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High-Pressure Liquid Chromatographic Determination of Salicylic Acid in Aspirin Powder and Pharmaceutical Dosage Forms

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Abstract □ A sensitive, simple, and rapid method for the quantitation of salicylic acid in aspirin powders and its dosage forms was developed. The method is based on reversed-phase high-pressure liquid chromatography using a mobile phase containing 20% methanol in aqueous phosphate buffer of pH 2.3. Other common ingredients present with aspirin such as acetaminophen, caffeine, codeine phosphate, phenacetin, and salicylamide do not interfere. Salicylic acid quantities as low as 0.1 μg can be assayed with a relative standard deviation of ±2.3%. Sensitivity can be increased by using lower sensitivity settings. The method was tried on numerous commercial products and an old aspirin powder. The results generally were excellent, except that all of the aspirin and salicylic acid could not be extracted from suppositories. The old aspirin powder failed the USP limit test for salicylic acid. The powder apparently absorbed moisture and contained salicylamide as an impurity.

Keyphrases □ High-pressure liquid chromatography—analysis, salicylic acid in aspirin powder and dosage forms □ Aspirin—powder and dosage forms, analysis of salicylic acid, high-pressure liquid chromatography □ Salicylic acid—analysis in aspirin powder and dosage forms, high-pressure liquid chromatography

Salicylic acid is the major decomposition product of aspirin. Because free salicylic acid can cause gastric upset, the USP (1) prescribes limit tests for salicylic acid in aspirin powder and in pharmaceutical dosage forms containing aspirin. The limits are 0.1% for aspirin powder, 0.3% for tablets without buffers, 3.0% for buffered aspirin dosage forms, and 1.0% for suppositories. However, the USP method for the quantitation of salicylic acid in aspirin dosage forms is tedious and time consuming. For aspirin powder, the test is qualitative.

A high-pressure liquid chromatographic (HPLC) method (2) was reported which analyzes small aspirin quantities in the presence of large salicylic acid quantities in plasma. However, in dosage forms and aspirin powder, the assay of small salicylic acid quantities in the presence of large aspirin quantities was a problem. A tedious and complicated method was used for the separation of salicylic acid from aspirin (3).

The simultaneous quantitation of acetaminophen, aspirin, caffeine, codeine phosphate, phenacetin, and salicylamide was reported (4). In this method, the salicylic acid did not interfere. Small salicylic acid quantities could not be determined due to a lack of sensitivity in the analytical method. The purpose of these investigations was to develop a simple and rapid HPLC method for the quantitation of small salicylic acid quantities in aspirin powder and its pharmaceutical dosage forms.

EXPERIMENTAL

Reagents and Chemicals—All reagents and chemicals were ACS, USP, or NF quality and were used without further purification.

Apparatus—The high-pressure liquid chromatograph[1] was connected to a multiple-wavelength[2] detector, a recorder[3], and an integrator[4].

Column—The nonpolar column[5] (30 cm × 4 mm i.d.) consisted of a monomolecular layer of octadecyltrichlorosilane permanently bonded by silicone–carbon bonds.

Chromatographic Conditions—The solvent consisted of 0.01 *M* KH_2PO_4 in water with 20% (v/v) methanol, and its pH[6] was adjusted to 2.3 with an 85% aqueous phosphoric acid solution (~1.1 ml/liter). The temperature was ambient. The flow rate was 2.0 ml/min. The detector sensitivity was 0.04 (300 nm), and the chart speed was 30.5 cm/hr.

Solution Preparation—A stock solution of salicylic acid was prepared by dissolving 0.100 g in enough ethanol to bring to 100.0 ml. This solution was diluted with ethanol for the preparation of the standard solution or other solutions.

A stock solution of a standard mixture was prepared by dissolving 48.6 mg of acetaminophen, 97.2 mg of aspirin, 32.4 mg of caffeine, 50.0 mg of phenacetin, 64.8 mg of salicylamide, and 25.0 mg of salicylic acid in 10 ml of ethanol and bringing the solution to 100.0 ml with water. A solution of the standard mixture was prepared by diluting 10.0 ml of the stock solution to 100.0 ml with water. Both the stock and standard mixture solutions were prepared immediately before use.

Preparation of Assay Solutions from Aspirin Powder—All solutions were prepared immediately before injection into the chromatograph. Aspirin powder, 250.0 mg, was dissolved in enough ethanol to bring to 25.0 ml. Another solution of identical strength was prepared from an old aspirin powder (~10 years), which appeared to have decomposed. These solutions were used for the free salicylic acid determination.

Two other aspirin solutions were prepared by diluting the first solution to 250 μg/ml. Solution 1 was prepared by diluting with water, and Solution 2 was prepared with ethanol. These solutions were allowed to stand for ~50 hr and were then analyzed for free salicylic acid. This analysis was done to compare the hydrolysis rate of aspirin in water *versus* ethanol.

