Fluorometric Determination of Salicylic Acid in Aspirin Products Including Noninterfering, Interfering, and Buffered Substances

NORMAN SHANE and DENNIS MIELE

Abstract □ A rapid, fluorometric method for the determination of salicylic acid in aspirin products in combination with noninterfering fluorescent substances, such as phenyltoloxamine citrate, glyceryl guaiacolate, and caffeine, interfering fluorescent materials, such as hydrogenated castor oil (Castorwax) and salicylamide, and buffering agents, such as aluminum glycinate and magnesium carbonate, gave accurate and precise results. The procedure is based on a simple dissolution of the crushed tablet in a pH 4.0 buffer solution and reading of the filtered solution at activation and fluorescent wavelengths of 310 and 410 mμ, respectively. An additional operation of a chloroform and buffer solution extraction is necessary when interfering substances are present. In the case of salicylamide, a constant background reading has to be subtracted; however, an accuracy of 95.5% was established. For nonaspirin salicylates in buffered-aspirin products, the procedure gave results similar to those reported by Guttman and Salomon.

Keyphrases □ Aspirin dosage forms—salicylic acid determination □ Salicylic acid determination—aspirin dosage form, buffered, unbuffered □ Interfering substances—salicylic acid determination □ Fluorometry—analysis

There are many methods in literature for the determination of small amounts of salicylic acid in aspirin and aspirin products. These involve colorimetry (1–3), chromatography (4–7), spectrophotometry (5–10), and titrimetry (11). Fluorometric determinations (12–14) have been described for the determination of salicylate in biological tissues by measurement of the characteristic fluorescence of the salicylate ion on exposure to UV light.

The present paper describes a simple, fast, fluorometric determination of salicylic acid in aspirin products. With no interference of other fluorescent compounds, separations are not necessary. The ingredients can be dispersed in a pH 4 buffer solution, filtered, and the salicylic acid content determined fluorometrically. With interfering substances that have solubility differences in organic systems, a simple extraction can be performed.

For buffered-aspirin tablets, a variety of methods (15–18) is described for the determination of total nonaspirin salicylates. By treating the sample with HCl–citric acid solution and extracting with chloroform and then with pH 4 buffer solution, the salicylate content can be determined fluorometrically. Results similar to the Guttman and Salomon method (18) were obtainable.

EXPERIMENTAL

Apparatus and Reagents—*Fluorometer*—A G. K. Turner Associates model 2 fluorometer with a 7-60 primary filter (band pass 310 mμ) and a No. 2A secondary filter (band pass 410 mμ) was used to obtain the fluorescent readings. A Beckman research pH meter was used to measure the pH of the buffer solutions.

A Beckman DK2 recording spectrophotometer was used to obtain spectra of the chloroform solution. The results for the buffered-aspirin tablets were obtained on the Aminco-Bowman spectrofluorometer (4-8100). The activation and emission wavelengths were set at 310 and 410 mμ, respectively.

Materials—Spectral grade, analytical reagent chloroform (Merck & Co.) was used. All analgesic compounds, *i.e.*, acetylsalicylic acid, USP grade; salicylamide, NF grade; acetaminophen, NF grade; phenyltoloxamine citrate, USP grade; glyceryl guaiacolate, USP grade; salicylic acid, USP grade, were checked by IR spectrophotometry. Castor oil[1] was also used. The filter units employed in the experiment were Whatman folded filter paper No. 2V and the Millipore Swinnex-13 using celotate (cellulose acetate) No. ECPO-1300 filter.

Solutions—(*a*) pH 4 buffer; prepared by combining a ratio of 7.71 ml. of 0.2 *M* disodium hydrogen phosphate and 12.29 ml. of 0.1 *M* citric acid. (*b*) Seventeen milliliters of concentrated hydrochloric acid and 1000 ml. of 0.1 *M* citric acid.

