GLC Analysis of Aspirin from Solid Dosage Forms

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Abstract □ A GLC method of determining aspirin and salicylic acid in pharmaceutical formulations is presented. Acetylsalicylsalicylic acid was detected in varying amounts in tablets and granulations from different manufacturers.

Keyphrases □ Aspirin formulations—GLC analysis □ Salicylic acid in aspirin formulations—GLC analysis □ Acetylsalicylsalicylic acid in aspirin formulations—GLC analysis □ GLC—analysis, aspirin, salicylic acid, and acetylsalicylsalicylic acid

The different behavior in pharmaceutical systems of aspirin from differing manufacturers has been the subject of considerable investigation. The most popular explanation of the differences in melting points (1, 2), heats of fusion (3), densities (2, 4), and rates of solution (1, 4, 5) has been the existence of polymorphs. This explanation of polymorphism was recently severely criticized (6), and another unlikely explanation of the proof of polymorphism, that of differing pKa's of aspirin from varying sources, was put forward (7). In an attempt to investigate these differences further, it became necessary to develop a new assay for aspirin and salicylic acid. The USP method (8) for aspirin tablets involves time-consuming chromatography followed by separate spectrophotometric determinations of aspirin and salicylic acid. The method cannot be used for small sample sizes.

GLC methods for aspirin alone have been reported (9, 10), and an attempt was made to determine aspirin and salicylic acid in a single run (11). This latter method showed considerable tailing of the salicylic acid peak, probably due to the interaction of the hydroxy group with the column packing; hence, quantitative interpretation was not satisfactory. A method of determining aspirin and salicylic acid quantitatively is reported here. Acetylsalicylsalicylic acid was also found to be present in most products.

EXPERIMENTAL

Materials—Salicylic acid crystals USP[1] were recrystallized from methanol. Aspirin USP[2] was recrystallized from benzene and washed with hexane. Gas Chrom Q[3] (100–120 mesh), *N,O*-bis(trimethylsilyl)acetamide[4], a mixture of three trimethylsilyl donors[5], OV-210[3] (methyltrifluoropropyl silicone), and OV-225[3] (cyanopropylphenyl silicone) were used as supplied by the manufacturers. All other reagents were analytical grade. Aspirin tablets were obtained from several manufacturers[6], as were granulations[7].

Preparation of Acetylsalicylsalicylic Acid—This two-stage synthesis (12, 13) involves the preparation of disalicylic acid and its subsequent acetylation.

Synthesis of Disalicylic Acid—One hundred grams salicylic acid, 104 g. dimethylaniline, 60 ml. benzene, and 45 ml. phosphorus trichloride were stirred constantly at room temperature for 4 days. Excess dilute hydrochloric acid was added, and the resultant precipitate was extracted with ether. After evaporation of the ether, the residue was washed with boiling water five times. The residue was dissolved in ether and dried over sodium sulfate. The ether was evaporated off, giving disalicylic acid, m.p. 147–148°.

Synthesis of Acetylsalicylsalicylic Acid from Disalicylic Acid—One gram disalicylic acid was dissolved in 30 ml. acetic anhydride and placed in an ice bath; to this was added a cooled mixture of 20 ml. acetic anhydride and 9 ml. concentrated sulfuric acid. After the addition, the mixture was heated to 50–60° and stirred for 24 hr. The mixture was poured onto ice and the precipitate was extracted with ether: the residue, following evaporation of the ether, was recrystallized twice from aqueous ethanol to give acetylsalicylsalicylic acid, m.p. 162–164°. The product had IR and NMR spectra in agreement with acetylsalicylsalicylic acid.

METHOD

GLC Column—The column used was glass, 4 mm. × 0.91 m. (3 ft.), 1% methyltrifluoropropyl silicone on acid–base washed, silane-treated, flux-calcinated diatomaceous earth, 100–120 mesh. It was conditioned at 250° with 50 μl. of a column conditioner[5].

Gas Chromatograph—The gas chromatograph[8] was used with dual-flame ionization detectors and a temperature-programmable column oven.

Carrier Gas—The carrier gas used was nitrogen (flow rate 52.6 ml./min.) at 100° column temperature for all columns.

