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# Aspirin—A National Survey II: Determination of Salicylic Acid in Bulk Aspirin and Aspirin Formulations by High-Pressure Liquid Chromatography Using a Fluorescence Detector

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Received October 29, 1979, from the *National Center for Drug Analysis, Food and Drug Administration, St. Louis, MO 63101.* Accepted for publication December 14, 1979.

**Abstract** □ A quantitative high-pressure liquid chromatographic method, using a reversed-phase column coupled to a fluorescence detector, was developed to determine salicylic acid in bulk aspirin and plain and buffered aspirin tablets. The aspirin was dissolved, filtered, and injected into the chromatograph; the fluorescence of the salicylic acid was measured at ~425 nm. Excipients and impurities did not interfere, and recoveries of 100% were obtained. The method was used to analyze 84 aspirin samples.

**Keyphrases** □ Salicylic acid—high-pressure liquid chromatography, fluorescence detection and measurement □ Analgesics—determination of salicylic acid in aspirin by high-pressure liquid chromatography □ Aspirin—determination of salicylic acid by high-pressure liquid chromatography □ High-pressure liquid chromatography—determination of salicylic acid in aspirin

The nonaspirin salicylate test in USP XIX (1) is required for the assay of bulk aspirin and plain and buffered aspirin tablets. The official monograph test for salicylic acid in bulk aspirin is a tube comparison based on the color of the complex formed by ferric ion and salicylic acid and has only a pass–fail requirement at a limit of 0.1%. The plain tablet test, based on the method of Weber and Levine (2), uses a ferric chloride–urea trap of the salicylic acid on a diatomaceous earth[1] column with subsequent elution and measurement of salicylic acid at ~306 nm. The absorbance of the standard is only ~0.1 absorbance unit; samples with low levels of salicylic acid give very low absorbance readings, making accurate measurements difficult. The limit for salicylic acid in plain tablets is 0.3%. The buffered tablet assay, based on the method of Guttman and Salomon (3), uses the same column but can accommodate higher concentrations of salicylic acid. The limit in buffered tablets is 3.0%.

[1] Celite.

Salicylic acid and other impurities in aspirin products have been determined by high-pressure liquid chromatography (HPLC) (4, 5). As part of a national survey, the screening of many aspirin samples for salicylic acid was desired; therefore, a suitable HPLC procedure was needed. The method of Jansson and Andersson (5) was not suitable because of the *in situ* column preparation, which could cause reproducibility problems. The method of Ali (4) was chosen because of the commercial availability of the reversed-phase column and because the solvent system was compatible with aspirin solubility. However, preliminary experiments showed that salicylic acid was not easily determined at 254 nm at levels of 0.3% or lower in aspirin products.

Shane and Stillman (6) determined salicylic acid in the presence of aspirin by fluorescence in chloroform solution. The work of Shane and Miele (7) indicated that salicylic acid could be determined fluorometrically in the presence of aspirin using a pH 4 aqueous buffer solution.

An aqueous–alcoholic solvent of pH 3.4 is obtained when acetic acid is added to the HPLC mobile phase used by Ali (4). This solvent causes salicylic acid to fluoresce and is an excellent solvent for aspirin formulations.

The procedure described in this paper combines HPLC with a fluorescence detector and accurately measures the salicylic acid content of bulk, plain, and buffered aspirin formulations. The samples were prepared in methanol–water–acetic acid and injected into the chromatograph, and the salicylic acid was measured by the fluorescence detector. The results were compared with those obtained using the official USP XIX procedure and a semiautomated colorimetric procedure (8). The procedure was used successfully to analyze 34 bulk and 50 tablet aspirin formulations.



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Table I—Comparison of Results (Percent of Label Claim) Obtained by USP XIX and Fluorometric HPLC Methods for Salicylic Acid in Aspirin Tablets

| Tablet | USP[a] | Fluorometric HPLC[a] |
|---|---|---|
| 324 mg, plain | 0.073 | 0.088 |
| 324 mg, plain | 0.036 | 0.048 |
| 324 mg, buffered | 0.580 | 0.420 |
| 81 mg, pediatric | 0.230 | 0.492[b] |
| 81 mg, pediatric | 0.264 | 0.372[b] |

[a] Average of four determinations. [b] Average of five to seven determinations.

## EXPERIMENTAL

**Apparatus**—The high-pressure liquid chromatograph[2] was coupled to a ratio fluorometer[3] equipped with a quartz flowcell[4], a primary 5 × 5-cm glass filter[5], and a secondary 5 × 5-cm glass filter[6]. A 100-mv recorder[7] was connected to the fluorometer. Standard 0.2-cm small-bore polytetrafluoroethylene[8] tubing connected the liquid chromatograph to the fluorometer.