Tablets—Powdered mixtures of the following composition were analyzed: (*a*) 325 mg. of aspirin; (*b*) 325 mg. of aspirin and 146 mg. of castor oil; (*c*) 195 mg. of aspirin, 130 mg. of salicylamide, 98 mg. of acetaminophen, and 65 mg. of caffeine; and (*d*) 325 mg. of aspirin, 97 mg. of magnesium carbonate, and 49 mg. of aluminum glycinate.

PROCEDURE

Absence of Interfering Substances—*Calibration*—To five 50-ml. volumetric flasks, 0.1, 0.2, 0.3, 0.5, and 1.0 mg. of salicylic acid were added. Buffer solution, pH 4, was added to each flask, which was then shaken for 30 sec. and filtered. Five milliliters of the filtered solution was added to a cell and placed in the fluorometer which was initially set to zero with the buffer solution. When a steady state was achieved (approximately 1 min.), readings were taken.

Method—A tablet was crushed and transferred to a 50-ml. volumetric flask. The contents were brought to volume with pH 4 buffer solution, shaken vigorously for 30 sec., and then filtered. Proceed as described in the previous calibration beginning: "Five milliliters of the filtered solution was added. . . ."

Presence of Interfering Substances—*Calibration for Tablets Coated with Castor Oil*—To five separators, 0.1, 0.2, 0.3, 0.5, and 1.0 mg. of salicylic acid were added. To each separator, 25 ml. of chloroform was added and shaken for 30 sec. The chloroform solution was extracted with three 15-ml. portions of buffer solution; the buffer extracts were transferred to a 50-ml. volumetric flask and adjusted to volume. Five milliliters of the solution was added to the cell and read in the fluorometer that had been previously standardized with buffer solution. Readings were taken at equilibrium.

A tablet was crushed and transferred to a 125-ml. separator. Proceed as described in the previous calibration beginning: "To each separator, 25 ml. of chloroform was added and shaken for 30 sec. . . ."

For tablets containing aspirin, acetaminophen, salicylamide, and caffeine, a tablet was crushed and put into a 50-ml. volumetric flask which was brought to volume with chloroform. The solution was shaken vigorously for 1 min. and transferred with as much residue material as possible to the separator, allowing a few seconds for the complete drainage of the flask. Fifty milliliters of buffer solution was then added, and the separator was shaken vigorously for 30 sec. The lower chloroform layer was completely drained, including as much filmy interface as possible. (The clarity of the division of the two layers is enhanced by gently swirling the flasks as the layers separate. This enables the trapped air bubbles in the chloroform layer to escape to the surface. If these bubbles do not disappear,

[1] Castorwax, The Baker Castor Oil Co., Bayonne, N. J.

Table I—Recovery Efficiency of Salicylic Acid from Recrystallized Aspirin[a]

Recrystd. Aspirin, mg.	Salicylic Acid Added, mg.	Total Wt. Salicylic Acid Found, mg.	Corrected Wt. Salicylic Acid Found, mg.	Recovery, %
305.8	None	0.097 (0.032%)	—	—
315.0	None	0.097 (0.031%)	—	—
325.1	None	0.109 (0.034%)	—	—
335.5	None	0.113 (0.034%)	—	—
345.3	None	0.117 (0.034%)	—	—
356.1	None	0.120 (0.034%)	—	—
300.2	0.127	0.225	0.126	99.2
325.3	0.127	0.238	0.131	103.2
345.5	0.254	0.371	0.257	101.2
301.1	0.254	0.351	0.252	99.2
324.4	0.508	0.617	0.510	100.4
345.0	0.508	0.625	0.511	100.6
355.9	1.016	1.135	1.018	100.2
325.1	1.016	1.126	1.019	100.3

[a] The average of salicylic acid found in recrystallized aspirin was 0.033%; this amount was used to calculate the weight found in the composition composed of both aspirin and added salicylic acid in Tables I and II.

they should be drained with the chloroform.) The buffer solution was transferred to a 100-ml. volumetric flask through the top of the separator. The separator was rinsed with an additional 10 ml. of buffer, which was then transferred to the previous flask that was brought to volume with additional buffer solution. The solution was filtered using the Millipore filter. Five milliliters of the filtered solution is read on the fluorometer that has been standardized with buffer solution. Two samples should be read, and if the readings differ by more than one unit, a third sample is required. Readings should be taken after allowing the instrument to equilibrate with the sample inside for 1 min.

