Direct Spectrophotometric Determination of Salicylic Acid, Acetylsalicylic Acid, Salicylamide, Caffeine, and Phenacetin in Tablets or Powders

By A. W. CLAYTON and R. E. THIERS*

A simple ultraviolet spectrophotometric method has been developed for the simultaneous determination of up to 5 different common analgesic compounds. The technique can be applied to mixtures of the compounds or to tablets or powders containing common excipients. A water–isopropanol mixture capable of dissolving chloroform is used as spectrophotometric solvent at 3 different wavelengths and under 3 different conditions of acid or base content. One absorbance measurement per constituent is required.

STANDARD analytical methods for the determination of commonly used analgesic and antipyretic compounds in tablets or powders generally involve multiple steps, and often include special separation methods. Thus in the method of the Association of Official Agricultural Chemists (1) for analysis of mixtures containing phenacetin, caffeine, and acetylsalicylic acid the compounds are separated by column chromatography prior to measurement. Other techniques of separation have been used, such as solvent extraction (2, 8), and many methods of measurement have been proposed, including infrared spectrophotometry (3), ultraviolet spectrophotometry (4, 5), titration (2), phosphorimetry (6), and nuclear resonance spectroscopy (7).

Of these approaches ultraviolet spectrophotometry seems to offer the greatest promise of accuracy combined with simplicity and the ability to measure a number of constituents. Mixtures of compounds have been analyzed in this fashion by several workers, in some cases after preliminary separation from each other (4, 5, 8–11). However, common excipients employed in the formulation of actual analgesic powders or tablets also possess ultraviolet absorption and can interfere.

A simple method has been developed which can simultaneously determine up to 5 common analgesic compounds together, in simple mixtures or in tablets and powders containing the common excipients used in manufacture. Separation of the 5 compounds is unnecessary. A mixture of isopropanol and water is used as solvent for spectrophotometry under 3 different conditions of acid or base content. Five absorbance measurements are made, and from the 5 values obtained the amounts of acetylsalicylic acid (ASA), salicylamide (SAL), salicylic acid (SAA), caffeine (CAF), and phenacetin (PHE) in the original sample can be calculated easily and accurately.

Received December 23, 1965, from the Control Laboratory, B. C. Remedy Co., Durham, N. C.
Accepted for publication February 7, 1966.
The authors acknowledge the technical assistance of Mrs. Margaret Durham.
* Duke University Medical Center, Durham, N. C.

EXPERIMENTAL

Apparatus and Reagents.—*Spectrophotometer.*—A Beckman DU 2 spectrophotometer was used, except for the acquisition of the spectra shown in Fig. 1, which were obtained on a Bausch & Lomb model 505 spectronic spectrophotometer. In all cases standard 1 cm. square fused silica cells were employed.

Solvents.—Spectral grade, analytical reagent chloroform and isopropanol (Mallinckrodt Chemical Co.) were used.

Mixed Solvent.—An acid solution of isopropanol and water was made by placing 400 ml. of isopropanol in a volumetric flask, adding about 500 ml. of water, adding 0.5 ml. concentrated hydrochloric acid, mixing, then diluting to volume with water.

Hydrochloric Acid.—Mallinckrodt, analytical reagent grade.

Sodium Hydroxide.—Harleco APHA, ammonia free, 50% solution (Hartman-Leddon Co., Philadelphia, Pa.).

Acetylsalicylic Acid.—Monsanto, U.S.P. grade.

Salicylamide.—S. B. Penick Co., N.F. grade.

Salicylic Acid.—Merck, reagent grade.

Phenacetin.—Monsanto, U.S.P. grade.

Caffeine.—Monsanto, U.S.P. grade.

All 5 analgesic compounds were checked by infrared spectrophotometry.

Spectrophotometric Reference Solution.—A reference or "blank" solution for spectrophotometry was made by pipeting 1.00 ml. of chloroform into a 200-ml. volumetric flask, adding about 80 ml. of mixed solvent, mixing carefully, then diluting to the mark with mixed solvent and remixing.

Procedure.—Weigh accurately about 1 Gm. of the dry powder or tablet to be tested. Place this sample into a 100-ml. volumetric flask. Add chloroform to volume and mix to dissolve all soluble matter. Set aside for 15 min., or until the undissolved material has settled, leaving a clear supernatant fluid.