Temperature Programs—*Aspirin, Acetylsalicylsalicylic Acid, and Salicylic Acid in Approximately Equal Quantities*—

injection temperature	175°
detector temperature	350°
column temperature	100° for 8.5 min. and then 15°/min. rise up to 230°
attenuation	9×10^{-9} amp.

Aspirin Tablets and Granulations—When assaying aspirin tablets, two sets of operating conditions are necessary because of the very small concentrations of salicylic acid and acetylsalicylsalicylic acid.

(*a*) Aspirin:

injection temperature	175°
detector temperature	350°
column temperature	145° (isothermal)
attenuation	3×10^{-9} amp.

[1] Fred Portz, Fine Chemicals, Waukesha, Wis.
[2] J. T. Baker Chemical Co., Phillipsburg, N. J.
[3] Applied Science Labs., Inc., State College, Pa.
[4] BSA, Pierce Chemical Co., Rockford, Ill.
[5] SILYL-8, Pierce Chemical Co., Rockford, Ill.
[6] Bayer Co., Division of Sterling Drug, Inc., New York, N. Y.; Eli Lilly and Co., Indianapolis, Ind.; Squibb Products Co., New York, N. Y.; St. Joseph Aspirin, Plough, Inc., Memphis, Tenn.; and Rexall Drug Co., St. Louis, Mo.
[7] Monsanto Co., St. Louis, Mo., and Dow Chemical Co., Midland, Mich.
[8] Barber-Coleman Series 5000.

Table I—Precision Studies: Calculation of Standard Deviations[a]

Run	X	X^2	Y	Y^2	Z	Z^2
1	20.4	416.16	18.3	334.88	10.0	100.00
2	20.8	432.64	18.4	338.55	11.4	129.95
3	21.6	466.56	18.2	331.23	11.2	125.43
4	21.2	449.44	18.0	324.00	11.0	121.00
5	21.4	457.96	18.1	327.60	11.3	127.68
6	21.0	441.00	18.0	324.00	11.2	125.43
7	21.4	457.96	18.8	353.43	11.2	125.43
8	21.7	470.89	18.4	338.55	11.5	132.24
9	21.3	453.69	18.4	338.55	11.0	121.00
10	21.0	441.00	18.2	331.23	11.5	132.24
	$\Sigma X = 211.8$	$\Sigma X^2 = 4487.30$	$\Sigma Y = 182.8$	$\Sigma Y^2 = 3342.02$	$\Sigma Z = 111.3$	$\Sigma Z^2 = 1240.00$

$$S_X = \sqrt{\frac{4487.30 - \frac{(211.8)^2}{10}}{10 - 1}} = 0.39 \pm 1.88\%^b$$

$$S_Y = \sqrt{\frac{3342.02 - \frac{(182.8)^2}{10}}{10 - 1}} = 0.2 \pm 1.09\%^b$$

$$S_Z = \sqrt{\frac{1240.40 - \frac{(111.3)^2}{10}}{10 - 1}} = 0.42 \pm 3.6\%^b$$

[a] X, Y, and Z represent the area generated in planimeter units for salicylic acid, aspirin, and acetylsalicylsalicylic acid, respectively. [b] Precision area.

(*b*) Salicylic acid and acetylsalicylsalicylic acid

injection temperature	175°
detector temperature	350°
column temperature	100° for 8.5 min. and then 15°/min. rise up to 230°
attenuation	3×10^{-10} amp.

Detection of Acetylsalicylsalicylic Acid—The unknown peak from aspirin tablets had similar retention times to the synthesized acetylsalicylsalicylic acid using the above column and conditions. This column was also used with the following conditions and the retention times were again similar.

(*a*) injection temperature	190°
detector temperature	390°
column temperature	180° programmed at 5°/min. rise up to 230°
attenuation	3×10^{-10} amp.
(*b*) injection temperature	195°
detector temperature	395°
column temperature	190° programmed at 5°/min. rise up to 230°
attenuation	3×10^{-9} amp.