For buffered-aspirin tablets containing aspirin, magnesium carbonate, and aluminum glycinate, a tablet was crushed and placed in a 125-ml. separator. HCl–citric acid solution, 25 ml., was added. After the effervescence ceased, two 25-ml. chloroform extractions of the mixture were made and saved. The combined chloroform extracts were then extracted with two 25-ml. portions of buffer. The buffer extracts were placed in a 100-ml. volumetric flask and brought to volume. Some of this aqueous solution was filtered, and a 5-ml. aliquot was added to the cell and read on the Aminco spectrofluorometer. Readings were taken after the instrument was equilibrated with the sample inside for 1 min.

RESULTS AND DISCUSSION

Studies of the fluorescent properties of salicylic acid as a function of pH show that the fluorescent spectra arise from different ionic forms (19). The stable spectrum of the acid arises from the singly ionized form. Figure 1 shows the plots of pH *versus* fluorescence

Table II—Recovery of Salicylic Acid from Recrystallized Aspirin and Castor Oil

Recrystd. Aspirin, mg.	Castor Oil, mg.	Salicylic Acid Added, mg.	Total Wt. Salicylic Acid Found, mg.	Corrected Wt. Salicylic Acid Found, mg.	Recovery, %
300.5	126.5	0.101	0.200	0.101	100.0
316.0	136.1	0.101	0.203	0.099	98.0
324.5	176.3	0.202	0.312	0.205	101.5
335.4	155.8	0.202	0.315	0.204	101.0
345.7	166.1	0.404	0.523	0.409	101.2
356.1	177.0	0.404	0.522	0.405	100.3
301.1	177.1	0.808	0.903	0.804	99.5
325.2	125.4	0.808	0.927	0.820	101.5

Table III—Recovery of Salicylic Acid in Powdered Mixtures of Aspirin, Salicylamide, Acetaminophen, and Caffeine

Addition Salicylic Acid to Powdered Mixtures, mg.	Fluorometric Reading Adjusted for Background	Reading/mg. Salicylic Acid
1.98	21.5	10.86
2.58	25.4	9.84
3.45	34.5	10.00
4.00	39.1	9.75
4.16	40.2	9.66
4.84	48.3	9.98
5.07	49.2	9.68
5.58	54.3	9.73

(19). At pH 4, the monosalicylate ion levels off in fluorescent intensity. The detection limit of the salicylic acid at pH 4 is around 0.1 p.p.m.

Aspirin shows a rate constant of about 0.1 day^{-1} at 17° at pH 4 (8). At room temperature during the time period of the above experiments, aspirin showed a negligible decomposition.

The determination of salicylic acid in aspirin products in these experiments is based on the fact that the aspirin compound does not fluoresce while salicylic acid does at pH 4. At this pH, aspirin decomposition is negligible. For the experiment on noninterfering substances, aspirin, having starch as the excipient, gave results as shown in Table I.

In the analysis of aspirin tablets coated with castor oil, the tablets were dissolved in chloroform and extracted with pH 4 buffer solution. The salicylic acid forms the monosodium salts with the buffer solution and remains in the aqueous layer. Results are shown in Table II.