Assay Solutions from Solid Dosage Forms—An appropriate quantity of the fine powder was mixed with enough ethanol to bring to 25.0 ml, and the mixture was shaken for 2–3 min and filtered. The first 5–8 ml of the filtrate was rejected, and samples were collected for analysis. The solutions were prepared immediately before analysis. The quantity of the powder weighed represented at least 80 mg (for all nonbuffered tablets) or 50 mg (for buffered tablets) of aspirin. These quantities were determined by considering USP (1) salicylic acid limits. Exact concentrations of the aspirin solutions are reported in Table I.

Suppository Assay Solutions—A suppository containing 600 mg of aspirin was transferred to a 150-ml beaker. The beaker was warmed by

[1] Waters ALC 202 equipped with a U6K universal injector, Waters Associates, Milford, Mass.
[2] Spectroflow monitor SF770, Schoeffel Instrument Corp., Westwood, N.J.
[3] Omniscribe 5213-12, Houston Instruments, Austin, Tex.
[4] Autolab minigrator, Spectra-Physics, Santa Clara, Calif.
[5] μBondapak C_{18}, catalog No. 27324, Waters Associates, Milford, Mass.
[6] Model 4500 digital pH meter, Beckman Instruments, Irvine, Calif.

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Table I—Assay Results of Aspirin Powders and Commercial Dosage Forms

Sample	Claim per Dosage Form, mg	Concentration of Aspirin in Assay Solution, mg/ml	Assay Results	
			Aspirin, % label claim	Salicylic Acid[a] Based on Percent Label Claim of Aspirin
Aspirin powder	—	10.0	—	0.07
Old aspirin powder	—	10.0	95.6[b]	0.61
Tablet 1	Aspirin, 325	10.0	99.8	0.06
Tablet 2	Aspirin, 325; magnesium-aluminum hydroxide, 300	2.6	100.4	0.41
Tablet 3	Aspirin, 600; magnesium hydroxide, 150; aluminum hydroxide dried gel, 150	2.4	99.6	0.54
Tablet 4	Aspirin, 227; phenacetin, 162; caffeine, 32	6.8	100.0	0.14
Tablet 5	Aspirin, 195; acetaminophen, 97; caffeine, 65; salicylamide, 130	7.8	100.1	0.13
Suppository	Aspirin, 600	1.0	31.4	0.64[c]
Aspirin solution (50 hr old)				
In ethanol	—	0.25	—	2.8
In water	—	0.25	—	6.6[d]

[a] Relative standard deviation based on five injections was ±2.3%. [b] This old aspirin powder apparently had absorbed moisture. It also failed the USP limit test for salicylic acid. [c] Calculated based on label claim of aspirin; percent found was divided by 0.314. Since the salicylic acid peak was small, the margin of error is ~3.8%. [d] To determine salicylic acid in a water solution, the standard salicylic acid solution also was made in water by diluting the stock solution. The peaks were slightly higher for the standard solution in water *versus* in ethanol.

immersion in a water bath (~50°) to melt the suppository, and the melt was mixed with enough ethanol to make 60.0 ml of mixture. The mixture was shaken for 4–5 min, and the decanted alcoholic solution was diluted (from 10.0 to 100.0 ml) with ethanol to prepare the assay solution.

Assay Method—A 20.0-μl aliquot of the assay solution was injected into the chromatograph using the described conditions. An identical volume of salicylic acid solution in ethanol (10.0 μg/ml) was injected for comparison after the assay solution eluted.

Two chromatograms (Fig. 1) were developed by injecting a 20.0-μl aliquot of the standard mixture. One chromatogram used a detector wavelength of 300 nm, and the other chromatogram used 254 nm.

Calculations—Since preliminary investigations indicated that peak heights were directly related to concentrations (0.10–0.6 μg) of salicylic acid, the results were calculated by:

$$\frac{Ph_a}{Ph_s} \times 10 = \text{micrograms of salicylic acid per milliliter of solution} \quad \text{(Eq. 1)}$$

where Ph_a is the peak height of the assay solution and Ph_s is the peak height of the standard solution. To ensure quantitative extraction of aspirin from the dosage forms, aspirin was assayed using the method reported previously (4) (Table I and Figs. 2–4).

