of influenza virions, the virions were coated to a certain extent by the polymer.

3. The extent of coating was dependent on the monomer concentration before polymerization.

4. Influenza virions were adsorbed by previously polymerized poly(methyl methacrylate) particles.

REFERENCES

(1) J. Kreuter and P. P. Speiser, *Infect. Immun.*, **13**, 204(1976).

(2) F. W. Sheffield, W. Smith, and G. Belyavin, *Br. J. Exp. Pathol.*, **35**, 214(1954).

(3) E. H. Riddle, "Monomeric Acrylic Esters," Reinhold, New York, N.Y., 1954, p. 15.

(4) K. Tessmar, in "Methoden der Organischen Chemie," (Houben-Weyl), vol. XIV/1, E. Müller, Ed., Makromolekulare Stoffe, G. Thieme-Verlag, Stuttgart, Germany, 1961, p. 1037.

(5) G. K. Hirst, *J. Exp. Med.*, **75**, 47(1942).

(6) O. Bonin, "Quantitativ-Virologische Methodik," G. Thieme-Verlag, Stuttgart, Germany, 1973, p. 203.

(7) O. Bonin, *ibid.*, p. 227.

(8) G. Birrenbach and P. P. Speiser, *J. Pharm. Sci.*, in press.

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Rapid Spectrophotometric Determination of Salicylamide in Analgesic Tablets

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Abstract □ An independent, simple, and rapid procedure is suggested for the routine analysis of salicylamide in analgesic tablets containing acetaminophen, phenobarbital, caffeine, codeine phosphate, prednisone, ascorbic acid, and chloroquine phosphate. The method does not require the preliminary separation of salicylamide from other constituents by the time-consuming solvent extraction technique or by chromatography prior to determination. The absorbance was linear for investigated concentrations of salicylamide from 0 to 4.0 mg/100 ml of solution at 308 nm.

Keyphrases □ Salicylamide—spectrophotometric analysis in tablets containing other drugs □ Spectrophotometry—analysis, salicylamide in tablets containing other drugs □ Dosage forms—multicomponent analgesic tablets, spectrophotometric analysis of salicylamide □ Analgesics—salicylamide, spectrophotometric analysis in tablets containing other drugs

Salicylamide, an analgesic, antipyretic, and antirheumatic drug, can be determined by visual titration in dimethylformamide against standard sodium methoxide solution with thymol blue indicator (1). Although this method is perfectly suitable for the determination of the drug in pure form, it is totally unsuitable for selective determination in the presence of such acidic substances as acetaminophen, phenobarbital, and codeine phosphate.

Salicylamide can be determined colorimetrically by several methods. The color produced when salicylamide reacts with ferric nitrate in the presence of nitric acid (2) has been the basis for colorimetric determination of the drug after its separation from sodium salicylate and sodium gentisate by extraction with ether. The same reaction was employed for colorimetric determination of salicylamide in serum and urine (3), but after hydrolysis with hydrochloric acid and extraction in ethylene dichloride. Measurements of absorbance were performed at 450 nm. Use of the color resulting from the condensation reaction of salicylamide with *p*-amino-*N*,*N*-dimethylaniline sulfate in the presence of potassium ferricyanide (4) was suggested.

A spectrophotometric procedure was described (5) for the simultaneous determination of five analgesic compounds including salicylamide. Absorbance was measured at three different wavelengths and under three different conditions of acid and base content. Salicylamide also was determined by differential spectrophotometry (6) in the presence of aspirin, acetaminophen, and caffeine. A spectrophotofluorometric method (7) was reported for the simultaneous determination of salicylamide and salicylic acid in blood serum and urine after acid hydrolysis of the salicylamide metabolites.

Salicylamide was determined bromometrically (8) in anhydrous acetic acid by adding aceteous bromine solution and determining unreacted bromine by adding potassium iodide and titrating against thiosulfate.

The objective of this study was to develop a rapid routine analytical method for salicylamide in analgesic tablets containing acetaminophen, phenobarbital, caffeine, codeine phosphate, prednisone, ascorbic acid, and chloroquine phosphate which would be useful for pharmaceutical control purposes.

EXPERIMENTAL

Chemicals—The following were used: salicylamide[1], acetamino-

[1] El-Nasr Pharmaceutical Chemicals Co., A.R.E.