For the determination of salicylic acid in tablets containing aspirin, salicylamide, acetaminophen, and caffeine, a fluorometric procedure was developed in which the ingredients were dissolved in chloroform, and aspirin and salicylic acid were extracted into a buffer solution. The interference of the major ingredient is limited by the chloroform extraction and the use of the buffer. Salicylic

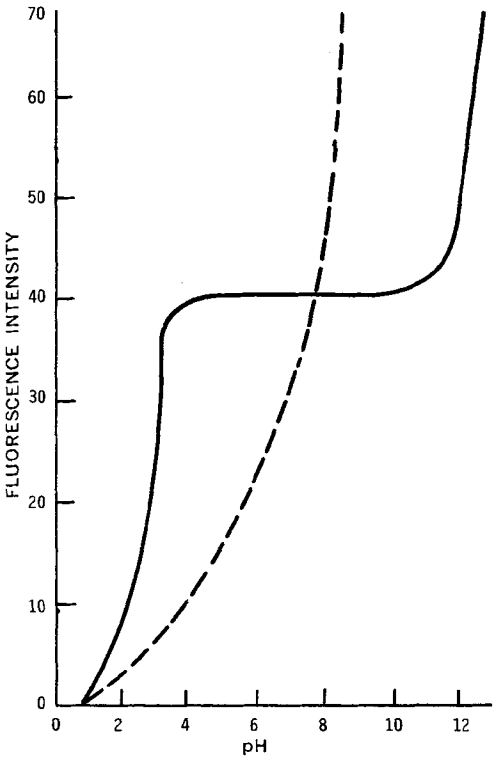

Figure 1—*Plot of pH* versus *fluorescence for salicylic acid and salicylamide. Key:—, salicylic acid; and - - -, salicylamide.*

acid can be determined with an accuracy of ±0.15 mg. at levels of 1 mg. The values may be simply calculated from a suitable calibration curve.

The fluorometer is especially useful in the determination of very small amounts of salicylic acid in the presence of large amounts of the ingredients of the tablet because its fluorescence per unit weight is much larger than that of the active ingredients. If the amount of salicylic acid in the tablet is as small as 2 mg., it would represent only 1.6% of the respective standard amount of salicylamide, the only ingredient which exhibits fluorescence at this pH. Figure 1 shows the effect of pH *versus* fluorescence of a solution containing 1 mg. of salicylic acid and 10 mg. of salicylamide. The optimum condition for determining salicylic acid in the presence of salicylamide was at pH 4. The actual amount of the fluorescence of salicylamide is limited by chloroform extraction and buffer operation. The reading of the salicylamide is depressed because 90% of it has been removed. The ratio of anionic to undissociated species is 10:1 in both aspirin and salicylic acid and only $1:10^4$ in salicylamide, and it is the anionic species which fluoresces to the largest extent.

The background reading of the tablet has been carefully determined by using a large number of runs. Tablet mixtures were simulated by weighing the standard amounts of the four components. The quantity of salicylic acid in each of the two components (aspirin and salicylamide, which gave readings) were carefully determined. Neither acetaminophen nor caffeine gave fluorescence under the conditions of this analysis. The salicylic acid content of aspirin was determined by using an iron (III) complexing technique (20)—0.128 mg./195 mg. of aspirin. Using a titration in nonaqueous medium with methanolic potassium hydroxide, it was determined that there was a negligible amount of salicylic acid in salicylamide, and the reading of salicylamide was a result of salicylamide fluorescence. The observed background of the composed tablet is 32.5. The salicylic acid content of the composed tablet gave a reading of 1.2; therefore, the adjusted background is 31.3 (four components with no salicylic acid).

The observed readings of fresh commercial tablets varied from 32.5 to 35.8. Individual batches were consistent among themselves, alternating between 0.13 and 0.45 mg. of salicylic acid in the tablets.

The data, summarized in Table III, were acquired through the addition of salicylic acid to a weighed portion, equivalent to one tablet, of a powdered mixture made from 10 tablets. The tablet background was obtained from the measurement of a given amount of the mixture and subtracted from the observed reading to determine the increase resulting from the addition of salicylic acid. The data are plotted in Fig. 2.

