and solving for k_{M_1} reduces to Eq. 11 of the text. This equation permits the calculation of the metabolic rate constant (k_{M_1}) for the oxidation of nalidixic acid to hydroxynalidixic acid.

REFERENCES

(1) Wagner, J., *J. Pharm. Sci.*, **50**, 359(1961).
(2) Wagner, J., and Nelson, E., *ibid.*, **53**, 1392(1964).
(3) Nelson, E., *ibid.*, **50**, 181(1961).
(4) Levy, G., *Arch. Intern. Pharmacodyn.*, **152**, 59(1964).
(5) Freeksen, E., ed., "Antibiotica et Chemotherapia," vol. 12, S. Karger, New York, N. Y., 1964.
(6) Moore, W. E., *et al.*, *J. Pharm. Sci.*, **54**, 36(1965).
(7) McChesney, E. W., *et al.*, *Toxicol. Appl. Pharmacol.*, **6**, 292(1964).
(8) Wagner, J. G., and Nelson, E., *J. Pharm. Sci.*, **52**, 610(1963).
(9) Portmann, G. A., *et al.*, *ibid.*, **55**, 59(1966).

Drug Standards

Determination of Free Salicylic Acid in Aspirin and Aspirin Products

By JOHN D. WEBER and JOSEPH LEVINE

In a previously described method for the determination of free salicylic acid in aspirin, the salicylic acid is isolated on a partition chromatographic column as its purple ferric–phenol complex, using ferric chloride solution as immobile phase. With the addition of a high concentration of urea to the ferric chloride, the method is significantly improved. The separation is more sharply defined, making feasible the analysis of larger samples of aspirin and permitting the use of a more easily prepared chromatographic column.

A PROCEDURE has been described (1) for the isolation and determination of small amounts of salicylic acid which occur in aspirin and aspirin products. The salicylic acid is retained on a Celite:2% ferric chloride partition chromatographic column as its ferric complex while the nonphenolic aspirin is eluted with chloroform. The ferric complex is then dissociated with acetic acid and the free salicylic acid eluted with chloroform.

Several investigators have encountered difficulty with the published method (2). During the elution of the aspirin, the salicylic acid migrates slowly down the column (as evidenced by the position of the purple complex) and spreads out into a diffuse band, which sometimes becomes difficult to discern. Unless the chromatographic column is packed with great uniformity, channeling may occur during both the elution of aspirin and the recovery of salicylic acid.

A radical improvement in the chromatographic separation is achieved with a modified ferric chloride reagent, which contains a high concentration of urea. The band of the ferric–salicylate complex obtained with this reagent is much more deeply colored than that obtained with the simple ferric chloride reagent. The dense, sharply delineated band migrates only very slightly during the elution of aspirin. The use of shorter columns, which do not require extraordinary care in packing, is therefore feasible. Columns prepared with the modified reagent will also accommodate much larger samples of aspirin than those prepared with ferric chloride alone.

Addition of urea to the ferric chloride reagent was suggested from the report of a urea–salicylic acid complex by Bolton (3). It is apparent that the formation of this binary complex does not account for the trapping of salicylic acid by the ferric chloride–urea reagent, however, since urea solutions alone, at any concentration, are completely ineffective in removing salicylic acid from chloroform solution.

Optimum results are obtained with an immobile phase containing 5% ferric chloride and which is 10 *M* with respect to urea. It must be maintained at a pH between 3.1 and 3.3. At lower pH levels the salicylic acid band becomes diffuse and more loosely retained, while at higher levels recovery of salicylic acid from the column may be incomplete using the specified volume of eluant.

The concentration of urea in the reagent must

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be 10 *M* to maintain dense salicylic acid bands during the elution of the aspirin. A quantity of salicylic acid which forms a zone 5 mm. deep with this reagent, for example, forms a zone spread throughout the column when the concentration of urea in the immobile phase is reduced to 7.5 *M*. In the absence of aspirin, the effect of the urea concentration is minor. Dense bands are obtained with urea concentrations over a range of 2.5 to 10 *M*.

It has been shown (1) that only dilute solutions of ferric chloride remove salicylic acid from chloroform solution; the U.S.P. ferric chloride test solution (9%) is virtually ineffective. In contrast, the effectiveness of the urea–ferric chloride reagent in maintaining a dense salicylic acid band increases with increase of ferric chloride concentration over a range of 1 to 15%. The color of the reagent at the higher concentrations deepens, however, lessening the visual contrast between its color and that of the salicylic acid complex. A concentration of 5% ferric chloride was selected as optimum for providing suitable color contrast while maintaining the compactness of the salicylic acid band on the column.

PROCEDURE

Reagent.—To 60 Gm. of reagent grade urea, add 8 ml. of 60% ferric chloride solution[1] and 42 ml. of 0.05 *N* HCl. Shake, without heating, to dissolve the urea. The pH of freshly prepared solution is about 3.2. It should be checked on a pH meter daily, and, as necessary, adjusted to between pH 3.1 and 3.3 by dropwise addition of 6 *N* HCl.

