Aspirin—A National Survey I: Semiautomated Determination of Aspirin in Bulk and Tablet Formulations and Salicylic Acid in Tablet Formulations

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Abstract □ A semiautomated assay to determine aspirin (acetylsalicylic acid) in aspirin tablets, powdered tablet composites, and pure drug substances is presented. The sample was dissolved in alcoholic buffer, diluted, and extracted with chloroform. The absorbance of the chloroform solution was read at 280 nm. Results obtained by the USP XIX and semiautomated methods are compared. The proposed method is accurate and precise, and common excipients do not interfere. Recoveries of 100% were obtained. A semiautomated assay for salicylic acid in aspirin tablets also is presented. The sample was dissolved in alcoholic buffer, ferric nitrate was added, and the absorbance of the resulting purple color was read at 532 nm. The method is suitable as a rapid screening procedure for testing the salicylic acid content of aspirin products. Results obtained by the USP XIX and semiautomated methods are compared, and the accuracy and precision of the proposed method are given. One hundred seventy tablet samples and 34 bulk drug substances were analyzed for aspirin and salicylic acid content. Approximately 5% of the tablet samples failed to meet the USP XIX limits for aspirin content, and 10% failed to meet the limits for salicylic acid.

Keyphrases □ Salicylic acid—semiautomated colorimetric determination in tablet formulations □ Analgesics—determination of aspirin and salicylic acid □ Aspirin—semiautomated UV determination in bulk and tablet formulations

A national survey of aspirin products and formulations was conducted in this laboratory. The survey consisted of the analysis of 170 samples of tablets, representing 58 formulations from 38 manufacturers, and 34 samples of bulk aspirin from 12 manufacturers. The purpose was to evaluate the quality of aspirin products on the market and the adequacy of present standards. Methodology was developed in these laboratories for the semiautomated analysis of the content uniformity test for aspirin (acetylsalicylic acid) and the limit test for salicylic acid, for the determination of impurities (1) and salicylic acid by high-pressure liquid chromatography (HPLC) (2), and for the determination of dissolution rates using semiautomated procedures (3). This paper presents the data on the determination of aspirin and salicylic acid.

Previously developed methods (4–11), including semiautomated procedures for the determination of aspirin (4, 5), were rejected for various reasons. The method of Hubin and Ganshirt (4) was not suitable because it involves the hydrolysis of aspirin itself. The method of Oydvin and Sapiraa (5) was not tried because it involves the use of chloroform, which is not a practical solvent to use in a semiautomated system with a liquid sampler. The Clayton and Thiers (6) manual spectrophotometric procedure determines up to five compounds simultaneously and thus appeared to be too complicated to automate. Other methods were eliminated because of the described problems or other practical considerations.

This paper describes a semiautomated UV method for the determination of aspirin and a semiautomated colorimetric method for the determination of salicylic acid. The aspirin method is applicable to plain and buffered tablets, powdered composites, and bulk drug substances and is free from the influence of excipient interferences. The salicylic acid procedure is based on the method of Cullen *et al.* (12) and can be used as a rapid screening assay. This method is applicable to plain, buffered, and pediatric aspirin tablets. Results are compared with the official USP XIX (13) procedures.

EXPERIMENTAL

Aspirin

Apparatus—An automatic analyzer with a sampler[1], pump[2], manifold, and timer[3] was used. The manifold was connected to a spectrophotometer[4] equipped with a quartz flowcell[5]. A 100-mv recorder[6] was connected to the spectrophotometer.

Reagents—Ethanol, 95%, was used. Chloroform, ACS grade, was washed with water and filtered through paper on the day of use. The pH 2.2 buffer solution was prepared by diluting 250.0 ml of 0.2 *M* KCl and 39.0 ml of 0.2 *M* HCl to 1 liter with water. The 0.2 *M* KCl was prepared by dissolving 14.911 g of potassium chloride in water and diluting to 1 liter with water. The 0.2 *M* HCl was prepared by diluting 17.0 ml of concentrated hydrochloric acid to 1 liter with water. The buffer–ethanol solution was prepared by mixing equal volumes of pH 2.2 buffer solution and ethanol.

Standard Preparation—About 324 mg of USP aspirin reference standard was weighed accurately and dissolved in 50.0 ml of buffer–ethanol (1:1). It was prepared fresh daily.

