Stabilized Normal-Phase High-Performance Liquid Chromatographic Analysis of Aspirin and Salicylic Acid in Solid Pharmaceutical Dosage Forms

ROBERT N. GALANTE ˣ, **ANTHONY J. VISALLI**, and **WAYNE M. GRIM**

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Abstract ☐ A simultaneous analysis of aspirin and nonaspirin salicylates in solid pharmaceutical dosage forms is described. Two separate extraction procedures are employed, one for plain aspirin tablets and one for tablets containing aspirin plus buffers or antacids. The analyses of the extracted samples are accomplished by a stabilized normal-phase high-performance liquid chromatographic (HPLC) procedure. Prepared samples and standards are stable for up to 24 h, and the methodology is suitable for an automated HPLC system.

Keyphrases ☐ Aspirin—stabilized normal-phase high-performance liquid chromatographic analysis, solid pharmaceutical dosage forms, salicylic acid ☐ Salicylic acid—stabilized normal-phase high-performance liquid chromatographic analysis, solid pharmaceutical dosage forms, aspirin ☐ Solid dosage forms—stabilized normal-phase high-performance liquid chromatographic analysis of aspirin and salicylic acid

Successful analyses of aspirin and free salicylic acid by reverse-phase high-performance liquid chromatography (HPLC) have been reported (1–13). Effective adsorption HPLC procedures have also been described (14–16). All of these methods require immediate injections of prepared samples and standards into the HPLC. We described a stable assay preparation for aspirin and salicylic acid in tablets containing buffers or antacids *via* a chromatographic siliceous earth column with subsequent GC (17). The methodology described here combines some of these effective techniques into a stabilized normal-phase HPLC analysis. None of the previously published HPLC procedures allow the determination of salicylic acid as nonaspirin salicylates in tablets containing buffers or antacids.

EXPERIMENTAL

Materials and Equipment—HPLC-grade chloroform, methylene chloride, and acetonitrile and reagent-grade chloroform, formic acid (88%), methanol, hydrochloric acid, citric acid monohydrate, and chromatographic siliceous earth (acid washed by the USP procedure) were used. A modular high-pressure liquid chromatograph equipped with a variable-flow pump[1], an automatic injector[2] with a 20-μL loop, a variable-wavelength detector[3], and an electronic integrator[4] was used. The HPLC adsorption column was 4.6 mm × 25 cm packed with a porous 5-μm silica[5]. Reagent-grade chloroform saturated with citric acid was used as diluent for all sample and standard preparations.

Standards Preparation—*Aspirin*—About 125 mg of aspirin USP reference standard was accurately weighed, transferred to a 50-mL volumetric flask, and dissolved in ~10 mL of diluent. After adding 0.25 mL of formic acid, the mixture was diluted to volume with diluent. About 200 mg of citric acid crystals was added to the flask and mixed.

Salicylic Acid—A 1-mg/mL stock solution of salicylic acid USP reference standard was prepared in the diluent containing 0.5% formic acid. This solution was diluted to make standards containing 7.5, 25.0, 50.0, and 75.0 μg/mL, corresponding to 0.3, 1.0, 2.0, and 3.0% salicylic acid in the assay preparation.

Sample Preparation—*Plain Aspirin Tablets*—The average tablet weight of 20 tablets was determined, and the tablets were ground to a fine powder. A portion of the ground tablets equivalent to 250 mg of aspirin was transferred to a volumetric flask, and 100 mL of diluent containing 0.5 mL of formic acid was added to the flask. About 500 mg of citric acid crystals was added to the flask, and the assay preparation was placed in an ultrasonic bath for 2 min, then centrifuged or filtered for subsequent injection into the liquid chromatograph.

Aspirin Tablets Containing Buffers or Antacids—A portion of 20 ground tablets equivalent to 500 mg of aspirin was transferred to a small beaker containing 3.0 g of acid-washed chromatographic siliceous earth. The powders were mixed with a glass rod, 2.0 mL of 6 M HCl was added, and the powders were mixed again with the glass rod. The mixture was transferred to a 20 × 2.5-cm chromatographic column, and the beaker was dry washed with 1.0 g of siliceous earth (glass wool was used at both ends of the column). The column was packed uniformly and eluted with successive portions of diluent *via* the sample beaker, at the rate of ~10 mL/min. About 150 mL of the eluate was collected in a 200-mL volumetric flask. The tip of the column was rinsed with diluent, and 1.0 mL of formic acid was added. The flask was made to volume with the diluent. Approximately 500 mg of citric acid crystals were added to the flask and mixed.

