus filler) in capsules. Table I also presents the data for absorbance of various amounts of added salicylic acid in the presence of tablets of the same aspirin containing medicinal.

The accuracy of the assay may be stated in terms of the fractional difference between the figures obtained for weighed amounts of salicylic acid added to various aspirin containing solutions and the values

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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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