Sample Preparation—*324-mg Tablets*—One tablet was placed in a snap-cap vial or erlenmeyer flask. Three or four drops of water were placed on each tablet, and 50.0 ml of buffer–ethanol (1:1) was added. The solution was shaken or treated ultrasonically until the tablet disintegrated. Samples were analyzed within 6 hr of preparation. For buffered tablets, 1 ml of dilute hydrochloric acid (15:85 v/v) was added.

Powdered Composites or Bulk Drug Substances—An amount equivalent to 325 mg of aspirin was dissolved in 50.0 ml of buffer–ethanol (1:1). The sample was shaken mechanically or placed in an ultrasonic generator for ~5 min.

Semiautomated Determination—The automated system was assembled as shown in Fig. 1. The solutions were sampled at a rate of 30 cups/hr with a sample-to-wash ratio of 2:1. A sampling pattern of three standards, five samples, one standard, five samples, *etc.*, was used. Two cups of standard were placed at the end. The first two standards and the last standard were not included in the calculations. A polytetrafluoroethylene[7] strip was inserted into the BO fitting to direct the organic phase downward.

To start the system, ethanol was pumped through the chloroform pump tube for 5 min and then the tube was pumped dry. The chloroform line

[1] AutoAnalyzer sampler II, 127A000, Technicon Instruments Corp., Tarrytown, NY 10591.

[2] AutoAnalyzer proportioning pump III, 133-A014-04, Technicon Instruments Corp., Tarrytown, NY 10591.

[3] Flexopulse, model HG93A603, Eagle Signal Timer Division, Gulf Western Industries, Davenport, IA 52803 (14).

[4] Model DBGT, Beckman Instruments, Fullerton, CA 92634.

[5] The length was 10 mm and the capacity was 0.25 ml; model 8495-L10, Arthur H. Thomas Co., Philadelphia, PA 19105.

[6] Servo-Riter II, PS01W6A, Texas Instruments Inc., Houston, TX 77001.

[7] Teflon.

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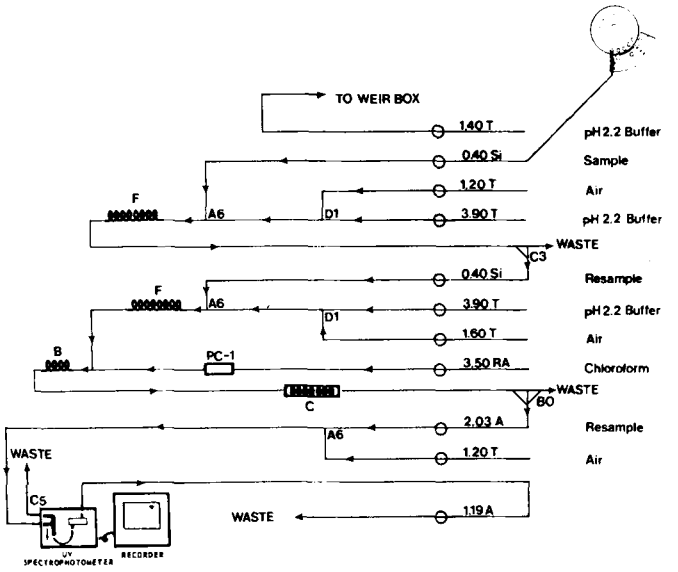

Figure 1—*Flow diagram of an automated system for aspirin. Key: Si, silicon pump tube; T, Tygon pump tube; RA, red Acidflex pump tube; A, Acidflex pump tube; F, 28-turn × 2.4-mm i.d. mixing coil; B, 28-turn × 2.4-mm i.d. mixing coil with one double end; and C, 5.5-turn settling coil.*

was placed in its solution and pumped until the solution reached the spectrophotometer cell. The pH 2.2 buffer line was placed in its solution, and the system was allowed to equilibrate. In the manifold, the sample stream was diluted twice with pH 2.2 buffer and chloroform was added. The chloroform containing the extracted drug was pumped through a flowcell, and the absorbance of the drug was read at 280 nm.

Salicylic Acid

Apparatus—The same type of automatic analyzer was connected to a spectrophotometer[8] equipped with a quartz flowcell[9] and a 100-mv recorder[6].

Reagents—Ethanol, 95%, was used. The pH 2.2 buffer solution was prepared as described. The ferric nitrate hexahydrate was prepared by adding 10 g of ferric nitrate and 5.0 ml of concentrated nitric acid to a 1-liter volumetric flask and diluting to volume with water.

