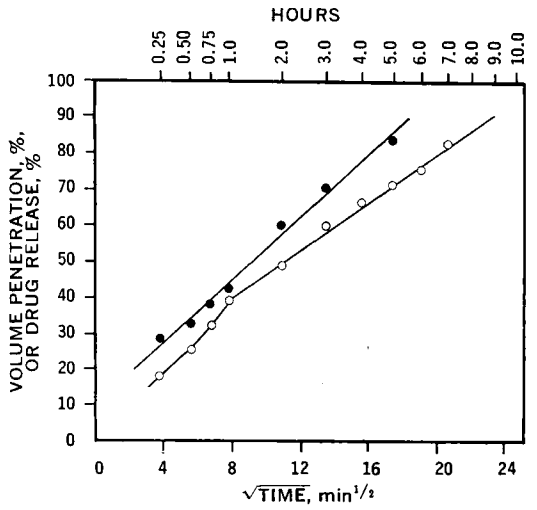

Figure 4—*Comparison of fluid penetration and percent drug release from a phenylpropanolamine hydrochloride 150-mg wax matrix tablet. Compression force was 544.3 kg. Key: ●, percent volume penetration of dissolution fluid; and ○, percent drug release.*

the lower tablet surface and the medium. This procedure assured that the upper surface was the only releasing surface. Adjustment of stirring height was made to provide the same tablet to propeller distance as used for the free tablet.

The pattern of drug release for the tablet-in-a-die was linear with the square root of time throughout the entire period (Fig. 3). A comparison of dissolution rates (Q *versus* t) was made for free tablets and the tablet-in-a-die compressed at 544.3 kg (Table IV). Comparisons in rates were made for the 1-hr time period and the average 2–4-hr time period. These rates for all conditions, various compressional forces, and various surface areas compare favorably, indicating that diffusion in free tablets is occurring through both top and bottom surfaces and the sides. Therefore, the dissolution apparatus does not restrict diffusion from any part of the tablet surface.

Penetration of Dissolution Media into Tablets—Wax matrix tablets are slowly permeated by the dissolution media as a function of time. The percent of volume penetration and percent drug release as a function of $t^{1/2}$ is shown in Fig. 4. It can be seen that some correlation exists between drug release and fluid penetration and that volume penetrated is proportional to the $t^{1/2}$. In the earlier time period, drug release lags about 1 hr behind fluid penetration. In later time periods, the lag time increases. An extrapolation of penetration and drug release curves results in a time period of about 7 hr for complete penetration and about 11.5 hr for complete drug release.

REFERENCES

(1) E. Stempel, *Drug Cosmet. Ind.*, **98** (1), 44(1966).

(2) *Ibid.*, **98** (2), 36(1966).

(3) W. A. Cressman, C. A. Janicki, P. C. Johnson, J. T. Doluisio, and G. A. Braun, *J. Pharm. Sci.*, **58**, 1516(1969).

(4) J. R. Robinson and S. P. Eriksen, *ibid.*, **55**, 1254(1966).

(5) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., 1970, p. 887.

(6) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 932.

(7) F. W. Goodhart, R. H. McCoy, and F. C. Ninger, *J. Pharm. Sci.*, **62**, 304(1973).

(8) L. Chafetz, *ibid.*, **60**, 291(1971).

(9) J. B. Schwartz, A. P. Simonelli, and W. I. Higuchi, *ibid.*, **57**, 274(1968).

(10) T. Higuchi, *ibid.*, **52**, 1145(1963).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 7, 1974, from the *Pharmaceutical Research and Development Laboratory, Warner-Lambert Research Institute, Morris Plains, NJ 07950*

Accepted for publication June 20, 1974.

[x] To whom inquiries should be directed.

GLC Determination of γ-Hydroxyphenylbutazone in Plasma

K. K. MIDHA [x], I. J. McGILVERAY, and C. CHARETTE

Abstract □ A recently described GLC method for the determination of phenylbutazone and its major metabolite, oxyphenbutazone [1-phenyl-2-(*p*-hydroxyphenyl)-3,5-dioxo-4-*n*-butylpyrazolidine], in plasma was extended to the estimation of the second major metabolite, γ-hydroxyphenylbutazone [1,2-diphenyl-3,5-dioxo-4-(3-hydroxybutyl)pyrazolidine]. The method is sensitive to 1.0 μg/ml.