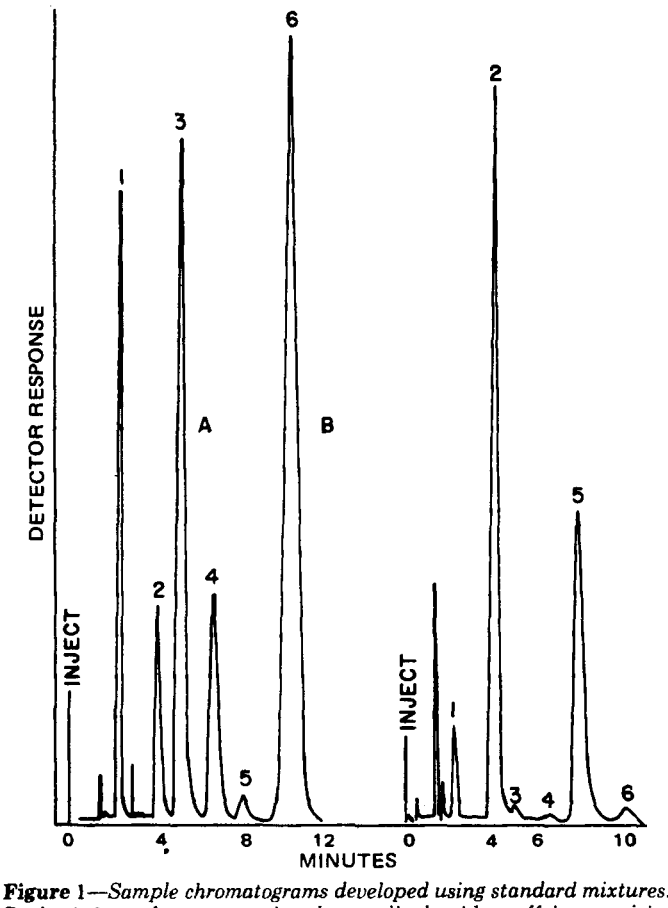

Figure 1—*Sample chromatograms developed using standard mixtures. Peaks 1–6 are from acetaminophen, salicylamide, caffeine, aspirin, salicylic acid, and phenacetin, respectively. Chromatogram A was developed at 254 nm, and B was developed at 300 nm.*

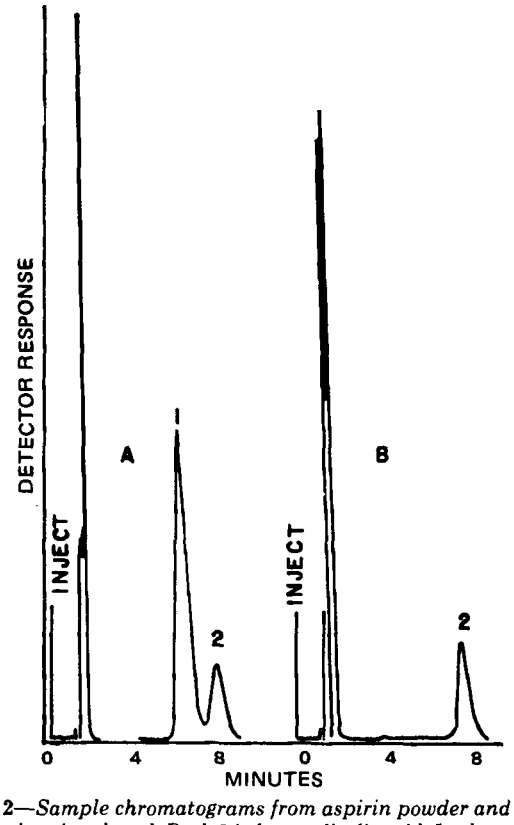

Figure 2—*Sample chromatograms from aspirin powder and salicylic acid solutions in ethanol. Peak 2 is from salicylic acid. In chromatogram A, peak 1 is from aspirin (0.2 mg). In B, 0.2 μg of salicylic acid was injected.*

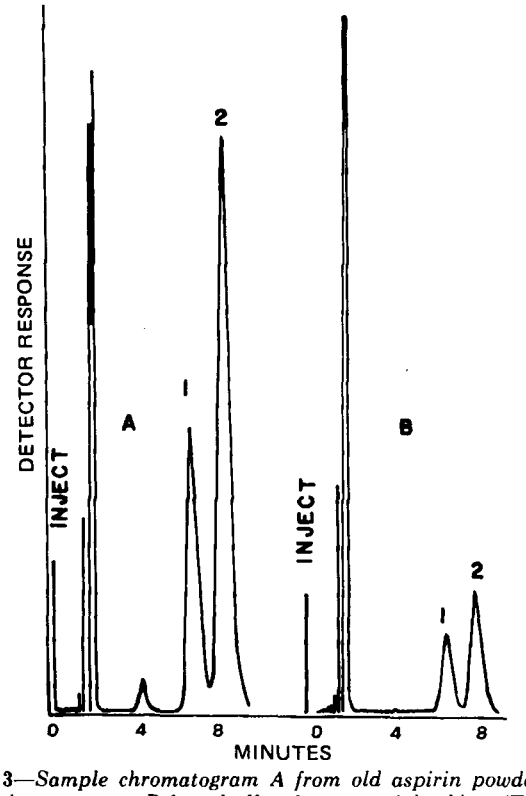

Figure 3—*Sample chromatogram A from old aspirin powder and sample chromatogram B from buffered commercial tablets (Tablet 3 in Table I). Peak 1 is from aspirin, and 2 is from salicylic acid. In A, there is an additional peak after ~4 min, which may be due to salicylamide impurity.*