Standard Preparation—*Plain Tablets*—About 20 mg of salicylic acid standard was weighed accurately and dissolved in 100 ml of pH 2.2 buffer–ethanol (3:1).

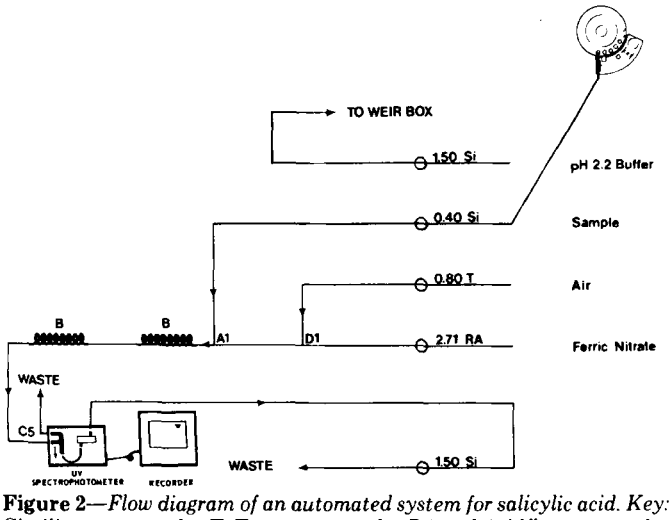

Figure 2—*Flow diagram of an automated system for salicylic acid. Key: Si, silicon pump tube; T, Tygon pump tube; RA, red Acidflex pump tube; and B, 28-turn × 2.4-mm i.d. mixing coil.*

[8] Model PM2DL, Carl Zeiss, Oberkochen, West Germany.

[9] The length was 10 mm and the capacity was 18 μl (model 886881), or the length was 10 mm and the capacity was 80 μl (model 886878); Beckman Instruments, Fullerton, CA 92634.

Table I—Comparison of Results (Percent of Label Claim) Obtained by USP XIX and Automated Procedures for Aspirin in Aspirin Tablets

Tablet	USP[a]	Automated[b]
324 mg, plain	101.2	101.5 (1.11, 1.09)[c]
324 mg, plain	97.9	99.0 (0.69, 0.70)
324 mg, buffered	98.2	100.1 (1.36, 1.36)
324 mg, buffered	95.3	94.2 (0.75, 0.79)
81 mg, pediatric	103.3	103.3 (0.75, 0.73)
81 mg, pediatric	105.4	106.1 (1.08, 1.01)

[a] Average of four results. [b] Average of 10 results. [c] Values in parentheses are standard deviations and coefficients of variation, respectively.

Buffered Tablets—About 200 mg of salicylic acid standard was weighed accurately and dissolved in 100 ml of pH 2.2 buffer–ethanol (3:1).

Sample Preparation—*Plain Tablets*—Ten tablets were placed in an erlenmeyer flask, 25 ml of buffer–ethanol (1:1) was added, and the flask was shaken for 5 min. Then 25 ml of pH 2.2 buffer was added and mixed. The solution was filtered through paper, and the first 10 ml was discarded.

Buffered Tablets—Ten tablets were placed in an erlenmeyer flask, 10 ml of ethanol was added, and the flask was shaken for 5 min. Then 40 ml of pH 2.2 buffer was added and mixed. The solution was filtered through paper, and the first 10 ml was discarded.

Semiautomated Determination—The automated system was assembled as shown in Fig. 2. The solutions were sampled at a rate of 30 cups/hr with a sample-to-wash ratio of 2:1. A sampling pattern of three standards, two samples, and two standards was used. The first two standards and the last standard were not included in the calculation.

To start the system, the pH 2.2 buffer and ferric nitrate reagent lines were placed in their respective solutions and the pump was started. The sample stream was introduced and then segmented with air, and ferric nitrate reagent was added. The absorbance of the colored solution was read at 532 nm.

RESULTS AND DISCUSSION

Aspirin—*Validation Tests*—A series of validation tests was performed on the automated system. A linear response was obtained when four standard solutions containing 3–13 mg of aspirin/ml (corresponding to 50–200% of declaration) were tested. A standard solution containing 6.5 mg of aspirin/ml gave reproducible peaks whose heights reached ~95% of the steady state. The assay of 20 individual cups of solution exhibited a relative standard deviation of 1.0%.