Assay Procedure—The mobile phase, chloroform–methylene chloride-acetonitrile–formic acid (700:300:30:4), was placed in an ultrasonic bath to expel dissolved gases, and ~100 mL was allowed to pass through the column prior to the start of the analysis. At the completion of a 1-d run the column was washed with ~200 mL of methanol.

The variable-wavelength detector was set at 300 nm to maximize sal-

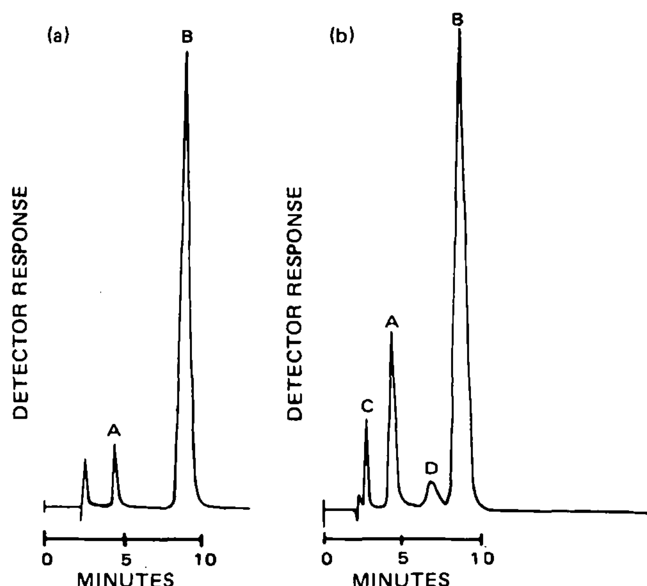

Figure 1—*Chromatograms of aspirin and salicylic acid (a) and a spiked aspirin sample (b) using a Zorbax-Sil column with chloroform–methylene chloride–acetonitrile–formic acid (700:300:30:4) at a flow rate of 2.0 mL/min. Detection was at 300 nm, 0.01 AUFS, with a recorder presentation of 128. Key: (A) salicylic acid; (B) aspirin; (D) acetylsalicylsalicylic acid; (C) acetylsalicylic acid anhydride.*

[1] Beckman Instruments, Model 110A.
[2] Micromeritics, Model 725.
[3] Laboratory Data Control, Spectromonitor III.
[4] Hewlett Packard, 3380A.
[5] Dupont Col, Zorbax-Sil.

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Table I—Assay Results on Commercial Aspirin Tablets

Product[a]	Aspirin, % of Claim		Salicylic Acid, %	
	HPLC	GC	HPLC	GC[b]
A	101.8	100.0	0.03	<0.30
B	98.9	97.8	0.05	<0.30
C	105.0	105.2	0.13	<0.30
D	99.7	100.3	0.06	<0.30
E	100.6	100.3	0.04	<0.30

[a] Products: A, Bayer; B, Empirin; C, Eckerd; D, Rite Aid; E, Treasury. [b] GC method determined by a 0.3% limit standard.

icylic acid detection. The detector sensitivity and the recorder presentation were adjusted so that the salicylic acid standard, containing 75 μg/mL, and the 2.5-mg/mL aspirin standard were both ~70% full scale. The pump flow rate was 2.0 mL/min. The relative standard deviation (n = 5) for the aspirin standard was <2.0%, and <2.0% for the salicylic acid standard at a concentration of 50 μg/mL. The retention times for salicylic acid, and aspirin were ~4 and 8 min, respectively (Fig. 1a).

Calculations—Integrated area counts were used to determine the amount of aspirin and salicylic acid in the assay preparation by external standard techniques. The aspirin was calculated as percent of label claim. The salicylic acid was calculated as a percentage of the theoretical aspirin content in the dosage form analyzed.

RESULTS AND DISCUSSION

The response linearity was suitable for the entire assay range, *i.e.*, aspirin 70–130% and salicylic acid 0.1–5.0%. The average relative standard deviation (n = 10) for the standard preparations was 0.48% for aspirin and 0.65% for salicylic acid. In most analyses a single point standard can be used for both aspirin and salicylic acid. Recoveries of salicylic acid and aspirin in spiked samples, standards, and placebos ranged from 97 to 102%. Recoveries of salicylic acid and aspirin from the chromatographic siliceous earth column have been previously reported (17). The rate of hydrolysis of aspirin to salicylic acid in the prepared samples and standards was 0.002%/h, which allows the preparation and storage of a large number of samples up to 24 h prior to injection without assay-induced hydrolysis. Since the aspirin and salicylic acid standards are injected separately into the liquid chromatograph, the rate of hydrolysis of the aspirin standard can be continuously monitored.