Keyphrases □ γ-Hydroxyphenylbutazone—GLC determination in plasma □ Phenylbutazone metabolites—GLC determination of γ-hydroxyphenylbutazone in plasma □ GLC—analysis, γ-hydroxyphenylbutazone in plasma

Since the classic work of Burns *et al.* (1) on the metabolism of the antiarthritic phenylbutazone, a number of methods for the determination of the drug and its major metabolite oxyphenbutazone have been published (2, 3). These workers also described the isolation of a second metabolite, γ-hydroxyphenylbutazone, from urine following repeated high doses but no assay of this metabolite apparently has been described. GLC procedures have been reported for the determination of phenylbutazone (3) and oxyphenbutazone (2), required for pharmacokinetic studies in these laboratories (4, 5). The method for oxyphenbutazone (2) has now been extended to allow simultaneous determination of γ-hydroxyphenylbutazone in human plasma following single and multiple doses of phenylbutazone.

EXPERIMENTAL

Reagents—All reagents and chemicals used were described previously (2). Complimentary samples of phenylbutazone[1], oxyphen-

[1] Ciba-Geigy, Canada.

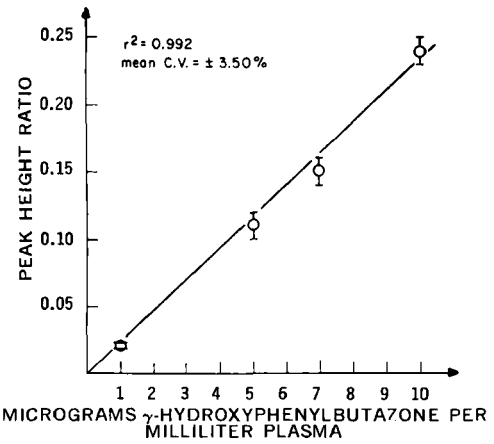

Figure 1—*Calibration curve of γ-hydroxyphenylbutazone extracted from human plasma. Each point is a mean of at least four determinations.*

butazone[1], and γ-hydroxyphenylbutazone[2] were obtained. All chromatographic materials[3] were purchased commercially. Stock solutions of γ-hydroxyphenylbutazone containing 50 μg/ml were prepared and diluted to the required concentrations with methanol. Stock solutions of oxyphenbutazone and the internal standard, 5-(4-hydroxyphenyl)-5-phenylhydantoin, were prepared as described previously (2).

Plasma Level Study—Phenylbutazone (2 × 100-mg tablets[4]) was administered to a healthy male volunteer (31 years, 75 kg). Three weeks after the single dose, this subject was given a 100-mg dose of phenylbutazone[4] every 12 hr for 7 days (9:00 a.m. and 9:00 p.m., 15 doses total). Samples of blood (10 ml) were withdrawn from the cubital vein using heparinized vacuum tubes[5] at 15 appropriate time intervals after dosing. The blood samples were centrifuged and plasma was removed and stored at −10°. Plasma phenylbutazone and oxyphenbutazone were determined as previously reported (2, 3).

GENERAL PROCEDURE

Extraction—The extraction procedure and GLC analysis, following flash-heater methylation with trimethylanilinium hydroxide, were similar to those described for oxyphenbutazone (2). The plasma sample (1 ml), to which 1 ml of internal standard [5-(4-hydroxyphenyl)-5-phenylhydantoin in 0.01 *N* NaOH (1.11 μg/ml)] was added, was first extracted with ether to remove interfering substances and then with *n*-heptane under acidic conditions to separate the phenylbutazone. The aqueous acidic residue from which the phenylbutazone had been selectively removed was shaken again with ether to extract oxyphenbutazone and γ-hydroxyphenylbutazone, which were analyzed by flash-heater methylation on a gas chromatograph.

GLC—GLC was effected on a gas chromatograph[6] equipped with a flame-ionization detector. A stainless steel column [1.8 m × 0.3 cm (6 ft × 0.125 in.) o.d.], packed with 5% phenyl methyl dimethyl silicone[7] on acid-washed, dimethylchlorosilane-treated, high performance flux-calcined diatomite support[8], 80–100 mesh, was used. The column was conditioned by injecting 25–50 μl of column conditioner[9] [mixture of *N,O*-bis(trimethylsilyl)acetamide, trimethylsilyldiethylamine, and hexamethyldisilazane] and maintaining the column at 300° for 18 hr with low nitrogen flow.