Table I—Absorbance of Salicylamide in Chloroform at 308 nm

Concentration, mg/100 ml	Average Absorbance[a]
0.5	0.158
1.0	0.318
1.5	0.473
2.0	0.628
2.5	0.776
3.0	0.932
3.5	1.060
4.0	1.236

[a] Average of three readings.

phen[2], phenobarbital[3], codeine phosphate[4], caffeine[5], prednisone[6], ascorbic acid[6], chloroquine phosphate[6], and chloroform[1].

Calibration Curve—Prepare a stock solution of salicylamide in chloroform containing 50.0 mg/100.0 ml. Transfer by pipets 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 ml of the stock solution to eight 100-ml volumetric flasks, dilute to volume with chloroform, and mix well. These working solutions contain 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mg of salicylamide/100.0 ml of solution, respectively.

Measure the absorbance[7] of each solution in 1-cm cells against a proper blank at 308 nm (Table I). These values give a straight line whose equation was determined by the method of least squares.

Sample Preparation and Assay—Weigh and powder 20 tablets. Weigh an aliquot of the powder corresponding to 50.0 mg of salicylamide and transfer quantitatively to a 100-ml volumetric flask. Add about 70 ml of chloroform, shake for about 30 min, and dilute to volume with the same solvent. Filter through paper, to remove excipients and chloroform-insoluble materials, into a suitable dry receiver, discarding the first portion of the filtrate.

Accurately measure 5.0–6.0 ml of the filtrate, transfer into a 100-ml volumetric flask, dilute to volume with chloroform, and mix. Measure the absorbance as described previously. Calculate milligrams of salicylamide in the aliquot of powder taken from the straight-line equation using the measured absorbance.

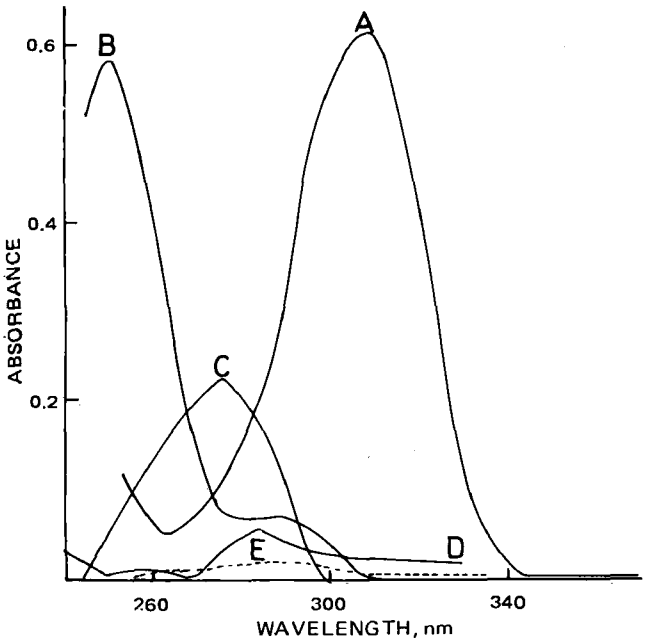

Figure 1—*Absorbance spectra. Key: A, salicylamide (2.0 mg %); B, acetaminophen (4.0 mg %); C, caffeine (0.5 mg %); D, phenobarbital (2 mg %); and E, codeine phosphate (0.125 mg %).*

[2] Rhone Poulenc, France.
[3] Alkaloid, Hungary.
[4] Macfarlan Smith, Germany.
[5] China.
[6] BP grade.
[7] Prolabo Jean et Constant UV spectrophotometer.

Table II—Recoveries of Salicylamide by the Proposed Procedure

Added, mg	Found, mg	Recovery, %
20.1	19.7	98.0
30.2	30.2	100.0
39.8	41.0	103.3
50.3	50.8	100.9
60.0	60.4	100.7
70.1	71.0	101.1
Mean percent recovery ± *SD*		100.7 ± 1.72

Bromometric Determination—Accurately weigh about 100.0 mg of salicylamide and place in a stoppered 250-ml erlenmeyer flask. Add 50.0 ml, accurately measured, of 0.1 *N* bromine solution and warm to dissolve. Cool, add 15 ml of concentrated hydrochloric acid, and stopper the flask immediately. Leave for 30 min, add 3 g of potassium iodide, and titrate against 0.1 *N* sodium thiosulfate. Carry out a blank experiment.

RESULTS AND DISCUSSION

Salicylamide is often compounded in a multicomponent system with several analgesic compounds such as acetaminophen, phenobarbital, caffeine, and codeine phosphate. The separation for analysis of salicylamide from such a system is difficult and time consuming. The complicated spectrophotometric methods, requiring the solution of several algebraic equations, may make the determination tedious because of computational problems. Therefore, an independent and simple spectrophotometric method is suggested for the determination of salicylamide in the presence of the previously mentioned drugs. Separation of the drug by solvent extraction or chromatography prior to analysis is avoided.