Tables I and II show the results of the analysis for plain and buffered tablets, respectively, by HPLC and GC. Results for the salicylic acid determination are reported as free salicylic acid in plain aspirin tablets and nonaspirin salicylates, in buffered tablets. Comparative analysis of the USP method to the GC method have been reported (17). Placebo tablet preparations of commonly used tablet excipients and binders showed no HPLC interferences.

The related compounds, acetylsalicylsalicylic acid and acetylsalicylic acid anhydride (Fig. 1b), are seldom seen in routine analysis. Salsalate has the same retention time as salicylic acid and, as in the current USP, is included as salicylic acid in the tablet analysis. Since the detector response for salicylic acid is ~20 times greater than aspirin at 300 nm, salicylic acid can be determined to the 0.02% level by use of integrated area counts.

The stability of aspirin in citric acid–chloroform has been previously described (15). One of the mechanisms for aspirin hydrolysis involves an intramolecular general base catalysis by the carboxylate anion. Citric acid, with a solubility in chloroform of 0.007 g/100 g, forms a more active carboxylate anion than aspirin, thereby competing more vigorously for trace amounts of water in the chloroform. In addition, a trace amount of hydronium ion from the citric acid has a stabilizing effect on the aspirin

Table II—Assay Results on Commercial Buffered Aspirin Tablets

Product[a]	Aspirin, % of Claim		Nonaspirin Salicylates, %	
	HPLC	GC	HPLC	GC
F	101.8	98.6	1.26	1.01
G	100.8	101.0	1.11	1.12
H	95.4	98.4	0.27	0.40
I	98.9	96.6	0.35	0.35
J	103.0	102.6	0.70	0.65

[a] Products: F, Treasury; G, Rite Aid; H, Bufferin; I, Bufferin Extra Strength; J. Eckerd.

Table III—Results of 10 Consecutive HPLC Analyses of the Same Assay Preparations, Spaced 20 Min Apart, for Plain and Buffered Aspirin Tablets

Run No.	Plain Tablets: Aspirin, %	Plain Tablets: Salicylic Acid, %	Buffered Tablets: Aspirin, %	Buffered Tablets: Nonaspirin Salicylates, %
1	102.2	0.12	97.8	0.80
2	102.6	0.16	97.6	0.81
3	102.1	0.12	97.8	0.81
4	102.1	0.13	96.9	0.81
5	102.0	0.12	97.7	0.81
6	103.0	0.13	98.2	0.81
7	101.6	0.12	97.5	0.81
8	102.5	0.12	97.1	0.81
9	101.4	0.12	97.1	0.82
10	101.4	0.13	97.4	0.81
SD	0.53	0.013	0.43	0.007

molecule. The results of 10 consecutive injections of the same assay preparations into the liquid chromatograph are shown in Table III. Recoveries of aspirin and salicylic acid from spiked placebos, samples, and standards are shown in Table IV.

Adsorption–Partition HPLC—Unlike bonded phases, silica columns are not subject to rapid deterioration. Silica columns used exclusively for a specific analysis can last for months, even years, as long as the column is kept clean. Washing the column with ~200 mL of methanol rids the active silanol sites of bound solutes. The composition of the mobile phase in the procedure described here requires no prior activation of the silica.

Chloroform and methylene chloride, the nonpolar solvents, are weakly held to the hydrogen-bonded water on the silica gel surface by disperson forces as a monolayer isotherm. Acetonitrile, a more polar solvent, forms a bilayer isotherm. The first layer is hydrogen bonded to water on the silica gel, and the second layer is formed by interacting with the first layer of the polar solvent (18–20). Under these circumstances the acetonitrile acts as the stationary phase proper. Therefore, the partitioning of the

Table IV—Aspirin and Salicylic Acid Recoveries from Spiked Placebos, Standards, and Assay Preparations