The injection port and detector temperatures were 320° and the column oven temperature was 270°. Nitrogen was used as a carrier gas at a flow rate of 30 ml/min. Hydrogen and compressed air flow rates were adjusted to give maximum response. The retention times of γ-hydroxyphenylbutazone (peak III) and the internal standard (peak IV) were 10.6 and 12.0 min, respectively.

Calculation—Peak height ratios were calculated by dividing the height of peak III (10.6 min), due to methylated γ-hydroxyphenylbutazone, by the height of peak IV (12.0 min), due to the methylated internal standard. By using spiked plasma, calibration curves were constructed by plotting the peak height ratios against their respective concentrations of γ-hydroxyphenylbutazone (micrograms per milliliter) (Fig. 1).

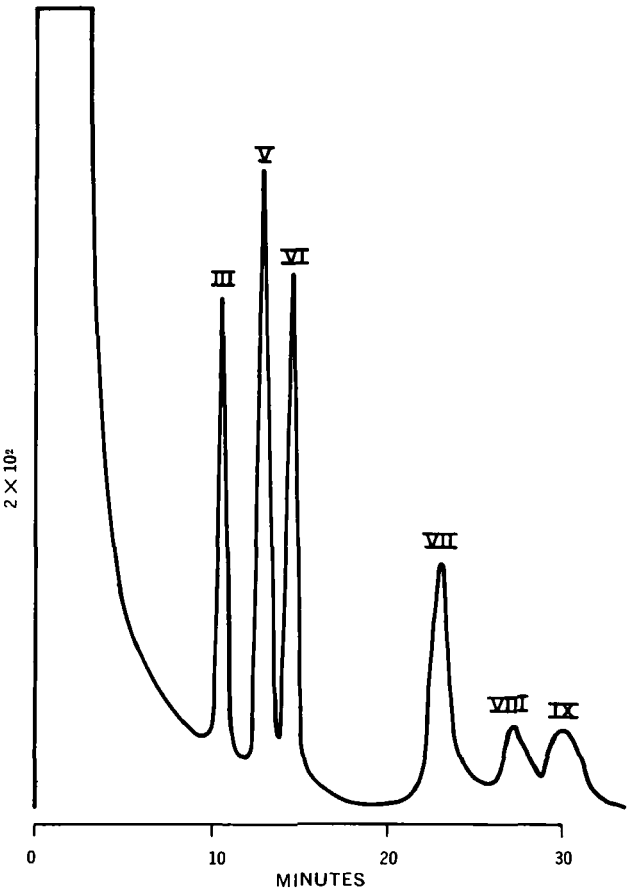

Figure 2—*Gas chromatogram of flash-heater methylated γ-hydroxyphenylbutazone (peaks III, V, VII, and VIII) and oxyphenbutazone (peaks VI and IX)—no extraction.*

RESULTS AND DISCUSSION

Flash-heater methylation of γ-hydroxyphenylbutazone with trimethylanilinium hydroxide gave four peaks with retention times of 10.6, 12.9, 23.1, and 27.1 min (Fig. 2, peaks III, V, VII, and VIII, respectively). To establish the structures of the compounds giving rise to these peaks, the eluates from the GLC column were fed directly into the mass spectrometer[10] and a mass spectrum of each peak was recorded. The mass spectrum of the compound giving rise to peak III showed a molecular ion at *m/e* 352 and other diagnostic ions, which indicated the structure of the methylated derivative to be 1,2-diphenyl-3,5-dioxo-4-methyl-4-(3-methoxy)butylpyrazolidine.

The mass spectra of the other peaks (V, VII, and VIII) showed them to be methylated breakdown products of γ-hydroxyphenylbutazone. The respective peak height ratios of the three breakdown products giving rise to peaks V, VII, and VIII varied with the concentration of flash-heater methylated γ-hydroxyphenylbutazone. Under the described GLC conditions, the height of peak III was observed to be proportional to the concentration of γ-hydrox-

[2] Dr. A. Sallmann, Ciba-Geigy, Switzerland.
[3] Chromatographic Specialties, Brockville, Ontario, Canada.
[4] Butazolidin, Geigy Pharmaceuticals, Montréal, Canada.
[5] Vacutainers, Becton Dickinson & Co., Mississauga, Ontario, Canada.
[6] Model F/11, Perkin-Elmer, Canada.
[7] OV-7, Chromatographic Specialties, Brockville, Ontario, Canada.
[8] Chromosorb W, Chromatographic Specialties, Brockville, Ontario, Canada.
[9] Silyl-8, Pierce Chemical Co., Rockford, Ill.
[10] Hitachi Perkin-Elmer model RMS4 mass spectrometer with Perkin-Elmer gas chromatograph model 990.