A 250 × 4.6-mm column, packed with octadecylsilane bonded to 10-μm silica[9], was used at ambient temperature. The flow rate was 1.5 ml/min, and column pressure was ~1600 psi. The volume of the sample and standard solutions introduced on the column was 40 μl. Methanol was used to rinse the column.

**Reagents**—Methanol and acetic acid were ACS reagent grade. The methanol and deionized water were filtered with suction through 0.6[10]- and 0.45[11]-μm filters, respectively, before use. The mobile phase, consisting of 54% methanol and 46% water that contained 2.5% acetic acid, was degassed on the instrument at 40° for 20 min before use.

The mobile phase for the liquid chromatograph can be made independently from the sample solvent. The mobile phase should not be left on the column overnight.

The same batch of mobile phase was used to dilute the sample and standard.

**Standard Salicylic Acid Solutions**—*Stock Solution*—Approximately 100 mg of salicylic acid[12] of known purity was weighed accurately, transferred to a 100-ml volumetric flask, and dissolved in and diluted to volume with methanol.

*Working Solution*—A 1.0-ml aliquot of the stock solution was transferred to a 50-ml volumetric flask and diluted to volume with the mobile phase. This concentration should be 0.972 mg/50 ml to be equivalent to the 0.3% limit for salicylic acid in plain 324-mg tablets.

**Sample Solution**—*Bulk and Plain Tablets*—An amount of aspirin or ground composite equivalent to 324 mg of aspirin was transferred to a 50-ml volumetric flask, and 25–30 ml of the mobile phase was added. Then the solution was either treated ultrasonically for 30–90 sec or shaken vigorously for 60–120 sec, diluted to volume with the mobile phase, mixed, and filtered[13,14]. After the first 5 ml was discarded, the HPLC vial was filled and capped, and the solution was injected. The total time for manipulation should be <3 min.

*Buffered Tablets*—An amount of aspirin formulation equivalent to 324 mg of aspirin was transferred to a 100-ml volumetric flask. Fifty milliliters of the mobile phase was added, and the sample was treated as outlined under *Bulk and Plain Tablets*. Then a 10.0-ml aliquot was transferred to a 100-ml volumetric flask, diluted to volume with the mobile phase, and mixed. The solution was filtered and injected as outlined under *Bulk and Plain Tablets*. The total time for manipulation should be <4 min.

The fluorometer response and/or the standard concentration were adjusted when salicylic acid levels greatly exceeded 0.3% for plain tablets or 3.0% for buffered tablets.

[2] Model 1084A, Hewlett-Packard Co., Avondale, PA 19311.
[3] Farrand Optical Co., Valhalla, NY 10595.
[4] No. 124374, 300 μl, 10 mm, Farrand Optical Co., Valhalla, NY 10595.
[5] No. UG-11, 325-nm peaking, Beckman Instrument Co., Fullerton, CA 92634.
[6] No. 3389, 80% transmission at 425 nm, Corning Glass Co., Corning, NY 14830.
[7] Servo-Riter II, Texas Instruments Inc., Houston, TX 77001.
[8] Teflon.
[9] Reversed-phase $C_{18}$, E. Merck, Darmstadt, West Germany; available as No. 906046-94 from Alltech Associates, Arlington Heights, IL 60004.
[10] BDWP04700, Millipore Corp., Bedford, MA 01730.
[11] HAWP04700, Millipore Corp., Bedford, MA 01730.
[12] Sigma Chemical Co., St. Louis, MO 63178.
[13] BDWP02500, 0.6-μm pore size, Millipore Corp., Bedford, MA 01730.
[14] BSWP02500, 2.0-μm pore size, Millipore Corp., Bedford, MA 01730.

Table II—Comparison of Results (Percent of Label Claim) Obtained by Fluorometric HPLC, USP XIX, and Semiautomated Colorimetric Methods for Salicylic Acid