	Aspirin: Initial Amount, mg/mL	Aspirin: Amount Added, mg/mL	Total Recovery, %
Placebo			
1	0	.7640	101.0
2	0	1.9628	101.8
3	0	.2340	98.9
4	0	.4318	99.7
Standard preparation			
1	2.856	0.2946	99.8
2	2.5404	0.3975	101.0
3	2.183	0.5239	98.7
4	2.5204	0.5159	98.7
Assay preparation			
1	2.5027	0.2419	98.3
2	2.6511	0.6517	96.9
3	2.5052	0.5203	97.7
4	2.4180	0.3819	97.8

	Salicylic Acid: Initial Amount, μg/mL	Salicylic Acid: Amount Added, μg/mL	Total Recovery, %
Placebo			
1	0	1.506	101.0
2	0	3.012	101.0
3	0	4.518	99.8
4	0	7.530	99.8
Standard preparation			
1	7.80	10.40	98.1
2	26.00	10.40	99.4
3	52.00	10.40	100.0
4	78.00	10.40	100.2
Assay preparation			
1	34.06	9.92	99.3
2	41.27	9.92	98.6
3	27.04	9.92	98.2
4	16.80	9.92	99.2

solutes passing through the column is dependent on the interaction or displacement of the acetonitrile, rather than displacement of hydrogen-bonded water on the silica gel surface. The mass of the three solvents produces a stable and self-activating chromatographic system in equilibrium. The presence of 1% ethanol or methanol, commonly used preservatives in chloroform and methylene chloride, does not adversely affect retention times or resolution of the solutes.

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Particle Size and Surface Area Distributions of Pharmaceutical Powders by Microcomputerized Mercury Porosimetry

F. CARLI [x] **and A. MOTTA**

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Abstract □ The Mayer–Stowe theory was applied to derive the particle size distribution of powders of pharmaceutical interest using mercury porosimetry. Particle size data obtained by this approach are fairly comparable with data derived by other, more popular, techniques such as the electrical sensing zone or the air jet sieving methods provided that the experimental value of the mercury–powder contact angle and the state of aggregation of the powder are carefully studied. Furthermore, by applying the Rootare–Prenzlow method a surface area distribution can also be derived from the same porosimetry data used to obtain the particle size distribution. All experiments were carried out with a microcomputerized mercury porosimeter, which allows storage of data during the analysis and a subsequent fast elaboration at the end of the run, with fully printed data on pore size, pore volume, surface area, and particle size of the powder sample.

Keyphrases □ Mercury porosimetry—application to particle size and surface area distributions, pharmaceutical powders □ Particle size—distribution in pharmaceutical powders, determination by mercury porosimetry □ Surface area—particles in pharmaceutical powders, distribution, determination by mercury porosimetry

The particle size of pharmaceutical powders plays a major role both in drug processing and bioavailability. Poorly soluble drugs are often rendered more available for absorption by reducing the particle size, *i.e.*, increasing the surface area (1–3). On the other hand, very important technological processes such as the compression (4) or mixing of powders (5) are strongly influenced by the particle size of the materials used. Not only the powdered drugs, but also the excipients, exhibit many particle size- or surface area-related properties. For example, (a) the lubricating efficacy of materials such as magnesium stearate is strongly influenced by the surface area of the powder (6), and (b) the disintegrating properties of povidone derivatives are dependent on particle size (7).

The growing need for particle size and surface area analyses has led to the introduction of such methods for particle size analysis as microscopic counting (8), the electrical sensing zone method (8, 9), the air jet sieving technique (8, 10), and simple sieving (8). The surface area analysis methods used range from gas adsorption techniques (11, 12) to gas diffusion or permeability (6).

Although mercury porosimetry is used mostly to characterize the porous structure of materials (13, 14), it has been used to determine the surface area (15) and the particle size of powders, both in the coarse region (16, 17) and in the submicron range (18). In pharmaceutics mercury porosimetry has been used almost exclusively to study the porous structure of tablets (19–21), granules (22, 23), or polymeric matrices (24, 25); no attempt has been made to characterize the micromeritics of pharmaceutical powders (26). In this paper, we use mercury porosimetry to measure the particle size and the surface area distributions of powdered drugs and excipients.

THEORETICAL

Particle Size Determination—The mercury porosimetry principle, based on the Washburn model, consists of registering the volume of pores penetrated at each intrusion pressure, which can be easily transformed into pore size *via* the Washburn equation (27) to give a complete pore size distribution. An alternative model describing the penetration of mercury was proposed by Frevel and Kressley (28) and subsequently developed by Mayer and Stowe (29). This treatment defines the solid being pene-

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