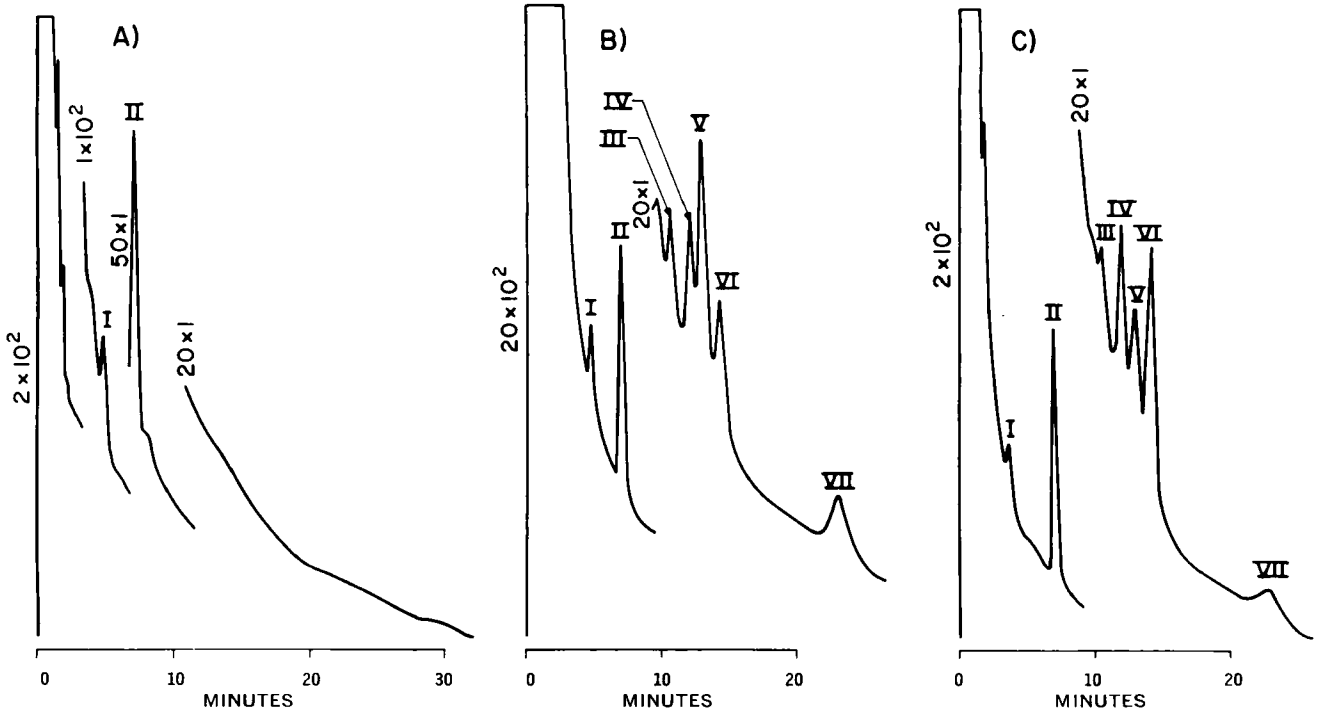

Figure 3—*Gas chromatograms of human plasma. Key: A, control plasma, endogenous material (I and II); B, plasma containing 11.2 μg/ml γ-hydroxyphenylbutazone (III, V, and VII), 7.76 μg/ml oxyphenbutazone (VI), and 1.11 μg/ml internal standard (IV); and C, plasma from a human volunteer who was given 15 × 100-mg tablets of phenylbutazone, 6.00 μg/ml of γ-hydroxyphenylbutazone, and 12.5 μg/ml of oxyphenbutazone.*

yphenylbutazone added to plasma. Therefore, peak III was used for quantitation.

Peaks VI and IX (Fig. 2) with retention times of 14.4 and 30.0 min, respectively, are due to flash-heater methylation of oxyphenbutazone, the other major metabolite of phenylbutazone.

The concentration of the compound giving rise to peak III varied with the injection port temperature. This temperature was optimized to give the maximum quantity of the methylated product (peak III).