Hydrolysis—There was concern that the aspirin might hydrolyze to salicylic acid in the solvent, thus giving erroneous results. A sample solution of powdered aspirin tablet composite was prepared and assayed with freshly prepared aspirin standard solutions for 6 hr, and the assay results did not change significantly.

Composite Assays—Portions of tablet composites equivalent to single tablets were analyzed by the proposed method and the USP XIX (13) method. The ground tablet composites were prepared from available commercial samples. Table I shows the close agreement between the two methods and the precision of the automated procedure.

Interferences—Separate tests were performed to check for interferences from impurities, dyes, and common excipients. Two known impurities and one postulated impurity from aspirin formulations were tested: acetylsalicylsalicylic acid, acetylsalicylic anhydride, and *O*-salicylsalicylic acid. The interferences by impurities were measured by spiking standard solutions of aspirin with weighed amounts of each im-

Table II—Comparison of Results (Percent of Label Claim) Obtained by USP XIX and Automated Procedures for Salicylic Acid in Aspirin Tablets

Tablet	USP[a]	Automated[a,b]
324 mg, plain	0.073	0.089 (0.01)
324 mg, plain	0.036	0.050 (0.01)
324 mg, buffered	0.580	0.420 (0.05)
324 mg, buffered	3.400	3.480 (0.14)
81 mg, pediatric	0.230	0.620 (0.02)
81 mg, pediatric	0.264	0.421 (0.01)

[a] Average of three or more determinations. [b] Values in parentheses are standard deviations.

Table III—National Survey Results (Percent of Label Claim) for Aspirin and Salicylic Acid Determined by Automated and USP XIX Procedures

