Spectrofluorometric Determination of Acetylsalicylic Acid, Salicylamide, and Salicylic Acid as an Impurity in Pharmaceutical Preparations

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Abstract □ Spectrofluorometry, either direct or in combination with a separation technique, provides a sensitive and accurate method for the determination of certain fluorescent analgesic drugs and the determination of impurities in many combination preparations. A critical examination of the UV spectra of common analgesics and related compounds indicates that the fluorescence inner filter effect should be negligible below 10^{-5} *M* and that selective excitation and emission wavelengths should minimize interference from other fluorescent species. Fluorometric procedures are presented for the determination of salicylamide, acetylsalicylic acid, and salicylic acid, as an impurity, in preparations containing salicylamide, acetylsalicylic acid, acetaminophen, caffeine, and phenacetin as major constituents. Inner filtering is the limiting factor only for the direct and indirect determination of salicylamide and the direct determination of acetylsalicylic acid. Results of fluorometric determinations compare favorably with other reference methods. Salicylic acid is determined in the 10^{-7} *M* concentration range after separation from salicylamide, acetaminophen, and caffeine.

Keyphrases □ Salicylic acid—analysis, spectrofluorometry, impurity in multicomponent analgesic tablets □ Salicylamide, aspirin—spectrofluorometry, as major constituent in multicomponent tablets □ Aspirin—spectrofluorometric determination of an impurity in drug combinations □ Spectrofluorometry—determination of salicylic acid, salicylamide, and acetylsalicylic acid in multicomponent analgesic tablets

Various methods for the determination of salicylamide, acetylsalicylic acid, and salicylic acid, as an impurity, are available. However, with the advent of new instrumentation, new drugs, and new drug combinations (Table I), many of these methods have become outmoded or are no longer specific. The present study developed new methods along with improvements in some older methods.

BACKGROUND

Methods for Acetylsalicylic Acid—Fluorometric (8, 9) and phosphorimetric (10) methods for the determination of acetylsalicylic acid in pharmaceuticals were reported. Other fluorometric methods involve fluorophore formation by conversion to salicylic acid (11, 12). Many of these methods have never been examined for the effect of other constitcylates that interfere with the direct determination of salicylamide. Additional steps to eliminate fluoresence interference from salicylic acid are needed for the many preparations containing acetylsalicylic acid and other fluorescent salicylates. Analyses by direct UV (4, 5) and differential UV (6) spectrophotometry are both subject to interferences. One visible spectrophotometric method (7) involves a chromophore-producing reaction with ferric chloride reagent.

Methods for Acetylsalicylic Acid—Fluorometric (8, 9) and phosphorimetric (10) methods for the determination of acetylsalicylic acid in pharmaceuticals were reported. Other fluorometric methods involve fluorophore formation by conversion to salicyclic acid (11, 12). Many of these methods have never been examined for the effect of other constituents commonly found in recent preparations, such as other fluorescent salicylates and substances that absorb large amounts of exciting radiation. Direct UV methods for acetylsalicylic acid are based on prior separation from other components in the preparation (13–15).

Methods for Salicylic Acid as Impurity—The salicylic acid content in acetylsalicylic acid used for pharmaceuticals is limited to 0.3–0.75%, depending on the type of preparation relative to the acetylsalicylic acid content. The USP/NF (16) methods of analysis require separation of salicylic acid from acetylsalicylic acid *via* column chromatography after reaction with ferric chloride reagent. Other automated methods use ferric chloride reagent directly (17). Although this method works well for pure acetylsalicylic acid preparations, salicylamide also undergoes the same reaction with ferric reagents used for colorimetric determination (18). The salicylamide content of newer preparations often is as high as 40% of the total salicylate content.

Schenk *et al.* (8) proposed a direct fluorometric method for the determination of salicylic acid in acetylsalicylic acid. Unfortunately, in their solvent system, both salicylic acid and salicylamide excite and fluoresce at virtually the same wavelengths. Other methods involving chromophore-forming reactions (19) and high-pressure liquid chromatographic separations (20, 21) were proposed but never evaluated for interference by other sample constituents common in multicomponent preparations.

EXPERIMENTAL[1]

Solvents—All fluorescence work was done with fluoro grade alcohol prepared by percolation through 30–40-cm columns of activated coconut charcoal[2] (40–80 mesh). After the first 50-ml portion (contaminated) was discarded, each column produced 5–10 liters of fluorescence quality alcohol.

The fluoro grade chloroform used was prepared by vigorous extraction with 0.45 *M* NaOH, two washings with small volumes of water, and filtration through chloroform-moistened filter paper.

Organic Reagents—The salicylamide[3] was freed from the salicylic acid impurity by recrystallization from hot concentrated chloroform solution. [Alternatively, salicylic acid could be extracted from salicylamide in chloroform by two washings with 5% sodium bicarbonate (22).]

The acetylsalicylic acid[4] was purified of an unacceptable amount of salicylic acid by two recrystallizations from hot saturated chloroform; fluorometric analysis (8) demonstrated that a third recrystallization was unnecessary.

