Fluorometric Determination of Salicylic Acid in Buffered Aspirin Products

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Abstract □ A rapid fluorometric method for the determination of total salicylic acid in buffered aspirin products has been developed and compared with existing methods. The new procedure is essentially a one-step operation; the complete analysis, exclusive of calibration, requires less than 10 min. The results obtained are in close agreement with those found using two alternative methods. All tedious operations (*e.g.*, extractions and liquid column chromatographic separations) are eliminated.

Keyphrases □ Salicylic acid in buffered aspirin products—determination □ Formic acid–chloroform solution—salicylic acid fluorescence □ Aspirin hydrolysis—salicylic acid assay effect □ Quinine sulfate—standard fluorescent species □ Fluorometry—analysis

Salicylic acid (SA) can be determined in the presence of acetylsalicylic acid (ASA) by measuring the fluorescence intensity of a chloroformic solution (1). In the application of this technique to commercial buffered aspirin products, the SA must be freed from the buffering components on which it may be physically adsorbed, and the chloroform-insoluble salicylate salts formed by free SA in the presence of buffer materials must be solubilized. Both of these objectives must be accomplished without appreciable hydrolysis of the ASA.

In a recent paper, Guttman and Salomon (2) reported the desorption of SA and aspirin by triturating powdered tablet mass with citric acid monohydrate (3). The SA and aspirin were dissolved in chloroform, separated by liquid column chromatography, and determined by UV absorption spectroscopy. The chloroform-insoluble residue was treated with an aqueous solution of strong mineral acid and extracted with chloroform. The chloroform solution was purified chromatographically and analyzed by UV spectroscopy. Thus, free SA and salicylate salts were determined separately.

A procedure for total nonaspirin salicylates was described by Levine and Weber (4). This method requires an initial treatment with 98–100% formic acid, which displaces SA from its salts, followed by the chromatographic separation and analysis.

The time-consuming chromatographic operation is circumvented in the fluorometric procedure of Shane and Miele (5). Release of SA and its salts was accomplished by treatment with a dilute HCl–citric acid solution, which caused minimal hydrolysis during the period specified for contact. The aqueous solution was then extracted into chloroform and reextracted with pH 4 buffer. Under these conditions, the aspirin hydrolysis was slow, and the SA concentration could be determined as a function of fluorescence intensity.

The present study was undertaken to determine the feasibility of combining the formic acid procedure with fluorescence assay, thus eliminating the chromatographic step and all extraction procedures. This has been accomplished by measuring the fluorescence intensity of the formic acid–chloroform solution directly. Since 98–100% formic acid is miscible with chloroform, no extractions are required. The direct fluorometric examination of the chloroform–acid solutions is permissible, because: (*a*) the reagent blank has no interfering fluorescence under the conditions specified in the procedure, and (*b*) the fluorescence emission spectra of synthetic mixtures of aspirin and SA in chloroform containing formic acid are essentially identical to those obtained in chloroform alone.

The validity of the formic acid procedure with respect to hydrolysis of aspirin was examined by Levine and Weber (4). If, after brief exposure of the powdered tablet mass to a small volume of formic acid, the sample was immediately diluted with chloroform, the hydrolytic action of the acid was sharply diminished (approximately 0.03%/hr.). Thus, hydrolysis was negligible in the 5 min. specified for dissolving the SA and the aspirin. During the period the sample was in contact with the formic acid (45 sec.), the total extent of hydrolysis was found to be on the order of 0.01% (4). These results were confirmed in this laboratory, and the extent of hydrolysis in chloroform was found to be appreciably less than reported.

As a check on the completeness of solution, the residual tablet mass was examined for the presence of SA and aspirin. The total residual salicylate was less than 0.03%, which is in close agreement with the published results of Levine and Weber (4).

EXPERIMENTAL

Instrument Calibration—An Aminco-Bowman spectrophotofluorometer (SPF), model 4-8202, with a solid-state photomultiplier was employed in these studies. The advantages of instruments of this type compared with the more common filter fluorometers may be summarized briefly.

1. The use of a continuous xenon arc source and activating monochromator provides high-intensity activating energy at the exact wavelength of maximum absorption, in contrast to the few wavelengths corresponding to the lines of the mercury spectrum available in conventional fluorometers.
2. The detection of fluorescence is not limited to the visible region, but is extended into the UV.
3. Specificity and elimination of interference from the fluorescence of impurities are enhanced by the greater resolving power or sharper bandwidths of monochromators as compared to filters.
4. The ability to scan the full fluorescence spectrum provides a convenient means of detecting the presence of extraneous materials which may cause errors due to light scattering, blank fluorescence, or absorption of either activating or fluorescent energy (quenching).