(*c*) In addition, a glass column, 4-mm. × 1.22-m. (4-ft.) was used, containing 1% cyanopropylphenyl silicone on acid–base washed, silane-treated, flux-calcinated diatomaceous earth, 100–120 mesh. It was conditioned at 250° with 50 μl. of a column conditioner[5].

injection temperature	200°
detector temperature	395°
column temperature	190° programmed at 5°/min. rise up to 230°
attenuation	3×10^{-9} amp.

Again the retention times of acetylsalicylsalicylic acid and the unknown were similar.

Preparation of Samples—*Standard Solutions*—Standard solutions of aspirin, salicylic acid, and acetylsalicylsalicylic acid were prepared in anhydrous ether. Either 100- or 200-μl. samples were taken, and the ether was removed with nitrogen. Two hundred microliters of *N,O*-bis(trimethylsilyl)acetamide was added, and the mixture was shaken on a mechanical shaker for a few seconds. Two microliters of this solution was then injected onto the column. Concentrations of unknowns were measured by comparing their peak areas with those of standards.

Material from Tablets or Granulations—The tablet (or equivalent of granules) was accurately weighed and extracted with anhydrous ether as quickly as possible. The solution was filtered and the final volume was adjusted to 50 ml. One hundred microliters of this solution was treated with 200 μl. *N,O*-bis(trimethylsilyl)acetamide as described for the analysis of aspirin. For analysis of salicylic acid and acetylsalicylsalicylic acid, 1 ml. of the solution was evaporated to dryness with nitrogen, and sufficient *N,O*-bis(trimethylsilyl)acetamide was added to make the total volume 100 μl. Four microliters of this solution was then applied to the column. For precision investigations, a solution containing 4.8 mg./10 ml. salicylic acid, 4.96 mg./10 ml. aspirin, and 4.98 mg./10 ml. acetylsalicylsalicylic acid was prepared and 2 μl. was injected onto

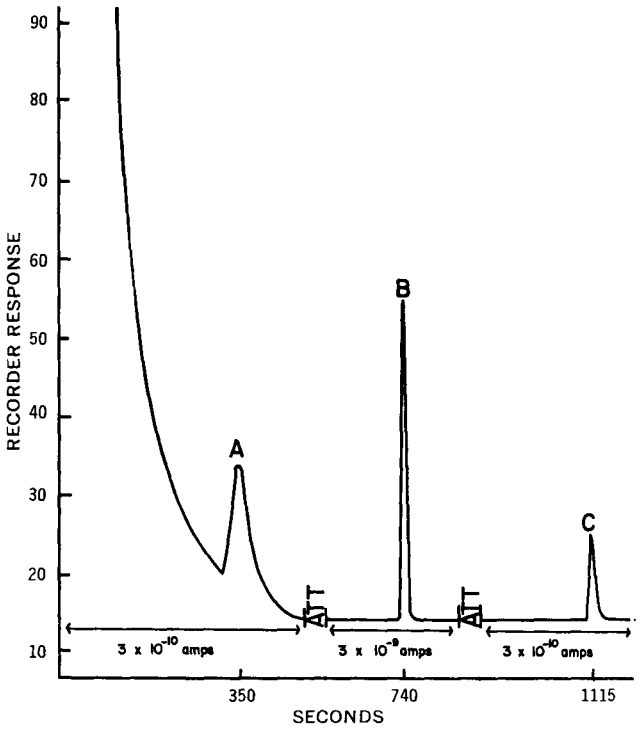

Figure 1—*Typical gas chromatogram of a mixture of: (A) salicylic acid, 0.5 mcg./μl.; (B), aspirin, 15 mcg./μl.; and (C) acetylsalicylsalicylic acid, 0.5 mcg./μl., at noted attenuation under GLC conditions as indicated in the text.*

Table II—Analysis of Commercial Aspirin Tablets and Granulations[a]