Data obtained for the construction of a calibration curve for salicylamide are listed in Table I. The calibration curve, obtained by plotting eight average values of the absorbance at 308 nm as a function of concentration, indicated that the Beer–Lambert law was obeyed. No trials, however, were made to extend the investigations to concentrations higher or lower than those listed, since the absorbance values obtained with the concentrations studied are most suitable for minimum experimental errors.

The straight-line equation for the calibration curve, as determined by the method of least squares, is $A = 0.0130 + 0.3043C$, where A is the absorbance and C is the concentration (milligrams per 100 ml). The concentration of salicylamide in an unknown sample may be calculated readily. According to the experimental results, the average molar absorptivity of salicylamide at 308 nm is $\epsilon = 4.28 \times 10^3$ liters/mole/cm. Data obtained from the determination of salicylamide in simple chloroform solutions of varying concentrations are listed in Table II.

Time–absorbance studies on standard solutions of salicylamide and on test solutions containing in addition acetaminophen, phenobarbital, caffeine, and codeine phosphate indicated that the solutions can be kept for 12 hr without appreciable change in absorbance.

The spectra of the compounds commonly encountered with sali-

Table III—Compounds Studied for Interference with the Determination of Salicylamide

Compound	Relative Concentration in Working Solution, mg %	Tested Concentration, mg %	Absorbance of Tested Concentration at 308 nm	Molar Absorptivity
Caffeine	0.625	5.0	None	—
Phenobarbital	0.313	1.25	0.010	186
Codeine phosphate	0.125	2.0	0.004	81
Acetaminophen	2.5	2.5	None	—
	3.75	3.75	0.010	40
Ascorbic acid	Insoluble	Insoluble	None	—
Chloroquine phosphate	Insoluble	Insoluble	None	—
Prednisone	0.01	0.75	0.002	95

Table IV—Recoveries of Salicylamide from Synthetic Tablets

Labeled Content in Assay Aliquot, mg	Found, mg	Recovery, %
	Formula A	
40.1	40.1	100.00
50.2	50.5	100.59
53.3	53.2	99.81
60.2	61.4	101.99
62.0	61.5	99.19
Mean percent recovery ± *SD*		100.32 ± 1.06
	Formula B	
41.1	42.7	103.89
51.4	52.2	101.56
61.6	61.1	99.19
Mean percent recovery ± *SD*		100.55 ± 2.35
	Product C	
50.6	50.4	99.60
50.8	50.8	100.00
61.0	61.4	100.65
61.2	60.9	99.51
Mean percent recovery ± *SD*		99.94 ± 0.52
	Product D	
50.3	50.0	99.40
50.7	50.6	99.80
59.7	60.4	101.17
60.9	62.0	101.80
Mean percent recovery ± *SD*		100.54 ± 1.13

cylamide in tablets were determined in chloroform (Fig. 1). However, the concentrations employed for determining the spectra of acetaminophen and phenobarbital were higher than their relative concentrations in analgesic tablets. As may be seen from Fig. 1, the absorbance of salicylamide at the working wavelength is high enough to make errors due to absorbance by other compounds, if any, minimal. The spectra of the other compounds tested for interference with the proposed procedure (ascorbic acid, chloroquine phosphate, and prednisone) also were determined in chloroform.

Table V—Results of Recoveries of Salicylamide from Commercial Tablets

Product[a]	Labeled Content in Assay Aliquot, mg	Found, mg	Recovery, %
A	40.0	41.1	102.75
	40.0	41.4	103.50
	50.0	49.6	99.20
	60.0	61.4	102.33
Mean percent recovery ± *SD*			101.94 ± 1.89
B	51.07	50.93	99.73
	61.29	61.78	100.80
	61.40	61.58	100.29
Mean percent recovery ± *SD*			100.27 ± 0.54
C	50.93	51.59	101.29
	61.11	61.45	100.55
	61.57	58.82	95.53
Mean percent recovery ± *SD*			99.12 ± 3.13
D	43.54	42.72	98.12
	54.42	53.17	97.70
	65.30	63.08	96.60
Mean percent recovery ± *SD*			97.47 ± 0.78

[a] Trade names, suppliers, and weights of components per tablet are: A, Dolviran, The Alexandria Co. for Pharmaceuticals under licence of Bayer, salicylamide (200 mg), acetaminophen (200 mg), caffeine (50 mg), phenobarbital (25 mg), and codeine phosphate (10 mg); B, Cidal Forte, CID, salicylamide (500 mg); C, Salestol, CID, salicylamide (200 mg), chloroquine phosphate (40 mg), and prednisone (0.75 mg); and D, Vicemide, Kahira, salicylamide (500 mg) and ascorbic acid (100 mg).