Caffeine citrate[5] was freed from citric acid by extraction with chloroform from a nearly saturated aqueous solution at pH 8.0 (ammonia). After filtration, the evaporated chloroform gave a white powder with a UV spectrum comparable to that of Sunshine and Gerber (23).

Samples of acetaminophen[6] and phenacetin[7] were used without further purification.

Determination of Salicylamide Alone—To determine salicylamide in the absence of acetylsalicylic acid, salicylic acid, or buffers, a tablet, capsule, or powder (equivalent to 50–100 mg of salicylamide) was placed in a 100-ml volumetric flask, and ~50 ml of methanol was added. The sample tablet form was crushed, shaken, and diluted to volume with methanol. After insoluble materials settled, a 0.1-ml aliquot was diluted to 100 ml with methanol and filtered through medium-speed filter paper. The first 20 ml of the filtrate was discarded, and the fluorescence of the next 50 ml was measured using the instrument settings in Table II. A blank reading was subtracted from the sample fluorescence reading by running a methanol blank for each lot of filter paper. A standard curve

[1] Fluorescence measurements were taken on a Perkin-Elmer MPF-2A spectrofluorometer. Absorption spectra were taken on either a Beckman DK-2A spectrophotometer or a Cary-14 spectrophotometer; a Coleman Jr spectrophotometer was used for quantitative measurement in the visible region. GLC analysis was done on a Perkin-Elmer 3920 chromatograph (flame-ionization detector, glass column packed with 3% OV-17 on Gas Chrom Q, 100–120 mesh).

[2] Fisher, 50–200 mesh (remeshed to above specifications).

[3] Practical grade, Eastman.

[4] Reagent grade, Baker Chemical Co.

[5] USP grade, Merck.

[6] NF sample, courtesy of Wyeth Laboratories.

[7] USP grade, American Pharmaceutical Co.

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Table I—Contents of Analgesics

Preparation	Unit Dosage, mg				
	Salicylamide	Acetylsalicylic Acid	Acetaminophen	Caffeine	Other
A	200	—	—	—	Methapyrilene hydrochloride, 25; scopolamine aminoxide hydrobromide, 0.25
B	250	—	—	—	Ascorbic acid, 50; ipecac and opium powder, 24; racephedrine hydrochloride, 5.0; pyrilamine maleate, 12; phenacetin, 120
C	150	—	150	100	—
D	—[a]	—	—[a]	—[a]	—
E	97	324	—	16	—
F	65	210	115	16	—
G	129.6	194.4	97	64.8	—
H	129.6	194.4	162	—	Methapyrilene fumarate, 25

[a] Unspecified quantity.

of fluoresence *versus* concentration was prepared using 0–1.4 × 10^{-5} *M* salicylamide in methanol.

Determination of Salicylamide in Acetylsalicylic Acid, Salicylic Acid, or Buffers—The tablet(s), capsule contents, or homogeneous powder (50–100 mg of salicylamide) was weighed into a 100-ml volumetric flask, and ~75 ml of chloroform was added. After agitation for 10 min, the solution was diluted to volume with chloroform and filtered through a medium-speed filter paper. [This solution also may be used to determine acetylsalicylic acid and salicylic acid (Scheme I).] A 10-ml aliquot was transferred to a 60-ml separator, and 20 ml of chloroform and 10 ml of pH 7.0 phosphate buffer (24) were added. Then the solution was extracted.

Before the chloroform extract was filtered, a filter paper blank was run by running 100 ml of chloroform through the filter paper and measuring the chloroform fluorescence (Table II). If the blank exceeded 3% of full scale, a different lot of filter paper[8] was used. The extracted chloroform layer was filtered through chloroform-wet filter paper into a 100-ml volumetric flask. The aqueous phase was extracted twice more with 30 ml of chloroform each time, and the extracts were combined and diluted to volume with chloroform. Dilution of 0.20 ml of chloroform solution to 25 ml with chloroform was performed, and the fluorescence was measured (Table II). The chloroform-wet filter paper blank was subtracted from each reading. A standard curve was prepared from standards of 0–10 × 10^{-3} *M* salicylamide treated in the same manner as the samples.

Determination of Acetylsalicylic Acid—The tablets or capsule contents were weighed and ground. The resulting homogeneous powder (50–300 mg of acetylsalicylic acid) was weighed into a 100-ml volumetric flask. Chloroform (75 ml) was added, and the solution was agitated for 10 min and diluted to volume with chloroform. The solution was filtered through medium-speed filter paper, and a 0.1-ml aliquot was diluted to 100 ml with 1% acetic acid–chloroform. The fluorescence was measured using the settings in Table II. A blank reading was subtracted from the sample fluorescence reading by running a chloroform blank for each lot of filter paper. A standard curve of fluorescence *versus* concentration was prepared using 0–1.7 × 10^{-5} *M* acetylsalicylic acid in 1% acetic acid–chloroform.