Manufacturer	Aspirin mg.	Aspirin %	Salicylic Acid mg.	Salicylic Acid %	Acetylsalicylsalicylic Acid mg.	Acetylsalicylsalicylic Acid %
			Tablets			
A	331.1	102	0.150	0.05	Traces	
	338.8	104	0.135	0.040	Traces	
	331.1	102	0.150	0.05	Traces	
B	330	101	0.274	0.08	0.185	0.06
	338	104	0.285	0.08	0.201	0.06
	334	103	0.222	0.06	0.159	0.05
C	331.5	102	0.204	0.06	0.061	0.02
	331	101.8	0.172	0.05	0.079	0.02
	338.6	104	0.168	0.05	0.091	0.03
	331	101.8	0.162	0.05	0.091	0.03
D	332.9	102	0.172	0.05	0.132	0.04
	322.2	99.1	Traces		0.130	0.04
	327.9	100.9	Traces		0.160	0.05
	336.5	103.5	Traces		0.140	0.04
E	320.7	98.7	0.302	0.09	0.076	0.02
	324.3	99.8	0.551	0.17	0.104	0.03
	336.4	103.5	0.272	0.08	0.091	0.02
	333.4	102.6	0.328	0.1	0.113	0.034
			Granulation			
1	339.2	92.8	0.220	0.06	0.119	0.04
	320.3	88.6	0.202	0.06	0.149	0.05
	339.2	92.6	0.147	0.04	0.149	0.04
	335.4	91.9	0.158	0.05	0.143	0.04
2	282.6	85	0.057	0.02	0.376	0.1
	305.5	87	0.114	0.04	0.376	0.1
	313	89	0.114	0.04	0.376	0.1
	287.7	87.2	0.100	0.04	0.376	0.1
			USP Method for Tablet A			
	329.95	101.5	0.159	0.05	—	—
	336.26	103	0.165	0.05	—	—
	333.2	102.5	0.201	0.1	—	—

[a] Aspirin percentage is expressed as percent of 325 mg. for the tablets and as percent of total granulation of the granulations. The salicylic acid and acetylsalicylsalicylic acid are expressed as percentage aspirin found.

the column. The calculation of the standard deviation is shown in Table I.

RESULTS AND DISCUSSION

The extraction of drug with anhydrous ether was performed as quickly as possible because there appeared to be some degradation on delay; this degradation stopped after filtration of the extract. Initially, an attempt was made to silylate the products in pyridine; however, this enhanced degradation and it was decided to silylate the residues from the ethereal extraction directly with *N,O*-bis(trimethylsilyl)acetamide. Rowland and Riegelman (10) found multiple peaks for salicylic acid following silylation with trimethylchlorosilane; however, in the method reported here, using *N,O*-bis(trimethylsilyl)acetamide, a single peak was obtained with salicylic acid. In the early experiments, a small peak of material of higher molecular weight than aspirin was consistently observed following the analysis of tablets and granulations. Acetylsalicylsalicylic acid was synthesized, and the peak of the unknown exhibited the same retention time as acetylsalicylsalicylic acid on the methyltrifluoropropyl silicone column under several operating conditions and also on the cyanopropylphenyl silicone column. It was assumed, therefore, that the compound was acetylsalicylsalicylic acid. A typical chromatogram obtained with aspirin, acetylsalicylsalicylic acid, and salicylic acid is shown in Fig. 1.

To determine whether or not acetylsalicylsalicylic acid was produced by acylation of the aspirin by salicylic acid on the high temperature column, the following experiment was carried out. A mixture containing 99 parts of pure aspirin and 1 part salicylic acid was chromatographed as reported for salicylic acid and acetylsalicylsalicylic acid. No trace of acetylsalicylsalicylic acid was found, suggesting that the impurity is formed during the aspirin manufacturing process.

The results from analyses of aspirin tablets and granulations are shown in Table II. The small percentages of salicylic acid and acetylsalicylsalicylic acid necessitate the separate determination of aspirin on the same column. Acetylsalicylsalicylic acid does not appear to have been previously reported to occur in aspirin formulations; however, all of the tablets and granulations investigated here appeared to contain the compound. One tablet only had traces, but one granulation had 0.13%. The method for tablets gave an average of 102.7% aspirin for Manufacturer A, and the USP method also gave 102.7% for the same batch of tablets. The proposed GLC method gave 0.043% salicylic acid and the USP method gave 0.050%. Any increase in salicylic acid found in the USP method may be due to the alkaline conditions used in the partition chromatography.

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