Pipet 1.000 ml. of this supernatant fluid into each

of three 200-ml. volumetric flasks labeled A (for acidic), B (for basic), and H (for hydrolyzed). Add about 100 ml. of mixed solvent to each and mix. Add 1.0 ml. of 50% NaOH to each of flasks B and H and mix. Wait 15 min. to ensure complete hydrolysis of ASA, then add to flask H 3.0 ml. of 12 *N* HCl to render the solution acid again, and mix. Dilute all 3 to the mark with mixed solvent.

If all 5 components are to be determined, read the absorbance against the reference solution, of solution A at 250, 273, and 301 mμ, of solution B at 333 mμ, and of solution H at 301 mμ. If fewer components are to be measured, appropriate omissions and short cuts may be introduced by inspection of the equations below in the light of the specific problem.

Calculations.—If the absorptivity, *a*, of phenacetin, for example, at 333 mμ is designated a_{333}^{PHE},

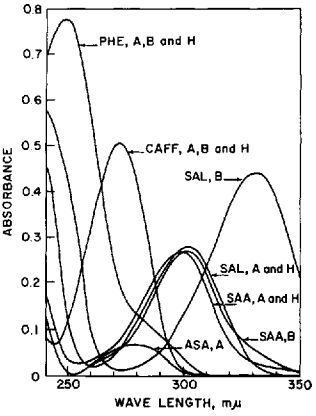

Fig. 1.—Spectra of 0.1 mg./L. solutions of the 5 compounds in mixed solvent.

$$A_{333}^{total} = a_{333}^{ASA}(ASA) + a_{333}^{SAL}(SAL) + a_{333}^{SAA}(SAA) + a_{333}^{CAF}(CAF) + a_{333}^{PHE}(PHE) \quad \text{(Eq. 3)}$$

Each of the 5 measurements made in the procedure provides an A^{total} for one particular set of conditions of wavelength and acid–base treatment. For each set of conditions a unique set of values of the various absorptivities exists. Five simultaneous equations may therefore be set up from which the 5 desired concentrations may be calculated. Other components of the original sample which are insoluble in chloroform do not interfere in the measurement. The chloroform itself is wholly soluble in the mixed solvent and its absorbance at the lower wavelengths is exactly cancelled by the reference solution.

Table I gives the value of *a*, the absorptivity, for each component under each condition and wavelength involved in the calculation. Figure 1 shows the spectra of 0.1 mg./L. solutions of the 5 compounds under the conditions shown.

As can be seen from both the figure and table, (*a*) the difference between the absorbance of solutions H and A at 301 mμ is wholly due to hydrolysis of acetylsalicylic acid to salicylic acid, (*b*) the absorbance of solution B at 333 mμ is predominantly due to salicylamide, (*c*) the absorbance of solution A at 301 is largely due to salicylic acid and the above two, (*d*) and finally, in solution A caffeine is the compound with by far the highest absorptivity at 273 mμ, while at 250 mμ phenacetin has this distinction. These facts permit a simpler but equally accurate approach to the calculations than the conventional solution of 5 simultaneous equations. In this approach a preliminary approximate calculation is made by equating to zero certain small absorptivities. This gives the following equations:

$$A_{301}^{H} - A_{301}^{A} = 2.10\,(ASA) \quad \text{(Eq. 4)}$$

which provides a value for (ASA)

TABLE I.—ABSORPTIVITIES[a]

Compd.	Condition: 333 mμ Flask B	301 mμ Flask A	301 mμ Flask H	273 mμ Flask A	290 mμ Flask A
ASA	0.11	0.05	2.15	0.63	0.52
SAL	4.40	2.81	2.81	0.57	1.73
SAA	0.17	2.75	2.75	0.48	0.96
CAF	0.02	0.05	0.05	4.92	1.57
PHE	0.03	0.28	0.28	1.81	8.02

[a] The absorbance values listed are those observed when 1.000 Gm. of each pure component is treated by the procedure described. Their units are therefore "absorbance units per Gm. in the original weighed sample." The values given are averages of triplicate measurements on each of triplicate weighed dry samples of the pure components repeated 4 separate times over a number of weeks.

and the concentration of phenacetin as (PHE), then the absorbance due to phenacetin at 333 mμ, A_{333}^{PHE}, will be given by:

$$A_{333}^{PHE} = a_{333}^{PHE}(PH) \quad \text{(Eq. 1)}$$

At any given wavelength, *e.g.*, 333 mμ the measured absorbance, A_{333}^{total}, is the sum of the absorbances of the various components.