Figure 3A shows a typical chromatogram obtained by processing fresh blank plasma but omitting the internal standard. The extraneous peaks I and II were observed in chromatograms of plasma from all human subjects (4), including pooled plasma. These extraneous peaks do not interfere with peaks due to methylated γ-hydroxyphenylbutazone (peak III) and internal standard (peak IV), as shown in the chromatogram for spiked plasma (1 ml) (Fig. 3B) containing γ-hydroxyphenylbutazone (11.2 μg) and oxyphenbutazone (7.76 μg). Phenylbutazone and oxyphenbutazone do not interfere in the assay, because phenylbutazone is removed by partitioning into *n*-heptane and flash-heater methylation of oxyphenbutazone gives peaks VI and IX separated from the methylated γ-hydroxyphenylbutazone. Figure 3C shows the chromatogram of a sample (175 hr after first dose) from a subject who had been given 100 mg phenylbutazone every 12 hr for 15 doses. The concentration of γ-hydroxyphenylbutazone was estimated to be 6.0 μg/ml.

The response of the flame-ionization detector to γ-hydroxyphenylbutazone was linear in the 1–10-μg/ml range (Fig. 1). Calibration curves were tested in the presence of various amounts of oxyphenbutazone, and no change in the slope of γ-hydroxyphenylbutazone was observed. The efficiency of extraction of γ-hydroxyphenylbutazone in this procedure was low but consistent. The overall recovery from plasma at 4 and 10 μg/ml was 20.8 ± 0.9% in the presence of oxyphenbutazone (1–20 μg). Several attempts were made to improve the recovery by employing different solvents, such as methylene chloride and ethylene chloride. However, there were large variations in the recovery, attributed either to the breakdown of γ-hydroxyphenylbutazone or to carryover of trace amounts of acid which would inhibit flash-heater methylation.

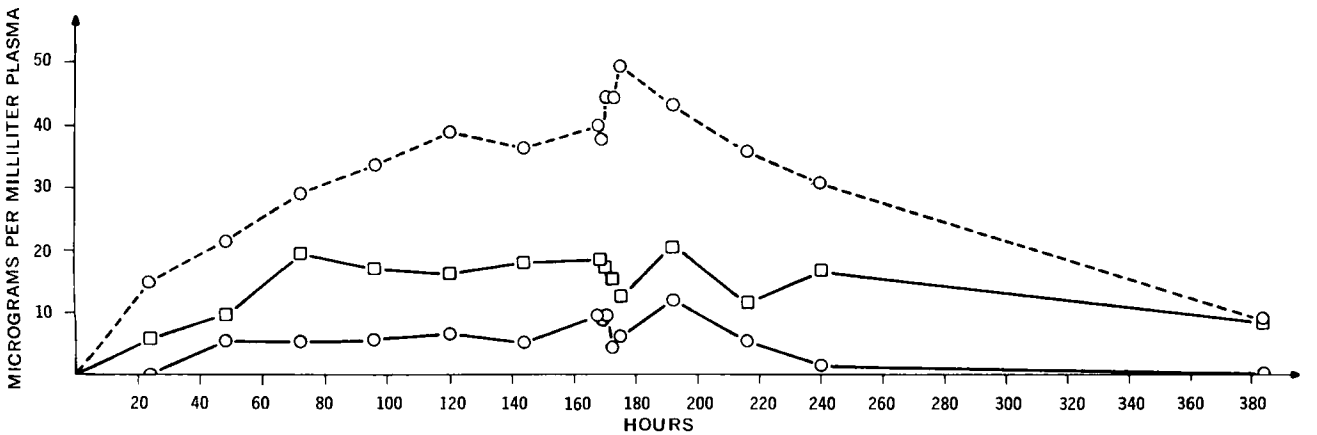

Figure 4—*Phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone concentrations in a human volunteer following multiple oral doses of 15 × 100-mg tablets of phenylbutazone. Key: O---O, phenylbutazone; □—□, oxyphenbutazone; and O—O, γ-hydroxyphenylbutazone.*

Application of the method to plasma level determination of γ-hydroxyphenylbutazone by GLC is shown in Fig. 4. This profile was assembled following multiple doses of phenylbutazone to this subject (15 × 100 mg). Following single doses of phenylbutazone (up to 400 mg) in six subjects, γ-hydroxyphenylbutazone could not be detected.

The described GLC method is sensitive and specific for γ-hydroxyphenylbutazone. It offers the advantage that both metabolites (γ-hydroxyphenylbutazone and oxyphenbutazone) can be determined simultaneously following phenylbutazone administration.