The best straight line through the data points is obtained by using a least-squares method (21). The calculated straight line is where $y = 9.63x + 0.85$, y = adjusted fluorescence reading, and x = milligrams of salicylic acid added.

Adjusting the background and initial salicylic acid content, the equation can be solved to find the total amount of salicylic acid per tablet to give

$$x = y_1 - \frac{32.1}{9.63}$$

where y_1 is the observed fluorometer reading.

The average deviation of calculated values from the actual values is 0.06 mg., representing an average relative error of 2.00% with a standard deviation of 0.074; one can be 95.5% confident of being accurate to within 0.15 mg. and 99.7% confident of being accurate within 0.22 mg.

However, the deviations in the observed data are not the only errors involved. An error of ±0.05 mg. is introduced by uncertainties in the fluorometer readings (readings are good only to ±0.5 division). The major error is a result of the tolerance limits of the ingredients. Limits of ±10% significantly alter the readings of the background. This introduces an error of ±0.3 mg. in the final determination of salicylic acid. However, as there are extremes, salicylamide and aspirin must both be either high or low by 10% to obtain such a large error; this large deviation is unlikely. This large error can only be eliminated by: (*a*) determining the salicylamide and aspirin contents of the tablets, and (*b*) adjusting the background accordingly.

By employing a high source intensity xenon lamp and a grating instrument rather than the one incorporating band filters and a mercury lamp source (as used in these experiments), a more monochromatic system for maintaining an exact activation and fluorescent spectrum would permit a lesser background effect and a higher intensified signal at the appropriate selected wavelengths.

Table IV—Results of Salicylic Acid Determination by the Method of Guttman and Salomon and by Fluorescence[a]

Batch	Salicylic Acid Found	$\bar{x}$[b]	SD[c]
	Guttman and Salomon Method, mg.		
A	0.6, 0.6, 0.7, 0.6, 0.95	0.690	0.1516
B	1.9, 1.6, 2.1, 1.8, 1.7	1.820	0.1924
C	4.2, 3.7, 4.3, 3.7, 3.8	3.940	0.2881
	Fluorescence, mg.		
A	0.7, 0.6, 0.8, 0.7, 0.6	0.680	0.0837
B	2.0, 1.8, 1.7, 1.9, 1.8	0.680	0.0837
C	4.3, 4.0, 3.7, 4.0, 3.7	3.940	0.0630

[a] Results obtained on the analysis of salicylic acid content on buffered aspirin composed of 325 mg. of aspirin, 97 mg. of magnesium carbonate, and 49 mg. of aluminum glycinate. The different batches contained buffered aspirin at various environmental conditions. [b] $\bar{x}$ = means of results. [c] SD = standard deviation.

Table IV shows the determination of salicylic acid in buffered-aspirin tablets by fluorescence and the Guttman and Salomon

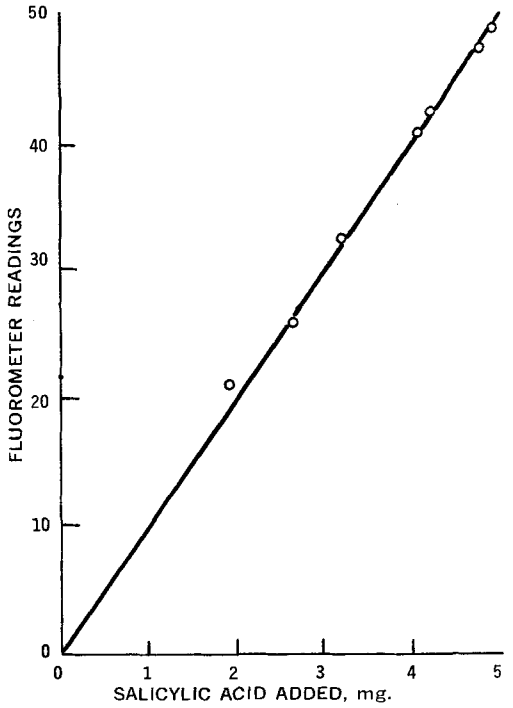

Figure 2—*Calibration curve for salicylic acid and powdered mixture containing aspirin, acetaminophen, salicylamide, and caffeine. Key:* O—O, *least-squares straight line.*

method. As shown in the table, there is no statistical difference between the methods with respect to the means and variances.