Determination of Salicylic Acid in Salicylamide (No Acetylsalicylic Acid)—Ten tablets or the contents of 10 capsules of samples without acetylsalicylic acid were weighed and ground to homogeneity. A portion of this powder (100 mg of salicylamide) was weighed into a 100-ml volumetric flask, and 75 ml of chloroform was added. The solution was agitated for 10 min and diluted to volume with chloroform. A suitable aliquot, such as 10 ml, was filtered through medium-speed filter paper and transferred to a 60-ml separator. Another 20 ml of chloroform was added with 10 ml of pH 7.0 phosphate buffer (24), the solution was extracted, and the chloroform layer was discarded. The process was repeated three more times with 30-ml chloroform volumes, and the chloroform was discarded.

The aqueous phase was extracted with 10 ml of chloroform and 2.0 ml of 2.0 *M* HCl. The chloroform layer was filtered through chloroform-wet filter paper into a 50-ml volumetric flask. The aqueous phase was extracted with two additional 10-ml portions of chloroform and filtered, and the three extracts were combined. The solution was diluted to volume, and the fluorescence of the solution was measured (Table II).

A blank was run on the filter paper using chloroform, and the blank reading was subtracted from the sample fluorescence reading. These readings were compared to a salicylamide control in the same concentration range as the sample (7.3 × 10^{-3} *M*) and salicylic acid standards (3.0–9.0 × 10^{-6} *M*), which were extracted in the same manner.

Determination of Salicylic Acid in Salicylamide and Acetylsalicylic Acid—The previous procedure was followed with the following changes: (*a*) the sample size contained 45 mg of acetylsalicylic acid; (*b*) on addition of the pH 7.0 buffer, the salicylic acid was extracted into chloroform as quickly as possible with a reproducible technique; (*c*) a suitable acetylsalicylic acid control in the 2.6 × 10^{-4} *M* range was substituted for the salicylamide control; and (*d*) salicylic acid standards in the 0.7–7.0 × 10^{-3} *M* range were used.

[8] Medium speed, Whatman.

DISCUSSION

UV Absorption of Salicylates and Related Compounds—The removal of a proton from salicylamide alters its UV spectrum. In alcohol, the salicylamide anion is produced by saturation of the solution with small quantities of sodium bicarbonate or sodium carbonate. In aqueous solution or in methanol, this anion has twice the molar absorptivity of molecular salicylamide, and its UV maximum shifts to 330 nm (23). In contrast, salicylic acid does not exhibit this effect in aqueous solutions (23).

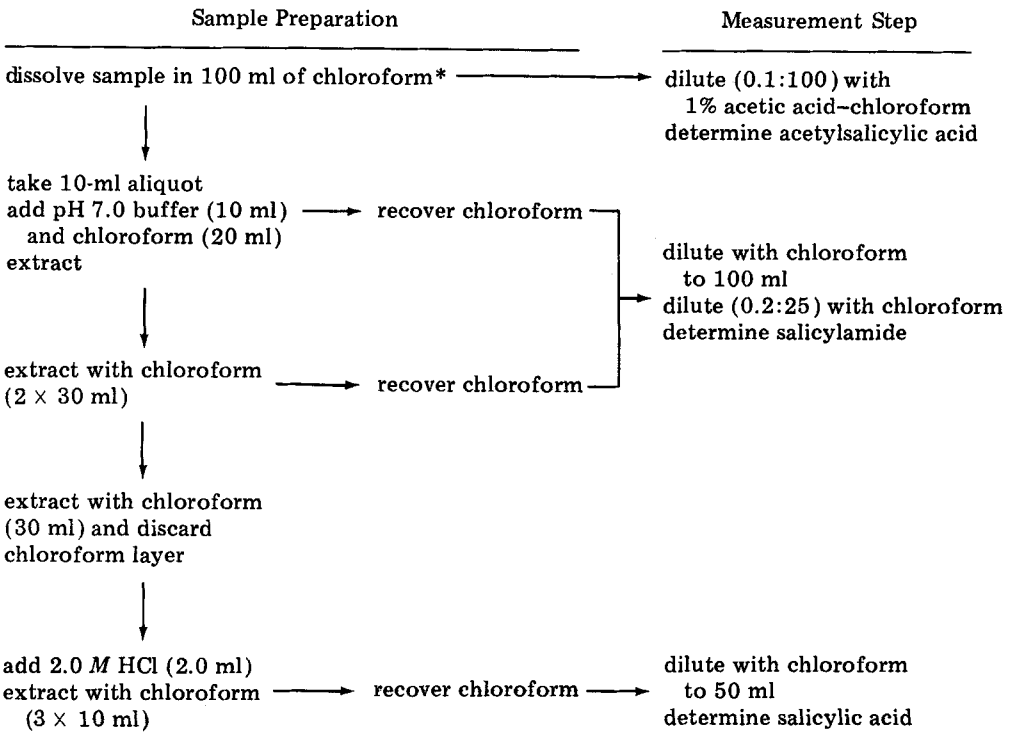
The molar absorptivities and wavelengths of maximum absorption of acetylsalicylic acid and salicylic acid in chloroform and 1% acetic acid–chloroform are not significantly different (8). Thus, the lowest energy electronic transition should be approximately the same in both solvent systems.