| Manufacturer[a] | Type of Sample | HPLC | USP | Colorimetric |
|---|---|---|---|---|
| AA | 300-mg tablet | 0.270 | 0.248 | 0.245 |
| BB | 324-mg tablet | 0.350 | 0.307[b] | 0.396 |
| C | 81-mg tablet | 1.200 | 1.270[b] | 1.230 |
| H | 81-mg tablet | 0.456 | 0.410 | 0.540 |
| H | 81-mg tablet | 0.240 | 0.197 | 0.239 |
| E | 324-mg tablet (buffered) | 4.400 | 3.360 | 3.400 |
| EE | 324-mg tablet | 0.048[c] | 0.030[c] | 0.050[c] |
| JJ | 81-mg tablet | 0.129 | 0.149 | 0.292 |
| JJ | 81-mg tablet | 0.148 | 0.142 | 0.275 |
| II | 324-mg tablet (buffered) | 0.428[c] | 0.581[c] | 0.420[c] |
| Q | 324-mg tablet | 0.375 | 0.370 | 0.330 |
| Q | 324-mg tablet | 0.461 | 0.511 | 0.400 |
| S | 324-mg tablet | 0.088[c] | 0.072[c] | 0.089[c] |
| T | 81-mg tablet | 0.350 | 0.240 | 0.262 |
| U | 81-mg tablet | 0.495 | 0.520 | 0.500 |
| U | 81-mg tablet | 0.400 | 0.400 | 0.500 |
| K | 81-mg tablet[d] | 2.245 | 4.400 | 2.100, 4.160, 2.160, 2.310, 3.790 |
| K | 81-mg tablet | 0.500 | 0.534[e] | 0.500 |
| V | 81-mg tablet | 0.600 | 0.524[b] | 0.600 |
| V | 81-mg tablet | 0.645 | 0.520[b] | 0.625 |
| V | 81-mg tablet | 0.580 | 0.520[b] | 0.629 |
| SM | Bulk | 0.020 | —[f] | —[g] |
| MA | Bulk | 0.020 | —[f] | —[g] |
| MA | Bulk (10% starch mix) | 0.320 | 0.300 | —[g] |

[a] C = Bowman Pharmaceuticals, Canton, Ohio; E = Chromalloy American Corp., Culver City, Calif.; H = Davis Manufacturing Co., Knoxville, Tenn.; K = Freeda Vitamins, New York, N.Y.; Q = Marshall Pharmacal Corp., South Hackensack, N.J.; S = Norwich-Eaton Pharmaceuticals, Norwich, N.Y.; T = Oak Park Pharmaceuticals, Fredonia, Wis.; U = Pennex Products Co., Pittsburgh, Pa.; V = L. Perrigo Co., Allegan, Mich.; AA = Sein-Mendez Labs, Rio Piedas, Puerto Rico; BB = Stanback Co., Salisbury, N.C.; EE = Sterling Drug, New York, N.Y.; II = Walgreen Co., Chicago, Ill.; JJ = West-Ward, Eatontown, N.J.; SM = Sigma Chemical Co., St. Louis, Mo.; and MA = Monsanto Chemical Co., St. Louis, Mo. [b] USP XIX modified; tablet material was mixed for 10 min with chloroform before being poured onto column. [c] Average of four or more results. [d] Results due to difference in salicylic acid content of individual tablets. Results reported are from separate prepared composites. [e] USP modified; formic acid was used to free salicylic acid from the formulation (see Ref. 11). [f] Method lacked sensitivity for proper analysis. [g] Passed USP XIX limit test of 0.1%.

**Quantitation**—Quantitation was achieved by measuring peak height with the 100-mv recorder. The column and detector were primed with two or three injections of a salicylic acid solution before a standard was injected. The first injection of the standard and sample was used to calculate the salicylic acid content.

## RESULTS AND DISCUSSION

Salicylic acid can be determined even if the HPLC column does not separate salicylic acid and aspirin. When the HPLC column fails to separate these compounds, any interference with the salicylic acid peak height due to aspirin should be checked. This problem did not arise in this study since the $C_{18}$ reversed-phase column separated the salicylic acid from aspirin; the retention times of aspirin and salicylic acid were 3.9 and 5.0 min, respectively.

Salicylic acid was detected at 40 pg/40-μl injection at a signal-to-noise ratio of 100:1, indicating a detection limit of ~1 pg/40 μl. This procedure is at least 100 times more sensitive than the procedures of Peng *et al.* (9) and Terweij-Groen *et al.* (10), who determined salicylic acid in plasma and deproteinized serum, respectively, by the use of HPLC with UV detectors at 237 and 235 nm.

Salicylic acid gave a linear response over almost the full range of the fluorometer, *i.e.*, 0–9 mg/50 ml. The fluorometer was normally operated on midrange with the salicylic acid peak height adjusted to ~75% full scale. Since the relative fluorescence of salicylic acid is pH dependent (6), the standard and samples must be prepared with the same mobile phase solution. The reproducibility of 11 salicylic acid standard injections calculated from the peak heights was ±1.2 fluorescence units with an average peak height of 67.3 fluorescence units. Recoveries of salicylic acid in the presence of aspirin from simulated formulations ranged from 101.0 to 105.5%. Recoveries of salicylic acid from buffered aspirin samples ranged from 100.0 to 117.0%. With some buffered tablet formulations,

it may be necessary to add a small amount of formic acid to the tablet material to release salicylic acid into the solution (3, 11).