After the HCl–citric acid solution is added to the crushed tablet, a pH of 0.9–1 is observed. Slight effervescence occurs but subsides in less than 1–2 min. The extraction of the undissociated salicylic acid and aspirin by chloroform is then accomplished. The aspirin and salicylic acid are extracted from the chloroform solution by the buffer solution. The whole experiment takes about 10 min.

To ensure complete extraction of salicylic acid and aspirin into the chloroform solution, some of the aqueous phase was treated with 1 *N* NaOH and tested for the absence of salicylate fluorescence. Also, to be assured of complete extraction of salicylic acid from the chloroformic solution, some of the organic phase was run on the spectrophotometer to observe the absence of salicylic acid.

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Gas-Liquid Chromatography of *d*-Biotin

V. VISWANATHAN, F. P. MAHN, V. S. VENTURELLA, and B. Z. SENKOWSKI

Abstract □ A rapid and specific gas chromatographic method has been developed for the detection and determination of *d*-biotin. This technique has been found applicable to agricultural premixes and pharmaceutical injectable preparations. After suitable preliminary extraction, the biotin silyl ester is prepared using bis-(trimethylsilyl)acetamide (BSA) reagent. The silyl ester of *d*-biotin exhibited good peak symmetry and a linear response when utilizing a hydrogen flame ionization detector with a 2% OV-17 column operated at a temperature of 190°. The conditions for the assay of *d*-biotin in several preparations are described employing *n*-octacosane as the internal standard. The standard deviation of the developed procedure under the conditions studied was ±2.7%.

Keyphrases □ *d*-Biotin—determination □ Parenterals, agricultural premixes—*d*-biotin analysis □ *n*-Octacosane—internal standard □ GLC—analysis

d-Biotin is widely distributed in animals and plants and was first isolated from egg yolk (1). It is required in comparatively small amounts for the growth of bacteria, plants, and animals and appears to be related to the process of cell development. In 1940, György *et al.* (2) published their work on the identity of biotin. In the following 2 years, du Vigneaud *et al.* (3, 4) established the empirical and structural formulas. In 1943, Harris *et al.* (5) synthesized *d*-biotin (the naturally occurring form) and in 1949, Goldberg and Sternbach (6) patented their findings of a more economical synthesis.

While several microbiological techniques are currently employed for the determination of *d*-biotin, a comprehensive survey of the literature indicated that GLC had heretofore not been employed for quantitative analysis. A considerable number of satisfactory GLC procedures have been reported for various water-soluble vitamins. These procedures involve the preparation of the appropriate trimethylsilyl derivatives (ester or ether) prior to GLC analysis.

Carboxylic organic compounds, such as biotin, are not sufficiently volatile for direct analysis employing GLC. However, the chemical structure of *d*-biotin indicated the possibility of substituting the active hydrogen of the carboxyl group with a silyl group, thereby making the gas chromatographic analysis possible. Horning *et al.* (7) mentioned the qualitative response of *d*-biotin silyl ester in their study of urinary acids and related compounds.

This paper describes the quantitative GLC determination of *d*-biotin after the conversion to its silyl ester with bis-(trimethylsilyl)acetamide (BSA). The derivative is easily formed and serves very well for quantitative analysis. The linearity of detector response with concentration injected, reproducibility, recovery data, and procedures for handling several types of dosage forms are described.