Fluorescence of Salicylates and Related Compounds—For salicylates and related compounds, emission in the 330–360-nm region of the spectrum has been postulated as arising from the neutral molecule (8, 25). Emission in the 400–450-nm region of the spectrum originates from the dipolar ion formed within the lifetime of the S_1 state *via* either inter- or intramolecular proton transfer (8, 25–28).

For salicylamide in methanol, the 340-nm emission is weak relative to that at 420 nm. The fluorescence emissions at 410 and 420 nm are postulated as arising from the zwitterion formed *via* intramolecular transfer within the lifetime of the S_1 state (26, 27). In methanolic solution, the 410-nm emission arising from the 333-nm excitation, which only occurs to a small extent, can be suppressed almost completely with small amounts of acid. In methanolic solution with traces of base, the UV maximum shifts from 300 to 330 nm and the emission at 410 nm is greatly enhanced because the 330-nm UV band is twice as intense as that of the neutral species and because the quantum yield for this form of the molecule is an estimated 20 times that of the neutral molecule. It then appears that the anion is more rigid than the molecular form of salicylamide,

Table II—Instrumental Conditions for Spectrofluorometric Determinations

Ingredient Determined	Wavelength, nm		Slit Width, nm	
	Excitation	Emission	Excitation	Emission
Salicylamide				
In methanol	300	425	10	10
In chloroform	315	445	10	20
Acetylsalicylic acid	280	335	10	20
Salicylic acid	315	445	10	10



Scheme I—Determination of salicylamide, acetylsalicylic acid, and/or salicylic acid in a multicomponent sample that may contain salicylamide, acetylsalicylic acid, acetaminophen, caffeine, and/or salicylic acid as an impurity. (Sample must contain appropriate amounts of salicylamide and acetylsalicylic acid for subsequent determination of salicylamide, acetylsalicylic acid, and salicylic acid as an impurity.)*

possibly because the negatively charged oxygen atom can form a strong hydrogen bond to the adjacent *ortho* group. The molecular form thus will be less rigid, allowing an increase in the overall rate of internal conversion to the ground state. In addition to fluorescing in methanol, salicylamide also fluoresces weakly at 325 nm and intensely at 445 nm in chloroform.

In designing a fluorometric method for salicylamide, excitation was done at 300 nm rather than at the 310-nm excitation maximum. Excitation at 300 nm seemed to produce more reliable fluorescence emission readings since the 310-nm excitation is more sensitive to small changes in the quality of methanol. At this wavelength, good quality spectrograde methanol (which gives a low fluorescence emission blank) could be substituted for fluorescent grade solvent. Partly because pharmaceutical preparations contain basic buffers to reduce GI bleeding from aspirin, a possible fluorometric method for salicylamide in basic methanol solvent was tested. Saturation of methanol with sodium bicarbonate produced only partial (75%) conversion to the highly fluorescent anion, while saturation of methanol with sodium carbonate produced complete conversion. Other buffers such as magnesium carbonate, dihydrogen citrate, and magnesium hydroxide, which frequently are incorporated in pharmaceutical preparations, probably also could cause large variations in the 425-nm fluorescence emission. Inner filtering by acetaminophen, caffeine, and phenacetin was negligible.

The methodology was tested on several preparations with sodium carbonate-saturated methanol and 330-nm excitation. A further dilution (1:10) of the samples was required to keep the fluorescence readings on scale and the UV absorbance at the excitation wavelength low enough for negligible inner filtering. Although salicylamide was 100% converted to its anion, acetaminophen was converted only partially to its anion in this buffer. Large variations in the acetaminophen content of the sample, particularly when in combination with other buffers, may alter the percent conversion of salicylamide and/or acetaminophen to their anionic forms even in sodium carbonate-saturated methanol. (The anion of acetaminophen absorbs strongly and at longer wavelengths than the neutral molecule.) In addition, a previous study (3) reported that salicylamide decomposes in strong alkaline solution.

For excitation of samples at 300 nm in methanol, all samples must be excited under uniform conditions. The fluorescence emission readings increased in a reproducible manner with respect to the excitation time. For the most reliable readings, the sample should not be excited unless the fluorescence emission is to be read immediately.

The fluorescence of acetylsalicylic acid in chloroform and chloroform–aliphatic acid mixtures was described prviously (8). In methanol and methanol–1% acetic acid, acetylsalicylic acid does not appear to fluoresce as in the chloroform–aliphatic acid solvent systems.

A single fluorescence emission peak in both chloroform and methanol was observed for salicylic acid. For salicylic acid, intramolecular proton transfer to the excited carbonyl oxygen occurs within the lifetime of the lowest excited singlet state (25). In chloroform, low concentrations (1%) of various aliphatic acids produce an additional fluorescence emission band in the 350–400-nm region. In methanol, the fluorescence emission at 400 nm is diminished almost completely by 0.5% acetic acid when excited at 295 nm.

Methodology—The National Formulary specifies that salicylamide-containing tablets contain 100 ± 5.0% of the labeled content. The acetylsalicylic acid content of acetylsalicylic acid-containing capsules (NF) must be within 100 ± 7.0% of the labeled content, and tablets (USP) must contain 100 ± 5% of the labeled content. Neither the USP nor the NF have established limits of purity for salicylamide with respect to salicylic acid as an impurity, but both specify limits for purity of acetylsalicylic acid with respect to salicylic acid as an impurity (0.3% in capsules and 0.75% in tablets).