Manufacturer[a]	Type of Sample	Aspirin		Salicylic Acid	
		Automated[b]	USP	Automated[c]	USP
A	324-mg tablet	100.2 (2.2)		0.06	
	324-mg tablet	99.7 (2.3)		0.08	
	324-mg tablet	99.4 (2.2)		0.08	
	324-mg tablet	100.3 (2.2)		0.09	
	Bulk (10% starch)	89.5		—[d]	
B	Bulk (10% starch)	90.1		—[d]	
	324-mg tablet	100.1 (1.1)		0.16	
	324-mg tablet	102.0 (2.2)		0.22	
	421-mg tablet	101.5 (2.7)		0.14	
	421-mg tablet	100.3 (1.9)		0.19	
C	Bulk	99.7		—[d]	
	Bulk (10% starch)	90.3		—[d]	
	81-mg tablet	104.0 (2.9)		1.23	0.6
	324-mg tablet	98.7 (2.1)		0.07	
	324-mg tablet	100.5 (1.1)		0.04	
	324-mg tablet	98.6 (3.1)		0.07	
	324-mg tablet	102.0 (1.2)		0.05	
	160-mg tablet	100.6 (4.1)		0.78	
	324-mg tablet	97.2 (2.4)		0.05	
	324-mg tablet	101.4 (2.1)		0.15	
D	486-mg tablet[e]	99.1 (2.8)		0.24	
	486-mg tablet[e]	96.6 (3.0)		0.22	
	486-mg tablet[e]	94.4 (2.5)	92.0	0.33	
	486-mg tablet[e]	94.4 (3.6)	90.5	0.43	
	486-mg tablet[e]	98.1 (2.4)		0.32	
	324-mg tablet	95.8 (2.4)		0.35	
	324-mg tablet	101.4 (3.0)		0.46	
	324-mg tablet	97.6 (2.3)		0.42	
	324-mg tablet	97.8 (2.7)		0.36	
	Bulk	100.0		—[d]	
E	324-mg tablet	98.4 (4.2)		3.30	
F	Bulk (10% starch)	89.5		—[d]	
	324-mg tablet[e]	95.8 (2.3)		5.30	7.8
	324-mg tablet[e]	95.0 (4.3)	94.6	5.00	4.0
	324-mg tablet[e]	94.9 (3.1)	94.5	4.30	3.2
G	324-mg tablet	98.4 (2.1)		0.03	
	324-mg tablet	99.4 (1.7)		0.04	
	324-mg tablet	99.4 (2.2)		0.04	
H	81-mg tablet	99.2 (4.5)		0.54	
	Bulk	100.0		—[d]	
	Bulk	99.8		—[d]	
	324-mg tablet	100.5 (2.4)		0.03	
	324-mg tablet	100.4 (3.8)		0.03	
	324-mg tablet	98.2 (2.6)		0.03	
	324-mg tablet	97.0 (2.9)		0.03	
	324-mg tablet	99.7 (5.0)		0.03	
	81-mg tablet	99.9 (4.7)		0.24	
	81-mg tablet	104.6 (3.2)		0.40	1.0
I	81-mg tablet	99.6 (2.9)		2.70	2.5
	324-mg tablet	100.2 (1.3)		0.10	
	324-mg tablet	100.7 (2.9)		0.09	
	324-mg tablet	100.8 (1.8)		0.05	
	324-mg tablet	100.0 (1.3)		0.07	
J	324-mg tablet	100.1 (2.4)		2.07	
	Bulk (10% starch)	90.4		0.46	
K	Bulk	100.3		—[d]	
	324-mg tablet	101.6 (1.1)		0.04	
	324-mg tablet	100.7 (1.4)		1.87	
	81-mg tablet	80.4 (3.6)	79.4	2.07	1.5
	81-mg tablet	104.1 (4.2)		0.46	0.5
L	324-mg tablet[e]	101.4 (5.4)		2.20	
	324-mg tablet[e]	97.8 (3.1)		1.51	
	324-mg tablet[e]	97.2 (2.7)		1.68	
M	324-mg tablet	98.6 (2.0)		0.04	
N	324-mg tablet	97.9 (2.9)		0.06	
	324-mg tablet	97.9 (1.9)		0.05	
	324-mg tablet	97.0 (2.1)		0.04	
	Bulk	100.3		—[d]	
	324-mg tablet	96.7 (2.2)		0.05	
O	324-mg tablet	99.3 (2.7)		0.07	
	Bulk (10% starch)	89.5		0.28	
	324-mg tablet[e]	97.4 (1.8)		0.28	
	Bulk (10% starch)	90.0		—[d]	
P	324-mg tablet	98.1 (2.3)		0.07	
Q	Bulk	99.8		—[d]	
	324-mg tablet	97.7 (1.6)		0.40	0.3
	324-mg tablet	99.3 (1.9)		0.10	
	324-mg tablet	98.1 (1.8)		0.33	
R	324-mg tablet	100.6 (3.0)		0.07	
	324-mg tablet	99.4 (3.0)		0.08	
	324-mg tablet	103.1 (3.6)		0.14	
	324-mg tablet	101.8 (1.6)		0.06	
	324-mg tablet	101.3 (1.8)		0.10	
	Bulk	99.5		—[d]	
S	Bulk	99.5		—[d]	
	324-mg tablet	98.8 (2.1)		0.08	
	324-mg tablet	103.1 (3.3)		0.07	
	324-mg tablet	100.5 (1.8)		0.06	
	324-mg tablet	101.1 (3.2)		0.07	
	324-mg tablet	100.3 (2.0)		0.06	
	324-mg tablet	100.2 (1.9)		0.08	
	324-mg tablet	100.2 (1.9)		0.09	
	324-mg tablet	99.1 (2.2)		0.07	
	324-mg tablet	101.8 (2.0)		0.08	
	324-mg tablet	100.7 (2.1)		0.06	
T	81-mg tablet	102.3 (1.9)		0.26	0.5
	Bulk	99.8		—[d]	
	324-mg tablet[e]	96.9 (2.9)		2.00	
	324-mg tablet[e]	98.1 (3.2)		1.60	
	324-mg tablet[e]	97.9 (3.8)		1.13	
	Bulk (10% starch)	90.9		—[d]	
	324-mg tablet	97.8 (3.9)		—[f]	
	81-mg tablet	103.5 (2.0)		0.36	
U	324-mg tablet[e]	98.7 (3.7)		2.10	
	324-mg tablet[e]	99.6 (3.0)		3.70	4.1
	324-mg tablet[e]	99.0 (3.3)		3.10	
	324-mg tablet[e]	105.4 (3.9)	104.8	1.10	
	324-mg tablet[e]	101.2 (3.4)		1.80	
	81-mg tablet	100.7 (3.1)		0.40	0.6
	81-mg tablet	103.1 (4.8)		0.50	0.5
	81-mg tablet	104.5 (5.0)	100.4	0.50	0.4
	81-mg tablet	100.9 (3.8)		0.50	0.4
V	324-mg tablet[e]	97.5 (2.3)		0.77	
	324-mg tablet[e]	96.7 (2.5)		0.44	
	324-mg tablet[e]	96.6 (2.3)		0.68	
	324-mg tablet[e]	96.7 (2.3)		0.61	
	324-mg tablet[e]	98.4 (2.0)		0.50	
	81-mg tablet	102.8 (3.2)		0.62	0.6
	81-mg tablet	101.0 (3.0)		0.64	0.6
	81-mg tablet	99.3 (2.1)		0.59	0.6
	81-mg tablet	102.1 (2.4)		0.58	0.6
	Bulk (10% starch)	89.8		—[d]	
	Bulk	100.4		—[d]	
W	324-mg tablet	95.3 (5.2)		0.99	1.2
	324-mg tablet	103.0 (3.2)		0.09	
	Bulk	99.7		—[d]	
	324-mg tablet	98.0 (1.3)		0.05	
X	324-mg tablet	100.7 (1.4)		0.04	
	324-mg tablet	100.9 (1.6)		0.03	
	324-mg tablet	100.9 (1.5)		0.04	
	324-mg tablet	101.8 (1.4)		0.04	
	81-mg tablet	100.7 (2.4)		0.21	
	81-mg tablet	101.1 (2.6)		0.20	
	81-mg tablet	100.5 (2.8)		0.21	
	81-mg tablet	98.2 (2.6)		0.21	
	81-mg tablet	98.2 (2.1)		0.20	
	Bulk	99.5		—[d]	
	Bulk	99.5		—[d]	
Y	81-mg tablet	97.9 (2.5)		0.30	
	648-mg tablet	100.8 (2.0)		0.14	
	324-mg tablet	99.6 (1.6)		0.19	
	Bulk	99.8		—[d]	
	648-mg tablet	99.6 (1.4)		0.14	
	324-mg tablet	98.9 (1.9)		0.11	
	486-mg tablet	95.8 (2.0)		—[f]	
	324-mg tablet	98.3 (2.2)		0.12	
Z	Bulk	99.9		—[d]	
	324-mg tablet	96.2 (3.2)		0.08	
	486-mg tablet	98.4 (3.4)		0.03	
	486-mg tablet	98.8 (3.8)		0.07	
AA	Bulk (10% starch)	89.3		—[d]	
	300-mg tablet	101.9 (2.6)		0.24	0.2
	81-mg tablet	97.8 (3.3)		0.31	
BB	324-mg tablet	101.6 (2.1)		0.40	
	Bulk	99.9		—[d]	
CC	Bulk (10% starch)	88.0		—[d]	
	324-mg tablet[e]	99.3 (4.7)		0.90	
	324-mg tablet[e]	97.7 (3.0)		1.20	