Salicylic Acid Standard.—Accurately weigh about 25 mg. of salicylic acid and dissolve in 100.0 ml. of chloroform. Transfer 5.0 ml. to a 100-ml. volumetric flask; add 2 ml. of glacial acetic acid, 20 ml. of ether, 20 ml. of methanol, and 4 drops of hydrochloric acid. Dilute to volume with chloroform.

Chromatographic Tube.—A 25- × 250-mm. test tube to which is attached a 50-mm. length of 6- or 8-mm. tubing. The tamping rod consists of a disk of stainless steel, aluminum, or glass, of a diameter 1 mm. less than that of the column, attached to a rod 12 to 18 in. long. Pack fine glass wool[2] in the base of the column as support.

Preparation of Trap Column.—To 3 Gm. of Celite 545[3] add 2 ml. of *Reagent*. Mix thoroughly, transfer to the column, and tamp, using gentle pressure, to a uniform mass. Cover with a pad of cotton about 20 mm. thick. (This insures uniform distribution of the solvent over the column cross section.)

Procedure.—Use water - saturated solvents throughout.

Aspirin and Aspirin Tablets.—Weigh a quantity of sample, equivalent to about 200 mg. of aspirin, into a beaker. Add 10 ml. of chloroform and stir for 3 min. to dissolve the sample. Transfer the solution to the column, rinsing the beaker with 10 ml. of chloroform in small portions. Wash the column successively with 10- and 50-ml. portions of chloroform to remove the aspirin. Rinse the tip of the column with a jet of chloroform. (If the purple zone reaches the bottom of the column, repeat with a smaller sample of aspirin.)

Place as receiver under the column a 50-ml. volumetric flask containing 10 ml. of methanol and 2 drops of hydrochloric acid. Elute the column with 10 ml. of ether containing 1 ml. of glacial acetic acid, followed by 30 ml. of chloroform, and dilute to volume with chloroform. Concomitantly determine the absorbance of this solution and of the standard solution at the maximum at about 306 mμ.

APC Tablets and Flavored Tablets.—Mount in series directly above the trap column a second column containing a pad of cotton about 20 mm. deep. Dissolve the sample as described above and transfer it to the upper column with the aid of 10 ml. of chloroform. Pass 20 ml. of chloroform over the columns; discard the upper column, and continue as described under *Aspirin and Aspirin Tablets*, beginning with "Wash the column successively with 10- and 50-ml. portions of chloroform."

DISCUSSION AND RESULTS

A moderate amount of urea is eluted together with the salicylic acid during the recovery of the latter from the column. Hydrochloric acid is added to insure acidity of the eluate, and methanol to achieve miscibility of the acid with the eluting solvent. The absorbance maximum of the resulting solution is at 306 mμ, as compared with the wavelength of 310 mμ in the absence of methanol. The standard reference solution is therefore prepared to have the same solvent composition as the eluate.

No measurable degree of hydrolysis of aspirin occurs during the analysis. The salicylic acid fraction obtained from 500-mg. samples of purified aspirin had an absorbance of 0.003, equivalent to about 0.001% of salicylic acid.

Measured amounts of salicylic acid were added to powdered aspirin U.S.P. and to powdered commercial tablets. Recoveries are presented in Tables I and II, respectively. The latter values show that the excipient materials present in these commercial formulations do not interfere with the procedure.

A number of samples of buffered aspirin tablets were assayed, employing the preliminary boric acid treatment described in the earlier procedure (1). Because of the precision of the analyses, it was thought that these represented the true content of free salicylic acid of the tablets. However, following a suggestion (4) that boric acid might not be fully effective in liberating salicylic acid from the buffering agents, it was indeed found that recovery of salicylic acid added to aluminum hydroxide gel, calcium carbonate, and magnesium trisilicate, which are commonly used in these formulations, was incomplete. A methanolic solution of oxalic acid effected complete recovery of salicylic acid from its calcium salt while producing only a negligible degree of hydrolysis of aspirin. However, neither this nor any other reagent thus

[1] Commercial reagent.
[2] Pyrex Filtering Fibre, Corning Glass catalog No. 3950.
[3] Johns-Manville Corp.