Factors Affecting Fluorescence Assay Procedures for Salicylamide in Preparations Containing No Acetylsalicylic Acid, Salicylic Acid Impurity, or Buffers—The salicylamide excitation band at 330 nm is extremely sensitive to solvent conditions. Thus, excitation at 330 nm was attempted in methanol, and then the specimen was reexcited after a trace of acetic acid was added. The difference between the two readings was linear in the 10^{-5} *M* range. Actual tablets contain ingredients that make this effect less useful for analytical purposes.

Other solvents were tested for analytical use. Although salicylamide fluoresced with greater efficiency in *n*-propanol and ethanol, *n*-propanol is not purified as easily as methanol, and ethanol is not as readily available. Chloroform was found to quench fluorescence.

Since acetaminophen, caffeine, and phenacetin also absorb in the region of salicylamide excitation, the possibility of an inner filter effect from any or all of these compounds was evaluated. At the levels normally present in pharmaceutical preparations, acetaminophen, caffeine, and phenacetin did not cause an inner filter effect in the present spectrofluorometric method.

RESULTS

Results of spectrofluorometric determinations compared favorably with those of several reference methods when corrections were applied and the reference methodology was modified for suitable sample handling

Table III—Determination of Salicylamide in Pharmaceuticals Containing No Acetylsalicylic Acid or Salicylic Acid Impurity

Preparation	Salicylamide Determined, mg: Reference Method	Salicylamide Determined, mg: Fluorescence	Percent Error
A[a]	221 ± 9[b]	218 ± 7[c]	−1.4
B[c]			
Lot X	239 ± 2[d,e]	234 ± 5[d]	−2.1
Lot Y	251 ± 6[d,e]	248.6 ± 2.3[f]	—
C[g]	150.7[h,i] (152.5[h,j])	151.1 ± 1.0[k]	+0.3 (−0.9[j])
	146.4[b]	—	+3.2
D[f]	96.5[h,j]	98.8 ± 0.4[k]	+2.4

[a] Unit dose analysis. [b] GLC using phenacetin as the internal standard. [c] Average of six determinations. [d] Average of four determinations. [e] Modification of Sunshine's salicylic acid method using Trinder's reagent (29). [f] Average of 12 determinations. [g] Aliquot representative of a unit dose taken from 10 unit doses combined and homogenized. [h] Differential UV. [i] Value corrected for error due to acetaminophen content. [j] Value not corrected for acetaminophen content. [k] Average of two determinations.

(Tables III–VI). Calibration curves were only linear to 100 mg/100 ml of salicylamide in the original sample (7×10^{-6} *M*) due to inner filtering; analytical results were most accurate when determined graphically with calibration standards that closely approximated the salicylamide concentration of the samples. When some of the same samples were excited at 313 nm in a filter fluorometer (mercury arc source), poor results were obtained due to the high amplification needed to measure the fluorescence emission.

Determination of Salicylamide in Preparations Containing Acetylsalicylic Acid or Salicylic Acid Impurity—If salicylic acid may be present, it is best to separate it from salicylamide before fluorescence analysis. Studies in this laboratory indicate that salicylamide is never completely recovered in chloroform at any pH by equal volume extractions (Fig. 1) and that better recoveries should result at a lower pH extraction than 5% sodium bicarbonate as previously reported (22). The behavior of acetylsalicylic acid and salicylic acid is similar; neither is extracted well with chloroform at pH > 5. At pH < 3, acetylsalicylic acid and salicylic acid extract significantly into chloroform. Therefore, any analytical separations must be done at pH 6–7.

The solubility of acetaminophen in chloroform is low (0.3 g/liter), and extraction into chloroform is not possible at any pH; therefore, accurate distribution ratio values, *D*, could not be determined. In contrast, caffeine is completely soluble in chloroform and is extracted so completely by chloroform over the pH range studied that accurate *D* values could not be determined for this compound either.

After three extractions, salicylamide was ~98% recovered from the aqueous phase. The salicylic acid, with ~50% of acetylsalicylic acid, remained in the aqueous phase at pH 7. After extraction with chloroform at this pH, all acetaminophen remained in the aqueous phase and all caffeine was in the chloroform with salicylamide. Solutions of molarities similar to those of the sample solutions were tested for inner filtering effects. No inner filtering by acetylsalicylic acid, acetaminophen, and caffeine was observed.

The effect of adding buffers to the pharmaceutical preparations was never critically evaluated. These compounds were assumed to be too insoluble in chloroform to alter the pH of the aqueous buffer (0.067 *M*) when equilibrated with it.

Although salicylamide has a larger molar absorptivity in chloroform than in methanol, it has a much lower quantum efficiency of fluorescence in chloroform. In spite of the former characteristic, accurate results are easily obtained in the 0–100-mg range. Self inner filtering begins at concentrations of >3×10^{-6} *M* salicylamide in the final solution. For accurate results, samples should be compared to extracted standards of similar salicylamide content. Comparison to extracted standards eliminates the need for extraction efficiency corrections; the extracted standards serve as controls.