(continued)

Table III—*Continued*

Manufacturer[a]	Type of Sample	Aspirin Automated[b]	Aspirin USP	Salicylic Acid Automated[c]	Salicylic Acid USP
DD	324-mg tablet[e]	98.9 (2.2)		2.03	
	324-mg tablet[e]	96.6 (3.2)		1.23	
	324-mg tablet[e]	98.1 (2.4)		2.90	
EE	81-mg tablet	100.7 (1.8)		0.14	
	81-mg tablet	100.6 (4.0)		0.15	
	81-mg tablet	100.9 (2.7)		0.19	
	81-mg tablet	100.8 (2.3)		0.18	
	325-mg tablet	101.6 (1.7)		0.06	
	325-mg tablet	101.9 (1.4)		0.06	
	325-mg tablet	101.2 (1.5)		0.05	
	325-mg tablet	100.6 (2.0)		0.04	
	325-mg tablet	100.0 (1.8)		0.05	
	Bulk	100.4		—[d]	
FF	324-mg tablet	100.0 (2.0)		0.08	
	324-mg tablet	100.2 (2.2)		0.06	
	324-mg tablet	97.8 (2.5)		0.06	
	324-mg tablet	98.9 (2.1)		0.09	
	Bulk (10% starch)	91.4		—[d]	
GG	324-mg tablet[e]	97.9 (3.1)		1.34	
	324-mg tablet[e]	97.0 (3.7)		2.03	
	81-mg tablet	104.0 (3.5)		0.27	
	Bulk	78.3		—[d]	
	324-mg tablet[e]	97.9 (1.9)		1.13	
	81-mg tablet	97.8 (2.7)		0.61	
	324-mg tablet	97.2 (2.0)		0.35	
HH	320-mg tablet	99.4 (2.0)		0.04	
	320-mg tablet	98.6 (2.0)		0.05	
	65-mg tablet	99.9 (2.5)		0.32	
	Bulk (10% starch)	89.5		—[d]	
II	324-mg tablet	97.4 (2.5)		0.51	
	324-mg tablet	99.8 (3.1)		0.65	
	Bulk	80.7		—[d]	
JJ	324-mg tablet	98.5 (2.3)		0.07	
	81-mg tablet	97.0 (1.8)		0.28	
	81-mg tablet	97.7 (1.8)		0.29	
	324-mg tablet	101.9 (3.0)		0.04	
	324-mg tablet[e]	99.4 (1.9)		0.04	
KK	486-mg tablet[e]	102.4 (3.4)		0.87	
	486-mg tablet[e]	101.7 (3.1)		0.96	
	486-mg tablet[e]	100.0 (2.0)		0.45	
	486-mg tablet[e]	99.4 (2.8)		0.71	
	486-mg tablet[e]	100.4 (2.6)		0.84	
LL	325-mg tablet[e]	95.9 (3.5)		1.13	
	Bulk	100.3		—[d]	
	325-mg tablet[e]	97.3 (2.4)		1.22	
	325-mg tablet[e]	97.4 (3.4)		1.03	