TABLE I.—STANDARD RECOVERIES OF SALICYLIC ACID ADDED TO ASPIRIN

Aspirin, mg.	Salicylic Acid Added, mg.	Total Wt. Salicylic Acid Found	Corrected Wt. Salicylic Acid Found, mg.	Recovery, %
218.5[a]	None	0.045 (0.020%)[c]	...	...
489.7[a]	None	0.093 (0.019%)	...	...
1005.4[a]	None	0.179 (0.018%)	...	...
1020.9[b]	None	0.225 (0.022%)	...	...
1005.6[b]	None	0.225 (0.022%)	...	...
1004.2[b]	None	0.225 (0.022%)	...	...
201.3	0.129	0.170	0.132	102.3
501.8	0.129	0.228	0.133	103.1
202.3	0.257	0.295	0.257	100.0
204.0	0.257	0.295	0.256	99.6
203.9	0.514	0.552	0.513	99.8
201.9	0.514	0.555	0.517	100.6
200.9	0.771	0.802	0.764	99.1
209.5	0.771	0.805	0.765	99.2
51.2	2.056	2.076	2.066	100.5
54.7	2.056	2.079	2.069	100.6

[a] Freshly opened container of powdered aspirin U.S.P. [b] Same sample 11 months after having been opened. [c] The accuracy at this level is limited by the reading of the spectrophotometer scale. The absorbance of the salicylic acid fraction in this analysis was 0.026; a deviation of ±0.001 would be equivalent to a deviation of ±4%. The accuracy at this level can be increased by increasing the size of the sample.

TABLE II.—STANDARD RECOVERIES OF SALICYLIC ACID ADDED TO COMMERCIAL TABLETS

Product	Sample Wt., mg.	Wt. Aspirin in Sample, mg.	Salicylic Acid Added, mg.	Total Wt. Salicylic Acid Found, mg.	Corrected Wt. Salicylic Acid Found, mg.	Recovery, %
5 gr.	234.0	196.9	...	0.096 (0.049%)	...	...
aspirin	253.8	213.6	...	0.105 (0.049%)	...	...
tablets	249.3	209.8	0.608	0.701	0.598	98.4
U.S.P.	251.8	211.9	0.608	0.701	0.597	98.2
	247.8	208.5	0.243	0.344	0.242	99.5
	250.0	210.4	0.243	0.341	0.238	97.9
1¼ gr.	548.7	195.5	...	0.111 (0.057%)	...	...
children's	555.0	197.8	...	0.113 (0.057%)	...	...
aspirin	538.3	191.8	0.489	0.596	0.487	99.6
	541.8	193.1	0.498	0.598	0.488	99.8
APC tablets	543.8	244.7	...	0.159 (0.114%)	...	...
N.F. (3.5	453.5	204.0	0.489	0.719	0.486	99.4
gr. aspirin)	420.3	189.1	0.489	0.693	0.477	97.6

far tested quantitatively releases salicylic acid from aluminum hydroxide gel without causing extensive hydrolysis of aspirin.

Analyses of buffered tablets using the oxalic acid reagent had a precision equal to that obtained with boric acid, and uniformly indicated a higher free salicylic acid content. Examination of the insoluble residue showed, however, that these analyses did not represent the entire nonaspirin salicylate content of the tablets. Despite this incomplete recovery, 4 of 7 samples analyzed gave values in excess of 1% salicylic acid.

TABLE III.—ANALYSIS OF COMMERCIAL SAMPLES

Sample	Salicylic Acid Found, %
Aspirin powder, U.S.P.	0.018, 0.019, 0.020
Aspirin tablets, 5 gr. U.S.P.	
Brand 1	0.045, 0.043
Brand 2	0.019, 0.020, 0.020
Brand 3	0.076, 0.078, 0.070
Brand 4	0.125, 0.122, 0.124
Brand 5	0.285, 0.280, 0.280
Aspirin tablets, flavored 1¼ gr.	
Brand 1*a*	0.199, 0.209, 0.196
Brand 1*b*	0.180, 0.168, 0.170
Brand 2*a*	0.094, 0.091, 0.091
Brand 2*b*	0.057, 0.057
Brand 3	0.534, 0.528, 0.531
APC tablets	
Brand 1	0.046, 0.044, 0.045
Brand 2	0.047, 0.045, 0.044
Brand 3	0.115, 0.120, 0.121
Brand 4	1.04, 1.06, 1.07
Brand 5	0.262, 0.264, 0.259

Aspirin products of various brands, both local and nationally advertised, were obtained from local retail pharmacies and food markets. The samples were purchased directly from the stock on the shelves; no information is available on their age. The analyses of these samples are presented in Table III.

Three of 5 samples of 5-gr. aspirin tablets were well within the U.S.P. XVII limits of 0.15% salicylic acid; 1 approached this value, and 1 exceeded it. Three of 5 samples of 1¼ gr. of children's flavored aspirin tablets exceeded this limit.

Four of the 5 samples of aspirin, phenacetin, and caffeine tablets were well within the N.F. XII limits of 0.75%, while a fifth, which had a loose bottle cap at the time of purchase, exceeded the limits.

REFERENCES

(1) Levine, J., *J. Pharm. Sci.*, **50**, 506(1961).
(2) Personal communication.
(3) Bolton, S., *J. Pharm. Sci.* **52**, 1071(1963).
(4) Auerbach, M. E., personal communication.