Table IV—Determination of Salicylamide in Pharmaceuticals Containing Acetylsalicylic Acid and Salicylic Acid as Impurity

Preparation	Salicylamide Determined, mg: Differential UV[a]	Salicylamide Determined, mg: Fluorescence	Percent Error[a]	Salicylamide[b], mg	Percent Error[b,c]
E	102	96.7	−5.4	96.1	+0.6
F	66.8	62.1	−7.0	64.1	+1.1
G	134	130.4	−2.7	129.6	+0.6
H	134	128.2	−4.3	127.8	+0.3

[a] Differential UV uncorrected for interference due to the presence of acetylsalicylic acid and acetaminophen. [b] Differential UV corrected for the presence of acetylsalicylic acid and acetaminophen. [c] Calculated by $\%E^{a} - E_{\text{due to acetylsalicylic acid}} - \%E_{\text{due to acetaminophen}}$.

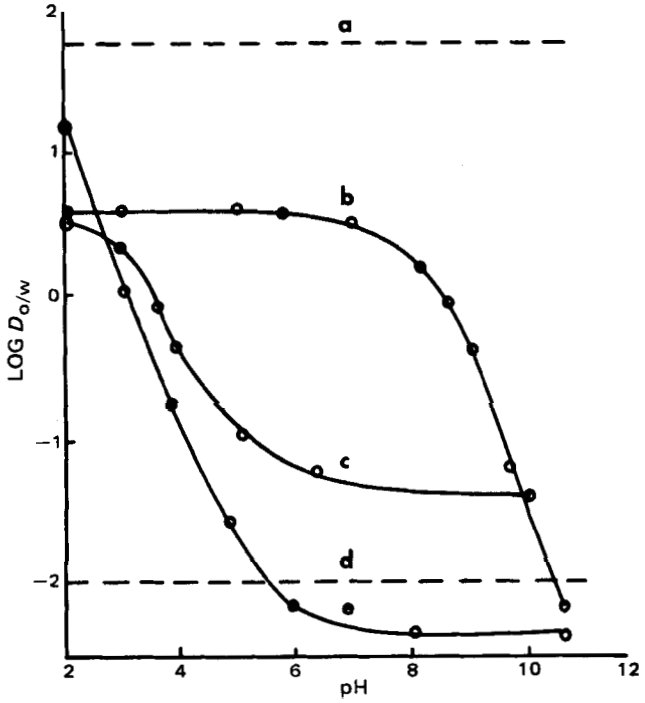


Figure 1—*Distribution of pharmaceuticals between chloroform and aqueous phosphate buffers. Key:* a, *caffeine;* b, *salicylamide;* c, *acetylsalicylic acid;* d, *acetaminophen; and* e, *salicylic acid.*

Determination of Acetylsalicylic Acid in Preparations Containing Salicylamide, Acetaminophen, and Caffeine—Many problems associated with the fluorometric determination of acetylsalicylic acid in aspirin tablets have been solved (8), including excitation and emission wavelengths, concentration ranges, choice of a suitable solvent (level of acetic acid for enhancement of fluorescence), and elapsed time between dissolution and analysis. However, the effects of other constituents found in recent complex pain-relief preparations were never evaluated fully.

For example, caffeine has its UV maximum at the same wavelengths as acetylsalicylic acid and the acetylsalicylic acid–acetic acid complex but has approximately six times the molar absorptivity. At low caffeine to acetylsalicylic acid ratios (expressed in milligrams) in pharmaceutical preparations, caffeine was not found to inner filter. However, at ratios greater than 1:1 in preparations containing large amounts of acetylsalicylic acid, the absorbance at 280 nm was so large that inner filtering became significant. The presence of acetaminophen was never a problem at low acetaminophen to acetylsalicylic acid ratios (expressed in milligrams) but became a problem at ratios greater than 2:1 where large amounts of aspirin were being determined. Typical content ratios for these compounds in newer preparations are 0.1 < acetaminophen (milligrams)/acetylsalicylic acid (milligrams) < 1.0 and 0.0 < caffeine (milligrams)/acetylsalicylic acid (milligrams) < 0.4.

For the determination of acetylsalicylic acid in the presence of sali-

Table V—Determination of Salicylic Acid in Pharmaceuticals Containing Salicylamide and No Acetylsalicylic Acid

Preparation	Salicylamide Content of Sample, mg	Determined by Fluorescence, mg	Correction for Extracted Salicylamide, mg	Salicylic Acid per Unit Dose, mg
A	109	0.006	−0.008	−0.002
B				
Lot X	164	0.013	−0.011	+0.002
Lot Y	169	0.013	−0.011	+0.002
C	92.0	0.004	−0.006	−0.002
D	84.0	0.004	−0.006	−0.002

Table VI—Determination of Acetylsalicylic Acid and Salicylic Acid as Impurity in Pharmaceuticals Containing Salicylamide