Table VI—Recoveries of Added Salicylamide to Commercial Tablets[a]

Labeled Salicylamide, mg	Added Salicylamide, mg	Recovery of Added Salicylamide, mg	Recovery, %
42.16	21.08	20.90	99.14
40.69	15.81	15.18	96.02
39.97	15.75	15.64	99.30
39.97	26.25	27.92	106.36
Mean percent recovery ± *SD*			100.20 ± 4.37

[a] Product A.

Table III lists all tested compounds and their relative concentrations in the working chloroform solution corresponding to their relative amounts in tablets. The table also includes the highest concentrations used to test the interference with the proposed procedure. The data indicate that the contributions to the absorbance of the working solution corresponding to the relative amounts of these drugs in tablets are negligibly small compared to the absorbance of salicylamide. Such a property may lend itself to the independent routine spectrophotometric analysis of salicylamide without serious interference from the tested drugs. Ascorbic acid and chloroquine phosphate were insoluble in chloroform. Codeine phosphate, however, dissolved with difficulty on heating, and the solution had to be filtered from the few undissolved particles before the absorbance was measured.

The suggested procedure was applied to the analysis of salicylamide in two types of synthetic tablets containing the same amounts of salicylamide (200.0 mg), caffeine (50.0 mg), phenobarbital (25.0 mg), and codeine phosphate (10.0 mg) and different amounts of acetaminophen (200.4 and 299.7 mg in Formulas A and B, respectively). The mean percent recoveries (Table IV) were 100.32 ± 1.06 and 101.55 ± 2.35. Further investigations indicated that, at a higher content of acetaminophen (400.0 mg/tablet) a positive error was introduced in the recovery of salicylamide. Recoveries of salicylamide from synthetic tablets containing the same amounts of active ingredients as Products C and D also are listed in Table IV. Common tablet excipients such as starch, talc, and magnesium stearate do not interfere.

The suggested method also was applied to the determination of salicylamide in a number of commercial tablets containing different components such as acetaminophen, phenobarbital, caffeine, codeine phosphate, ascorbic acid, chloroquine phosphate, and prednisone. The results obtained agree with the labeled amounts of salicylamide (Table V).

To differentiate between experimental errors and errors due to bulk production, recoveries of known amounts of added salicylamide to one of the commercial tablets were determined (Table VI).

For purity determination and comparative purposes, salicylamide was determined by two different methods: the nonaqueous titration method of NF XIII (1) and a bromometric titration procedure. The results (Table VII) show that the mean percent purities of salicylamide used in this study were 98.63 ± 0.73 and 99.41 ± 0.25, respectively.

The suggested procedure is simple and rapid and may be quite useful for routine analysis of salicylamide in analgesic tablets.

Table VII—Determination of Purity of Salicylamide by the NF XIII Nonaqueous Titration Method and Bromometric Titration

NF Assay			Bromometric Assay		
Weight Taken, mg	Weight Found, mg	Recovery, %	Weight Taken, mg	Weight Found, mg	Recovery, %
205.4	204.6	99.62	101.3	100.8	99.51
209.5	207.9	99.23	103.1	102.5	99.42
211.7	207.3	97.92	104.3	103.2	98.94
223.4	219.1	98.07	100.2	99.6	99.45
249.7	245.6	98.33	100.7	99.9	99.26
			100.8	100.4	99.64
			100.6	100.3	99.66
Mean percent recovery ± *SD*		98.63 ± 0.75	Mean percent recovery ± *SD*		99.41 ± 0.25

REFERENCES

(1) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 627.

(2) R. Rutkowski, *Arzneim.-Forsch.*, **4**, 209(1954); through *Anal. Abstr.*, **2**, 1313(1955).

(3) J. M. Crampton and E. Voss, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 470(1954); through *Anal. Abstr.*, **2**, 139(1955).

(4) O. Hardy and J. Cerny, *Cesk. Farm.*, **8**, 73(1959); through *Anal. Abstr.*, **6**, 4947(1959).

(5) A. W. Clayton and R. E. Thiers, *J. Pharm. Sci.*, **55**, 404(1966).