To utilize effectively the spectrophotofluorometer, careful attention to several factors that can vary the instrument sensitivity (*e.g.*, changes in ambient temperature, photomultiplier instability, and deterioration of the xenon arc source) is required. These factors are accounted for by standardizing the instrument sensitivity from day-to-day (and morning-to-afternoon) by means of

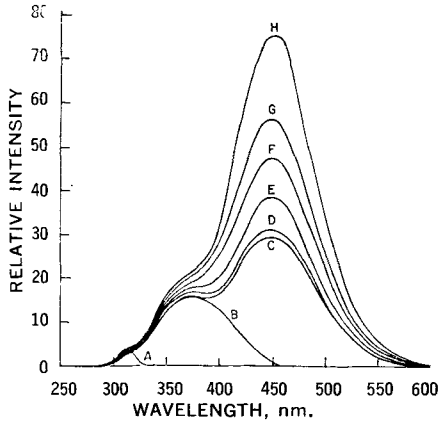

Figure 1—*Fluorescence emission spectra of: (A) formic acid–chloroform solvent; (B) pure ASA; (C) buffered aspirin tablets with no added SA; (D) buffered aspirin tablets with 0.1 mg. SA added; (E) buffered aspirin tablets with 0.5 mg. SA added; (F) buffered aspirin tablets with 1.0 mg. SA added; (G) buffered aspirin tablets with 1.5 mg. SA added; and (H) buffered aspirin tablets with 2.5 mg. SA added.*

a standard solution of a stable fluorescent species. In these experiments, quinine sulfate, 1.0 mcg./ml., in 0.1 *N* H_2SO_4 was used. This solution is stable for at least 1 month if stored in the dark to avoid photodecomposition. Quinine sulfate is intensely fluorescent, and its maximum emission at 450 nm. coincides with that of SA in chloroform. (This is desirable since the responses of light sources and detectors are not uniform over the entire spectral range.)

Reagents—The following were used: chloroform (Merck spectrophotometric grade); formic acid, 98–100% (Matheson Coleman & Bell); and salicylic acid USP standard.

Tablets—Most of the data reported here pertain to commercial buffered aspirin tablets which had been stored in plastic containers for 1 year prior to analysis. For comparison purposes, a synthetic mixture to simulate the tablets was formulated as follows: 325 mg. ASA (Monsanto, 80 mesh), 97 mg. magnesium carbonate, and 49 mg. aluminum glycinate.

ANALYTICAL PROCEDURE

Calibration—In the initial calibration experiments with production tablets, 20 tablets from a given batch were finely powdered and an amount equivalent to 325 mg. aspirin was weighed into each of seven 100-ml. volumetric flasks. A 5-ml. aliquot of 98–100% formic acid was added to the first flask, and the contents were swirled for 45 sec. Afterwards, 70 ml. chloroform was added, and the flask was placed on a mechanical shaker. This process was repeated for the remaining samples, and the assembly was shaken until the final flask had been on the shaker for 5 min. During this period, known increments of SA were added in 5-ml. aliquots from appropriate standard solutions. The standards were as follows: 0 (no added SA; run in duplicate, *i.e.*, no SA was added to the first and last samples), 0.1, 0.5, 1, 1.5, and 2.5 mg. The stock solution was 50 mg. SA dissolved in 100 ml. chloroform (0.5 mg./ml.), 5 ml. of which delivered 2.5 mg. SA. A 30-ml. aliquot diluted to 50 ml. with chloroform (0.3 mg./ml.) served for the 1.5-mg. addition, 20 ml./50 ml. (0.2 mg./ml.) for the 1-mg. addition, 20 ml./100 ml. (0.1 mg./ml.) for the 0.5-mg. addition, and 2 ml./50 ml. (0.2 mg./ml.) for the 0.1-mg. addition.

After the volume was adjusted with chloroform, the contents of each flask were filtered through a loose plug of glass wool, and 1-ml. aliquots of the filtrates were diluted to 100 ml. with chloroform. The relative fluorescence intensities were obtained (excitation, 310 nm., emission, 450 nm.).

Routine Analysis—For single-tablet assays, a tablet was crushed in a double thickness of glassine weighing paper and transferred to a 100-ml. volumetric flask. The previously described procedure was followed, except that addition to standards was omitted.