Preparation	Acetylsalicylic Acid per Unit Dose, mg		Percent Error	Salicylic Acid Determined by Fluorescence	
	Fluorescence[a]	Titration		Salicylic Acid per Unit Dose, mg	Salicylic Acid with Respect to Acetylsalicylic Acid Content, %
E	328.0 ± 0.8	323.4 ± 1.4	+1.4	1.64	0.49
F	221.0 ± 3.5	213.3 ± 0.3	+3.5	0.94	0.43
G	198.4 ± 0.6	—	—	0.46	0.23
H	210.2 ± 1.0	207.0 ± 0.5	+1.7	1.15	0.55

[a] Triplicate analysis.

cylamide, several simultaneously competing effects are possible. Although salicylamide exhibits a UV minimum at 260 nm, it has a band at 270–340 nm with a molar absorptivity at 307 nm, which is approximately six times that of acetylsalicylic acid at its maximum. Hence, inner filtering of excitational radiation is possible where large amounts of acetylsalicylic acid are to be excited at 280 nm with a high weight ratio of salicylamide to acetylsalicylic acid. Since the 307-nm absorption band of salicylamide is extremely wide, inner filtering of the 335-nm acetylsalicylic acid emission also is possible. In addition, the 445-nm salicylamide emission band is broad so that a small positive interference from salicylamide emission actually is observed when measuring the acetylsalicylic acid emission at 335 nm. At smaller ratios of salicylamide to acetylsalicylic acid and small amounts of acetylsalicylic acid, the sum of these three effects is negligible. Typical content ratios in newer preparations are $0.2 \leq$ salicylamide (milligrams)/acetylsalicylic acid (milligrams) ≤ 1.3.

Any pharmaceutical preparation containing acetaminophen and caffeine probably can be analyzed rapidly and accurately for acetylsalicylic acid content spectrofluorometrically. Any inner filtering can be avoided, at least in theory, by diluting the sample to <0.05 absorbance unit at 280 nm and using higher signal amplification. However, any positive error from salicylamide cannot be removed by dilution. At lesser content ratios, salicylamide does not contribute more than 1% positive error to the determination. Since the two emission peaks of the salicylamide–acetylsalicylic acid system are well resolved and can be read on approximately the same instrument sensitivity, multicomponent system calculations should be applicable if a large salicylamide to acetylsalicylic acid ratio is encountered.

Results of spectrofluorometric determinations compared favorably with nonaqueous titrations using chlorophenol red indicator. This indicator was not stable in the sample, and additional indicator was required as the titration proceeded; the end-point was still indistinct. In the end-point region, the kinetics of the titration became slow. The combination of these two titrimetric effects may have caused part of the overall positive error observed (Table VI). (Tablet Preparation F had the largest positive error but the lowest salicylamide to acetylsalicylic acid content ratio; the percent error due to salicylamide content was ~+0.3% for Preparations E and F and +0.6% for Preparations G and H.)

Determination of Salicylic Acid in Salicylamide-Containing Pharmaceuticals—Since salicylamide is separated so easily from salicylic acid, the salicylic acid content of salicylamide-containing pharmaceuticals should be negligible. Analysis of reagent grade salicylamide proved that the reagent contained significant amounts of salicylic acid; the salicylic acid content was not homogeneously distributed. Consequently, a method for the determination of salicylic acid content of salicylamide is necessary to ensure the purity of the salicylamide standard used for the direct spectrofluorometric analysis of salicylamide in methanol. This method also is applicable to pharmaceuticals containing salicylamide.

For the determination of salicylic acid in pharmaceuticals not containing acetylsalicylic acid, the only constituent of the sample solution prepared for analysis should be salicylic acid. In practice, small amounts of salicylamide appear in the final sample solution but can be compensated for by running a salicylic acid-free salicylamide control with the samples. The correction is linear from 50 to 300 mg of initial salicylamide content. Due to the reduced recovery of salicylic acid (89.4 ± 2.3%), samples should be compared to extracted standards to eliminate the need for percent recovery corrections. These standards also can serve as controls. With correction for residual salicylamide content, the detection limit for the determination of salicylic acid in samples containing 100 mg of salicylamide is ~0.05% of the total salicylamide content.

Determination of Salicylic Acid in Pharmaceuticals Containing Salicylamide and Acetylsalicylic Acid—One important problem in analyzing any preparation containing acetylsalicylic acid is that acetylsalicylic acid is hydrolyzed to salicylic acid in aqueous solution. At high pH, the acetylsalicylic acid is hydrolyzed immediately; in neutral and acid solution, the hydrolysis rate is slow enough that it can be compensated for accurately ($0.007 \pm 0.001_0$ mg/min and $0.001_2 \pm 0.000_3$ mg/min, respectively). In the present analyses, the hydrolysis rates were constant over the time required for the separation of acetylsalicylic acid from salicylamide, acetaminophen, and caffeine by extraction. The rates were not determined for various concentrations of acetylsalicylic acid so that the acetylsalicylic acid content of the initial sample in this method was limited to 45 ± 2 mg of acetylsalicylic acid. For best results, a previously analyzed USP grade acetylsalicylic acid control containing the same amount (within ±2 mg) of aspirin as in each sample should be processed with the samples and standards. Standards should be within the 0.01–0.1 mg of salicylic acid concentration range in the original sample. Any samples not within this range after extraction should be diluted and then remeasured.