(6) N. Shane and M. Kowblansky, *ibid.*, **57**, 1218(1968).

(7) S. A. Veresh, F. S. Hom, and J. J. Miskel, *ibid.*, **60**, 1092(1971).

(8) B. Miszczuk-Lucka and H. Taborska, *Przem. Chem.*, **11**, 706(1955); through *Anal. Abstr.*, **4**, 1016(1957).

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In Vitro and *In Vivo* Availability of Spironolactone from Oral Dosage Forms

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Abstract □ Tablet formulations of spironolactone with hydrochlorothiazide were studied *in vitro* and *in vivo* to evaluate the effect of formulation parameters on the bioavailability of spironolactone. The time required for 50% tablet dissolution (T_{50}) in simulated gastric fluid was linearly correlated with the disintegration times of four experimental formulations and one commercial tablet of spironolactone and hydrochlorothiazide. Bioavailability studies were conducted in four healthy, female beagle dogs. The mean time to peak concentration of canrenone, the major metabolite of spironolactone, was proportional to the T_{50} dissolution parameter. A study of spironolactone administered orally with and without hydrochlorothiazide showed that the bioavailability of spironolactone is not affected by hydrochlorothiazide. No significant differences in the bioavailability of spironolactone from one 100-mg and four 25-mg tablets were observed. Estimates of some pharmacokinetic parameters for canrenone closely agreed with those previously reported.

Keyphrases □ Spironolactone—dissolution and bioavailability, tablet formulations with and without hydrochlorothiazide, effect of formulation parameters □ Dissolution—spironolactone tablet formulations with and without hydrochlorothiazide, effect of formulation parameters □ Bioavailability—spironolactone, tablet formulations with and without hydrochlorothiazide, effect of formulation parameters □ Hydrochlorothiazide—effect on dissolution and bioavailability of spironolactone in tablet formulations □ Dosage forms—tablets, spironolactone with and without hydrochlorothiazide, dissolution and bioavailability, effect of formulation parameters □ Diuretic agents—spironolactone, dissolution and bioavailability, tablet formulations with and without hydrochlorothiazide

Bioavailability of a drug from a dosage form is of public health interest (1–4). Several investigations have documented the importance of formulation factors affecting drug bioavailability (4–7). Consequently, it has become increasingly important to recognize and evaluate the *in vitro* availability of existing drug products and to determine the influence of formulation on *in vivo* availability from oral dosage formulations. With this approach, effective *in vitro–in vivo* correlations can be established so that improvements in the drug bioavailability from a given drug combination can be achieved (6–10).

Spironolactone (I), a potent aldosterone antagonist, in combination with hydrochlorothiazide (II) is an effective antihypertensive agent. It is also used for the treatment of edema and ascites of congestive heart failure (11–13).

The objectives of this study were: (*a*) to test whether differences exist in the bioavailability of tablet formulations of I given orally with and without II, (*b*) to assess the *in vitro–in vivo* relationship for four experimental and one commercial tablet preparations of I and II, and (*c*) to determine the pharmacokinetics of canrenone (III), a major metabolite of I, with and without II.

EXPERIMENTAL

Materials—Starch USP[1], microcrystalline cellulose[2], povidone NF[3], calcium sulfate dihydrate NF[4], spironolactone USP[5] (I), hydrochlorothiazide USP[6] (II), magnesium stearate USP[4], polyethylene glycol 400[7], and methanol, analytical reagent grade, were used.

Methods—*Tablet Preparation*—Batches (2 kg) of each formulation, except the commercial tablets[8] containing 25 mg each of I and II, were processed. The drugs and excipients, except magnesium stearate, were mixed and wet granulated with distilled water.

The granulations were oven dried, comminuted, and then lubricated with 0.5% magnesium stearate. Each batch was compressed on a rotary tablet press[9] equipped with 16 sets of 0.95-cm (0.375-in.) standard concave tablet punches. Each batch was analyzed for the content of I per tablet (discussed later).

[1] Anheuser-Busch Inc., St. Louis, Mo.
[2] FMC Corp., Newark, N.J.
[3] Plasdone, GAF Corp., New York, N.Y.
[4] Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Searle & Co., San Juan, Puerto Rico.
[6] Ciba-Geigy Corp., Summit, N.J.
[7] Matheson, Coleman & Bell, Norwood, Ohio.
[8] Aldactone, 25-mg tablets (Lot 273-438), and Aldactazide, 25-mg tablets (Lot 21).
[9] Stokes B-2, Pennwalt Corp., Philadelphia, Pa.