The average result for 10 individual weighings from a prepared 325-mg commercial sample composite was 0.048% salicylic acid with a relative standard deviation of 3.9%.

Table I shows a comparison of the results of the fluorometric HPLC procedure with the official USP XIX procedure. The samples are the same as those reported in Table II in Ref. 8. The results were obtained on composites prepared from commercial tablet samples.

Table II shows a comparison of the fluorometric HPLC procedure with the USP XIX method and with a semiautomated colorimetric procedure (8) for samples collected during the aspirin survey. The results listed for each manufacturer were not determined on the same composite.

Baum and Cantwell (12) recently reported the simultaneous determination of salicylic acid in aspirin formulations by HPLC with UV detection. The Baum and Cantwell method was noted only after the HPLC procedure was being used routinely on manufactured formulations in this laboratory.

The hydrolysis of aspirin to salicylic acid (12) is well recognized. For this reason, a time limit was placed on the sample preparation in the procedure. Sample preparation times exceeding 10 min were unsatisfactory for accurate and precise recoveries.

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# Aspirin—A National Survey III: Determination of Impurities in Bulk Aspirin and Aspirin Formulations by High-Pressure Liquid Chromatography and Spectrophotometry


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**Abstract** □ A quantitative high-pressure liquid chromatographic method, using a reversed-phase column and an aqueous acetic acid–methanol solution as the mobile phase, was employed for the determination of *O*-acetyl-*O*-salicylsalicylic acid and *O*-salicylsalicylic acid in pharmaceutical aspirin preparations. The aspirin was dissolved, filtered, and injected into the chromatograph. The absorbance of the impurities was measured at 254 nm. Acetylsalicylic anhydride was determined by a spectrophotometric method. The aspirin was dissolved in pH 11.3 buffer and extracted with benzene. An aliquot of the benzene was evaporated, and the residue was dissolved in α-benzamidocinnamate–pyridine reagent. The acetylsalicylic anhydride was measured using the difference between the absorbance at 362 and 372 nm. Possible interference of aspirin with the procedure is discussed. Thirty-four bulk aspirin and 172 tablet formulations were examined. Results for *O*-acetyl-*O*-salicylsalicylic acid, *O*-salicylsalicylic acid, and acetylsalicylic anhydride are given.

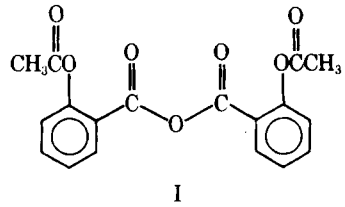


**Keyphrases** □ *O*-Acetyl-*O*-salicylsalicylic acid—high-pressure liquid chromatographic determination □ Analgesics—determination of impurities in aspirin □ Aspirin—determination of impurities □ *O*-Salicylsalicylic acid—high-pressure liquid chromatographic determination □ Acetylsalicylic anhydride—spectrophotometric determination


Acetylsalicylic anhydride (I), *O*-acetyl-*O*-salicylsalicylic acid (II), and salicylic acid have been determined in aspirin tablets by high-pressure liquid chromatography (HPLC) (1–5), GLC (1, 3, 6–9), TLC (10, 11), and spectrophotometry (12, 13).

The HPLC procedure of Ali (1) was used in these laboratories to determine impurities in aspirin products. However, certain reversed-phase HPLC columns did not separate I from another impurity, which was isolated and identified as *O*-salicylsalicylic acid (III) (14). Compound I also is decomposed rapidly while in contact with the mobile phase. Therefore, this procedure was used only for the measurement of II and III. Additional impurities were found in commercial samples but have not been identified.

Bundgaard (5) postulated the presence of III and *p*-acetoxybenzoic acid in aspirin formulations based on their HPLC retention times. Bundgaard indicated that these



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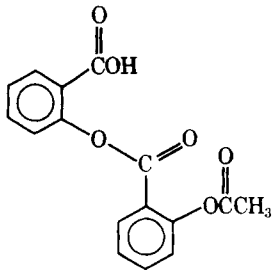

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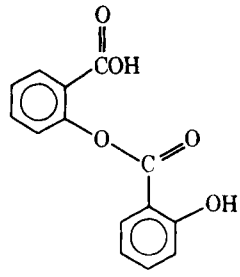

III




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