[a] A = Bell Pharmacal, Greenville, S.C.; B = Block Drug Co., Memphis, Tenn.; C = Bowman Pharmaceuticals, Canton, Ohio; D = Bristol-Myers Co., New York, N.Y.; E = Chromalloy American Corp., Culver City, Calif.; F = Otis Clapp & Sons, Boston, Mass.; G = Cord Laboratories, Detroit, Mich.; H = Davis Manufacturing Co., Knoxville, Tenn.; I = Dewey Products Co., Grand Rapids, Mich.; J = Ferndale Laboratories, Ferndale, Mich.; K = Freeda Vitamins, New York, N.Y.; L = ICN Pharmaceuticals, Cincinnati, Ohio; M = Lannett Co., Philadelphia, Pa.; N = Eli Lilly & Co., Indianapolis, Ind.; O = Mallard, Detroit, Mich.; P = Manhattan Drug Co., Hillside, N.J.; Q = Marshall Pharmacal Corp., South Hackensack, N.J.; R = McKesson Laboratories, Fairfield, Conn.; S = Norwich-Eaton Pharmaceuticals, Norwich, N.Y.; T = Oak Park Pharmaceuticals, Fredonia, Wis.; U = Pennex Products Co., Pittsburg, Pa.; V = L. Perrigo Co., Allegan, Mich.; W = Pill Mill, Grand Rapids, Mich.; X = Plough, Memphis, Tenn.; Y = Rexall Drug Co., St. Louis, Mo.; Z = Richlyn Laboratories, Philadelphia, Pa.; AA = Sein-Mendez Labs, Rio Piedas, Puerto Rico; BB = Stanback Co., Salisbury, N.C.; CC = Standard Pharmacal Co., Chicago, Ill.; DD = Stanley Laboratories, Portland, Ore.; EE = Sterling Drug, New York, N.Y.; FF = E. R. Squibb & Sons, New York, N.Y.; GG = Sun Laboratories, Portland, Ore.; HH = Vale Chemical Co., Allentown, Pa.; II = Walgreen Co., Chicago, Ill.; JJ = West-Ward, Eatontown, N.J.; KK = Whitehall Laboratories, New York, N.Y.; and LL = Zenith Laboratories, Hoboken, N.J. [b] Average of 30 results for tablets and one assay for bulk formulations. Numbers in parentheses are standard deviations. [c] Composite assays, 10 tablets. [d] Not determined by automated method. [e] Buffered tablets. [f] No result obtained.

purity and comparing the response with that from the standard solution. No interferences were found.

Colorimetric interference from the dye in a manufactured tablet was checked by adding chloroform to an aqueous sample solution of the tablet in a separator and shaking manually. All of the color from the dye remained in the aqueous layer. Since the drug is extracted into the chloroform layer on the automated system, there is no interference from the dye at 280 nm.