In processing samples for salicylic acid determination, each sample must be extracted completely before the next sample is started rather than processing many samples simultaneously. (However, once in the final chloroform solution, the sample can be read within 1 hr.) This process keeps acetylsalicylic acid corrections small. The upper limit was set at 10 min for the extraction of each sample.

All of the preparations analyzed were within NF specifications with respect to the salicylic acid content.

CONCLUSIONS

Fluorometric analysis of pharmaceutical preparations for salicylamide and acetylsalicylic acid and salicylic acid as an impurity in salicylamide-containing preparations is relatively free of interference from other associated compounds. This may not be the case with other reported methods that have never been evaluated for combination preparations. Analysis time is short, and results of determinations compare favorably with other reference methods for a wide variety of combination preparations.

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Continuous Flow Bead-Bed Dissolution Apparatus for Suppositories

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Abstract □ A bead-bed dissolution apparatus for suppositories was evaluated by measuring the release of benzocaine from various vehicles. During dissolution, suppositories soften, deform, disintegrate, and eventually pass through a phase change from a solid to an oil. The control of the interfacial area during dissolution is a key factor in obtaining experimentally reproducible release data. The proposed suppository dissolution apparatus was designed to provide greater constancy of the exposed suppository area for dissolution. The apparatus consisted of a glass bead-bed containing the suppository. A continuous flow of liquid was passed through the bead-bed at a constant rate. Direct contact of the suppository was maintained with the dissolution medium, confining the suppository within the beads.

Keyphrases □ Suppositories—evaluation of a bead-bed dissolution apparatus, drug release rate □ Dissolution—bead-bed apparatus for suppositories, evaluation, drug release rate □ Drug release—suppositories, evaluation of a bead-bed dissolution apparatus

Benzocaine was selected as a model compound with both a low melting (33.5–35.5°) and a high melting (37–39°) glyceride-type suppository base. *In vitro* release of benzocaine decreased as the melting point of the suppository increased. Reproducibility of the complete release curves was acceptable. Drug release also was affected by the temperature of the dissolution media, increasing, decreasing, and increasing again at certain temperatures. This finding was related to the ability of the beads to penetrate the surface of the suppository when the suppository softened. Release profiles, however, were reproducible at the temperature studied. The proposed bead-bed dissolution apparatus should offer an improved means for measuring drug release from suppositories.

BACKGROUND

Suppositories, administered either vaginally or rectally, are utilized as a dosage form for various drugs (1). Recent reports investigated the use of vaginally administered prostaglandins for fertility control and also emphasized certain advantages of this mode of drug therapy, *e.g.*, self-administration and single-dose therapy (2–9). This continued interest in suppositories and suppository bases (10) has led to the recognition that a dissolution test would be helpful during the initial phase of dosage form design. In addition, such a test would provide valuable information on the effect of storage time and temperature on the subsequent *in vitro* release profile.

The methods used for testing drug release rate characteristics of suppositories *in vitro* can be classified in terms of five general types (Fig. 1). The first type consists of simple placement of the suppository in a flask or beaker (11–15). The second type utilizes an existing tablet dissolution apparatus that provides a wire mesh basket for holding the sample (16–22). The third and fourth types employ a membrane; the third consists of a sample chamber separated from a reservoir by a membrane (23–33), whereas the fourth employs dialysis tubing or a natural membrane (33–46). The fifth type involves a flow system in which the sample is placed on cotton or a wire screen (47, 48).

One basic problem in testing for drug release from a suppository is that the suppository softens, deforms, melts, or disintegrates during the test, exposing a variable interfacial area to the dissolution medium. Because the release rate is dependent on the interfacial area, the variability of this factor leads to poor test reproducibility.

Membranes have been used to control the interfacial area on the principle that when the suppository softens, it would spread over the entire membrane, restricting the area exposed to the dissolution fluid. Bhavnagri and Speiser (24) designed a type three apparatus with a relatively small sample chamber. Others (39, 42) used a relatively small bag in a type four apparatus to restrict the interfacial area.

The need to control the interfacial area is important, but the intro-

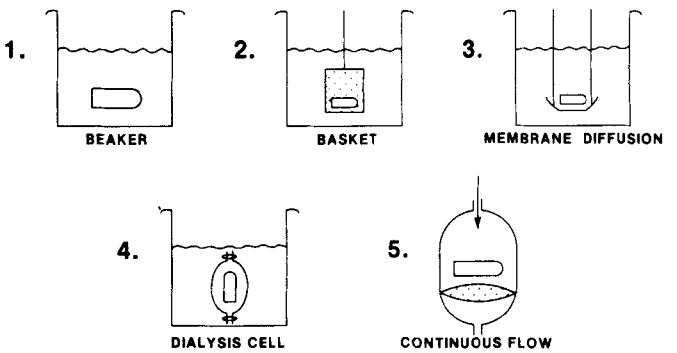

Figure 1—*Suppository dissolution methods.*

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