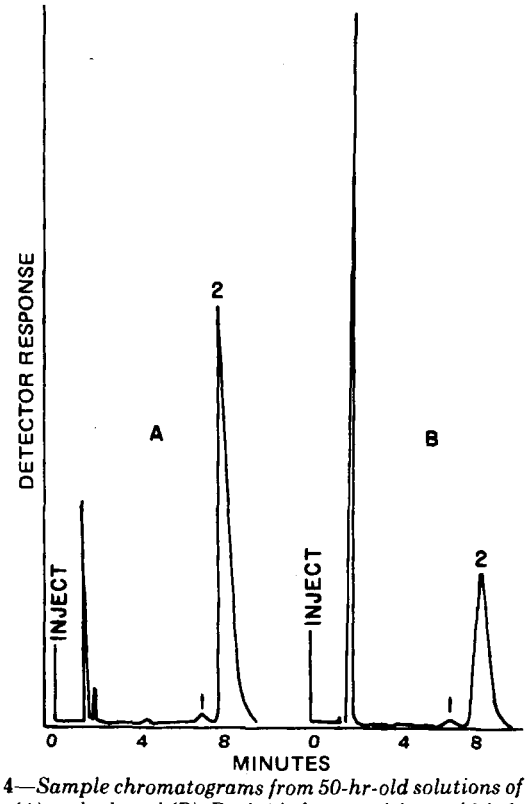

Figure 4—*Sample chromatograms from 50-hr-old solutions of aspirin in water (A) and ethanol (B). Peak 1 is from aspirin, and 2 is from salicylic acid.*

RESULTS AND DISCUSSION

It was reported (4) that it was not possible to assay salicylic acid using the solvent system developed for the simultaneous quantitation of acetaminophen, aspirin, caffeine, codeine phosphate, phenacetin, and salicylamide due to the lack of sensitivity at 254 nm (sensitivity 0.04). Otherwise, separation of salicylic acid from the six active ingredients was excellent (4). Preliminary investigations subsequently indicated that the spectral absorption of salicylic acid solution in the mobile phase was very high at 300 nm, the wavelength of maximum absorption. When considering weight per weight concentrations, the absorption of aspirin solution at 300 nm was almost negligible (Fig. 1B, peak 4). For comparison, chromatograms A and B (Fig. 1) were developed at 254 and 300 nm, respectively. In A, peak 1 was obtained at a sensitivity of 0.2; the sensitivity for peak 1 in B was 0.04. A sensitivity of 0.04 was used otherwise. Even with a sensitivity of 0.04, peak 1 (from acetaminophen) in chromatogram B was very small as compared with peak 1 in chromatogram A (Fig. 1).

The results (Table I) and the sample chromatograms indicate that very small quantities of salicylic acid can be assayed in aspirin powder and its dosage forms using a simple HPLC method. The mobile phase pH (2.3) was almost the optimum pH for aspirin stability (5). Therefore, the adverse effect from the mobile phase was minimal. Aspirin could not be extracted from the suppositories quantitatively with ethanol (Table I). Chloroform was not used for this extraction because it is not miscible with the mobile phase.

Salicylic acid determinations were made at a sensitivity of 0.04. The sensitivity of this method can be increased by changing the setting to 0.01; however, there was some noise problem at this low sensitivity. In the proposed assay, salicylic acid concentrations as low as 5 μg/ml can be determined with a relative standard deviation of ±2.3%. If the salicylic acid assay results are less than 5 μg/ml, the error could be higher. Therefore, the assay should be repeated either with a higher aspirin concentration or with a lower sensitivity.

The old aspirin powder did not pass the USP limit test (Table I) of 0.1% for salicylic acid. Moreover, the old aspirin powder assayed only 95.6%, which may have been due to the moisture absorption. Also, it showed an additional peak (Fig. 3A) after 4 min, which may have been due to salicylamide as an impurity since the retention time of the peak was identical to that of salicylamide (Fig. 1). Although no internal standard was used, one of the following (if not present in the dosage form) may be used as an internal standard: caffeine, 1–2 mg/ml; phenacetin, 1–2 mg/ml; and salicylamide, ~20 μg/ml.

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