Analysis of Residual Tablet Mass—The residue from the formic acid–chloroform procedure was retained on a fine sintered-glass funnel; the material remaining in the flask was rinsed onto the funnel with 1 *N* HCl, and the residue was washed with a total volume of

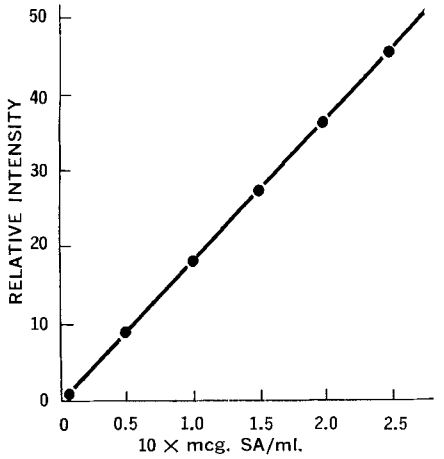

Figure 2—*Fluorescence of SA as a function of concentration in formic acid (5×10^{-5} %)–chloroform.*

50 ml. 1 *N* HCl. The filtrate was transferred to a 125-ml. separator and extracted three times with 25-ml. portions of chloroform. The chloroform extracts were drawn into a 100-ml. volumetric flask, brought to volume with chloroform, and examined for SA with the SPF at the same sensitivity used in the tablet analysis.

Determination of Extent of Hydrolysis—The increase of fluorescence intensity of the SA emission peak at 450 nm. provides a convenient means to monitor the hydrolysis of ASA. For a tablet treated with formic acid and chloroform, the initial intensity is noted and the increase with time observed. Typically, no increase in fluorescence can be detected until at least 2 hr. have elapsed.

RESULTS AND DISCUSSION

The fluorescence emission spectrum of a sample of powdered buffered aspirin tablets equivalent to 325 mg. ASA and treated with formic acid and chloroform is shown in Fig. 1, Curve C, together with the spectra obtained by adding known increments of SA to samples similarly treated (Curves D–H). The excitation wavelength, 310 nm., is evident as a small Raman scattering peak; the constant background due to ASA appears as a shoulder (maximum emission, 370 nm.) on the broad SA band (maximum emission, 450 nm.). That the fluorescence at 450 nm. of Curve C arises entirely from SA present in the tablet is confirmed by the spectrum of 325 mg. ASA (USP Standard) in formic acid–chloroform solution, Curve B, which has negligible fluorescence at 450 nm. (However, almost all samples of unpurified ASA do show a peak at 450 nm., indicating the presence of SA in varying amounts.)

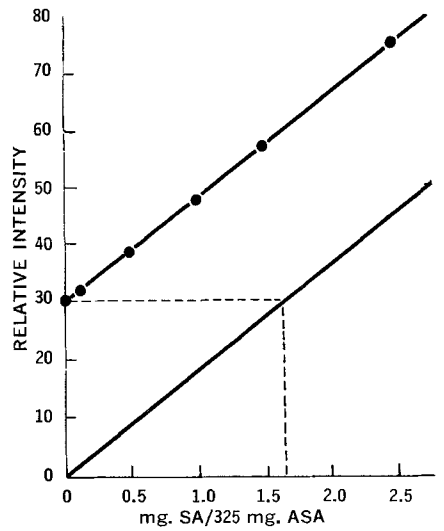

Figure 3—*Calibration curve for SA in buffered aspirin tablets.*

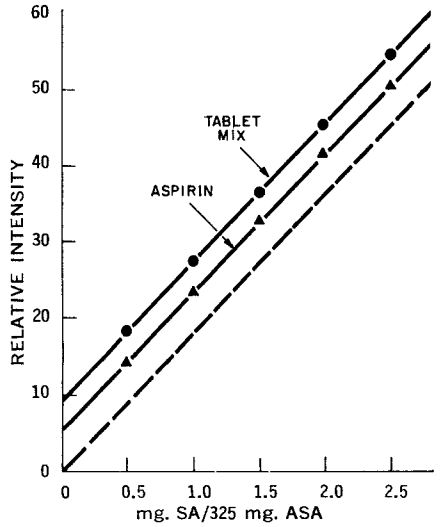

Figure 4—*Calibration curves for SA in ASA and synthetic tablet mixture.*

The linear proportionality of fluorescence to concentration for pure SA in formic acid–chloroform solutions at the dilutions used in the analysis (0.01–0.25 mcg./ml.) is shown in Fig. 2. Thus, a calibration curve can be obtained from the data in Fig. 1 (Curves C–H) by plotting fluorescence intensity at 450 nm. *versus* milligrams of added SA and extrapolating as shown in Fig. 3 to obtain the amount of SA corresponding to the initial reading (no added SA, Curve C, Fig. 1).