REFERENCES

(1) J. J. Burns, R. K. Rose, T. Chenkin, A. Goldman, A. Schulert, and B. B. Brodie, *J. Pharmacol. Exp. Ther.*, **109**, 364(1953).

(2) K. K. Midha, I. J. McGilveray, and C. Charette, *J. Pharm. Sci.*, **63**, 1234(1974).

(3) I. J. McGilveray, K. K. Midha, R. Brien, and L. Wilson, *J. Chromatogr.*, **89**, 17(1974).

(4) I. J. McGilveray, J. C. K. Loo, and K. K. Midha, *Pharmacologist*, **15**, 213(1973).

(5) J. C. K. Loo, K. K. Midha, and I. J. McGilveray, "Abstracts of Academy of Pharmaceutical Sciences," APhA Academy of Pharmaceutical Sciences, Washington, D.C., 1973, p. 72.

ACKNOWLEDGMENTS AND ADDRESSES

Received March 4, 1974, from the *Pharmaceutical Chemistry Division, Drug Research Laboratories, Health Protection Branch, Department of National Health and Welfare, Ottawa, Ontario, Canada K1A OL2.*

Accepted for publication June 11, 1974.

The authors express their appreciation to Mr. J.-C. Ethier for running the mass spectra.

[x] To whom inquiries should be directed.

Simultaneous GLC Estimation of Salicylic Acid and Aspirin in Plasma

L. J. WALTER, D. F. BIGGS [x], and R. T. COUTTS

Abstract □ Plasma salicylates, salicylic acid and acetylsalicylic acid (aspirin), were estimated as their respective trimethylsilyl derivatives by GLC. The method involved the use of fluoride and cooling to minimize the hydrolysis of aspirin in whole blood. Following solvent extraction, internal standards were added to each plasma extract. The extracts were evaporated to dryness and the residues were trimethylsilylated with hexamethyldisilazane in acetone. Small portions of the reaction mixture were chromatographed and the salicylates were quantitated. The components eluting from the chromatograph were identified by IR spectrophotometry and mass spectrometry.

Keyphrases □ Salicylic acid and aspirin—simultaneous GLC determination in plasma □ Aspirin and salicylic acid—simultaneous GLC determination in plasma □ GLC—analysis, simultaneous, salicylic acid and aspirin in plasma

Numerous methods have been described for the determination of salicylic acid and aspirin in pharmaceuticals and in biological media. Of these published techniques, surprisingly few have been developed for the simultaneous and direct determination of aspirin and salicylic acid in a single sample.

Among the techniques employed in the past is a large group of colorimetric estimations involving complexation with ferric ion (1–9). Other colorimetric assays involve diazotization with *p*-nitroaniline and nitrous acid (10), use of Folin and Ciocalteu phenol reagent (11), complexation with cupric ion in nitrous acid (13–15), or estimation as the nitro derivative (16).

Collectively, colorimetric assays are quite nonspecific. Plasma blank values are often high; blanks equivalent to 240 μg of salicylic acid/ml have been reported (11). Common plasma constituents such as salicyluric acid (1, 12), gentisic acid, uric acid, tyrosine, and tryptophan (12) interfere with colorimetric assays.

Furthermore, this entire group of assays determines salicylic acid only; to estimate aspirin, hydrolysis to salicylic acid and a second determination are required. The difference between the two salicylic acid concentrations is assumed to be due to aspirin.

A second analytical method is the group of UV assays. By these techniques, either salicylic acid alone or salicylic acid plus aspirin may be determined. Salicylic acid has been estimated, either in combination with aspirin (17) or individually following a physical separation step (18–21).

Both salicylates have been determined simultaneously by use of the pH-dependent shift in their individual absorption spectra (22–24). This hypsochromic shift technique results in some overlap of the absorption spectra of salicylic acid and aspirin, and corrections may be required for these spectral interferences (23, 24). The individual determination of salicylic acid is subject to interference from aspirin, gentisic acid, and 2,6-dihydroxybenzoic acid (19).

Salicylates have also been estimated fluorometrically. Aspirin may be estimated directly (25) or, more commonly, as salicylic acid. This has been accomplished by direct hydrolysis (26) or after a separation step followed by hydrolysis (27–30). Fluorometric assays generally require a hydrolytic procedure and a dual determination to estimate aspirin. Another problem is the high and variable background fluores-