Common excipients were examined and found not to interfere. A recovery of 99.9% was obtained with a mixture of 90% aspirin and 10% starch. A mixture of aspirin, cornstarch, lactose, and magnesium stearate gave a recovery of 99.8%. With a mixture of aspirin and magnesium carbonate, the recovery was 101.0%.

Evaporation Test—Since 50% ethanol was the sample solvent, the possibility of evaporation during the time required to analyze a number of solutions on the automated system was investigated. Twenty cups of a sample solution were loaded on the system and analyzed, and then three fresh cups were loaded just after sampling the 20th cup. There was no difference in absorbance between the first 20 cups and the last three cups. Thus, there was little evaporation over a 1-hr period.

Solvent Selection—Several solvents were tested for this method, including 95% ethanol, pH 2.2 buffer–ethanol (1:1), and buffer–ethanol (3:1). Results indicated that the buffer–ethanol (1:1) was the best solvent. If the alcohol concentration was too low, the aspirin did not completely dissolve. If the pH of the sample solution was too high, aspirin was hydrolyzed to salicylic acid.

Buffered Aspirin Preparation—With some buffered aspirin formulations, it was necessary to add acid to decrease the pH of the solution to that of the standard solution to prevent low recoveries. Usually 1 ml of dilute hydrochloric acid was sufficient.

Salicylic Acid—*Validation Tests*—A series of validation tests was performed on the automated system. A linear response was obtained when solutions of salicylic acid standard containing 0.1–0.4 and 1.0–4.0 mg/ml were tested. The absorbance of 30 individual cups of solution exhibited a relative standard deviation of 1.4%.

Composite Assays—Portions of tablet composites equivalent to 10 tablets were analyzed by the proposed method and the USP XIX method. The ground tablet composites were prepared from available commercial samples. Table II shows the results obtained by both methods and the precision of the automated procedure.

Recovery of Standards—Recovery assays, using the proposed method, were made on simulated mixtures of aspirin based on the manufacturers' formulations. Recoveries were obtained by spiking standard solutions of salicylic acid with weighed amounts of excipient materials and comparing the results with those from the standard solution. A recovery of 101.0% salicylic acid was obtained from a mixture of 90% aspirin and 10% starch. A mixture of aspirin, cornstarch, lactose, and magnesium stearate gave a recovery of 100.7% salicylic acid.

Interferences—Interference from the impurities acetylsalicylsalicylic acid, acetylsalicylic anhydride, and *O*-salicylsalicylic acid was measured by spiking standard solutions of salicylic acid with weighed amounts of each impurity and comparing the response with that of the standard solution. No impurity interference was found.

Solvent Selection—Aspirin is stable at pH 2.2 but is not very soluble in a pH 2.2 buffer; ethanol is needed to increase the solubility. When a solvent containing >25% ethanol was used, a brown hazy solution resulted when the ferric nitrate reagent was added. Cullen *et al.* (12) reported that a violet color resulted with the addition of ferric nitrate. A series of tests using different percentages of ethanol was performed to obtain the violet color. As a result, 25% ethanol in pH 2.2 buffer was selected as the solvent. Buffered tablets first were dissolved in 95% ethanol. When they were dissolved in 50% ethanol, a mass of undissolved flocculent material formed, which made it difficult to obtain sufficient filtered solution for analysis on the automated system.

Sensitivity Adjustment—Different levels of salicylic acid were found in plain as opposed to buffered tablets. Therefore, it was necessary to use a spectrophotometer with adjustable sensitivity.

Filtering—All solutions of plain or buffered tablets were filtered through paper since insoluble excipient materials present in the sample solutions interfered with the automated procedure.

Hydrolysis—Because of possible hydrolysis of the aspirin to salicylic acid in the solvent, tests were performed to check the hydrolysis. Aspirin was added to a standard salicylic acid solution, and this solution was compared to the unspiked standard solution. There was no detectable hydrolysis on the automated system. To limit the hydrolysis that would occur with time, all solutions were analyzed within 30 min after the solvent addition to the tablets.

Table III lists the results obtained from the national survey by the automated methods for aspirin and salicylic acid. It also shows comparisons with the USP methods for some samples. One hundred seventy tablet samples were analyzed for aspirin and salicylic acid content, and 34 bulk drug substances were analyzed for aspirin content. Four tablet samples failed to meet the USP XIX limits for aspirin content. Eighteen tablet samples failed to meet the USP XIX limits for salicylic acid content.

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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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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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