In a region of concentration where fluorescence is proportional to concentration, the measurement of fluorescence is made, essentially, in the absence of any significant absorption. This represents a limiting case of the Beer–Lambert law, where the energy available for excitation is uniformly distributed throughout the solution (6). At higher concentrations, an increasing proportion of energy is absorbed by that part of the solution nearest to the excitation monochromator. The resulting nonuniform distribution of fluorescence, in turn, leads to the failure of Beer's law. The effect, known as concentration quenching, is evidenced by marked deviations from linearity, especially as concentration is increased. Thus, for amounts of SA exceeding 3 mg./325 mg. aspirin, additional dilutions are required to obtain the linear range.

The results obtained with the synthetic tablet mix are shown in Fig. 4, together with the results for a concurrent run with pure aspirin (the same material used to prepare the mixture). The value for pure aspirin, expressed as mg. SA/325 mg. aspirin, is in accord with previous experience (7, 8) and incidentally demonstrates that the extent of aspirin hydrolysis resulting from the formic acid procedure is not significant. (In an actual analysis of pure aspirin or aspirin tablets not containing buffering materials, the initial treatment with formic acid is superfluous, because the aspirin can be dissolved directly in chloroform.) The slightly higher SA level in the tablet mix probably results from hydrolysis induced by atmospheric moisture during the period of preparation and grinding. This value for SA in the synthetic mix compared quite closely with that found for freshly prepared tablets of the same formulation, indicating that the tableting process does not contribute to aspirin decomposition in this preparation.

The formic acid procedure has been used to assay several additional aspirin products, both buffered and unbuffered. Satisfactory results are obtained with tablets containing noninterfering fluorescent substances, such as phenylephrine HCl, chlorpheniramine maleate, phenylpropanolamine HCl, phenyltoloxamine citrate, glyceryl guaiacolate, caffeine, and acetaminophen.

Table I—Results of SA Determination by Three Methods[a]

Run	SA Found, mg.					$\bar{x}$[b]	SD[c]
Guttman and Salomon (2)							
1	1.6	1.8	1.5	1.7	1.9	1.7	0.15
2	1.9	2.0	1.8	1.8	1.5	1.8	0.19
3	1.9	2.0	2.3	1.8	1.7	1.9	0.23
Shane and Miele (5)							
1	1.5	1.8	1.7	1.7	1.6	1.7	0.12
2	1.7	1.9	1.8	1.7	1.9	1.8	0.10
3	1.8	1.9	1.6	1.9	2.0	1.8	0.15
Formic Acid							
1	1.7	1.7	1.8	1.9	1.9	1.8	0.10
2	1.6	1.9	2.0	1.8	1.9	1.8	0.15
3	2.0	1.7	1.8	1.7	1.9	1.8	0.13

[a] Results obtained on the analysis of SA content on buffered aspirin composed of 325 mg. of aspirin, 97 mg. of magnesium carbonate, and 49 mg. of aluminum glycinate. The different runs were made on three successive days. [b] $\bar{x}$ = means of results. [c] SD = standard deviation.

To confirm the accuracy of the results obtained with the formic acid procedure, SA in buffered aspirin tablets was determined by two independent methods. The results are summarized in Table I for the Guttman and Salomon (2) and Shane and Miele (5) procedures as well as for the formic acid method. There is good agreement among results obtained with all three methods.

The desirability of employing the formic acid method on a routine or control basis is apparent from considerations of convenience, speed, accuracy, and repeatability. Once a calibration curve is established, a single tablet can be analyzed in less than 10 min., half of which is consumed by mechanical shaking; the analyst is spared the laborious extractions of existing procedures and can run many tablets concurrently. The suitability of the method is limited only by the requirement of an expensive monochromatic instrument (which does not lend itself to careless handling and which demands periodic calibration and maintenance) and the scrupulous attention that must be given to instrumental factors affecting sensitivity. A filter instrument for quantitative comparison of an unknown to a standard would probably be more appropriate for routine use. However, the band filters conventionally employed are not suitable, since in chloroformic solutions aspirin is weakly fluorescent and the broad emission band contributes an undesirable background fluorescence. The feasibility of having narrow band interference filters prepared to specification is under consideration.

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