$$A_{333}^{total} = A_{333}^{ASA} + A_{333}^{SAL} + A_{333}^{SAA} + A_{333}^{CAF} + A_{333}^{PHE} \quad \text{(Eq. 2)}$$

and therefore

$$A_{333}^{B} = 0.11\,(ASA) + 4.40\,(SAL) \quad \text{(Eq. 5)}$$

which provides a value for (SAL)

$$A_{301}^{A} = 0.05\,(ASA) + 2.81\,(SAL) + 2.75\,(SAA) \quad \text{(Eq. 6)}$$

which provides a value for (SAA)

$$A_{273}^{A} = 0.63\,(ASA) + 0.57\,(SAL) + 0.48\,(SAA) + 4.92\,(CAF) + 1.81\,(PHE) \quad \text{(Eq. 7)}$$

$$A_{250}^{A} = 0.52\,(ASA) + 1.73\,(SAL) + 0.96\,(SAA) + 1.57\,(CAF) + 8.02\,(PHE) \quad \text{(Eq. 8)}$$

TABLE II.—MIXTURE NUMBER[a]

Compd.	1 Taken	1 Found	2 Taken	2 Found	3 Taken	3 Found	4 Taken	4 Found	5 Taken	5 Found
ASA	0.200	0.194	0.350	0.353	0.300	0.294	0.250	0.247	0.300	0.301
SAL	0.300	0.297	0.000	0.000	0.300	0.302	0.150	0.149	0.100	0.097
SAA	0.000	0.000	0.000	−0.002	0.000	0.000	0.100	0.096	0.150	0.148
CAF	0.000	0.000	0.050	0.050	0.040	0.042	0.200	0.209	0.050	0.048
PHE	0.250	0.254	0.250	0.253	0.000	−0.001	0.050	0.052	0.100	0.103
Total	0.750	0.745	0.650	0.654	0.640	0.637	0.750	0.753	0.700	0.697

[a] Results are in Gm.

Equations 7 and 8 can easily be solved for (CAF) and (PHE).

The final calculation is made by rewriting Eqs. 5 and 6 to include all terms, as follows, then recalculating in the same manner as above but including the preliminary values for the terms missing in the first approximation.

$$A^{A}_{333} = 0.11\,(ASA) + 4.40\,(SAL) + 0.17\,(SAA) + 0.02\,(CAF) + 0.03\,(PHE) \quad (Eq.\ 5a)$$

$$A^{A}_{301} = 0.05\,(ASA) + 2.81\,(SAL) + 2.75\,(SAA) + 0.05\,(CAF) + 0.28\,(PHE) \quad (Eq.\ 6a)$$

For concentrations usually encountered in practice a third approximation is seldom necessary; and if any prior knowledge of approximate composition exists, it can usually be employed to make the first calculation the only one required.

If fewer than 5 constituents are known to be present, fewer readings and equations are obviously sufficient. The known absence of salicylamide makes the B flask and 333 mμ reading unnecessary, and of acetylsalicylic acid, the H flask and its reading. If salicylic acid is known to be absent it is possible to omit one step, but the omission is not recommended, since this step provides a measure of any hydrolysis of acetylsalicylic acid which might have occurred during storage or manufacture of the product analyzed.

The value $A^{H}_{301} - A^{A}_{301}$ can, of course, be obtained directly by making solution A the reference or "blank" sample at 301 mμ for solution H.

Discussion.—Three features of the method described combine with the known advantages of ultraviolet spectrophotometry to provide a simple and accurate procedure. First, the utilization of isopropanol–water as a solvent (as distinct from chloroform, etc.) permits spectra to be measured under either acidic or alkaline conditions, and permits the utilization of the solitary 333 mμ absorbance of basic salicylamide. This mixed solvent also dissolves sufficient chloroform to enable one to dilute a chloroform solution with an aqueous solution and still have a one-phase system. The lower limit of the concentration of isopropanol in the mixed solvent is in fact set by the amount of chloroform which must be dissolved. An important practicality—the mixed solvent handles like water and is more convenient than organic solvents. Second, the technique of rapid hydrolysis of acetylsalicylic acid to salicylic acid in basic solution followed by a return to acid conditions (solution H) provides a direct assay for the former compound in the presence of any other material unaffected by the treatment. Experiments were performed to determine the optimal concentration of base and time of hydrolysis. The values chosen provide complete hydrolysis in a conveniently short time interval without causing decomposition. Third, it is a fortunate fact that the excipients generally used in the manufacture of tablets and powders are not only insoluble in chloroform but settle out of suspension very rapidly when shaken with chloroform. Centrifugation, originally designed as part of the procedure, proved unnecessary. A variety of excipients were treated by the procedure described and in no case was any absorbance observed in the mixed solvent. These include talc, cornstarch, lactose, sucrose, microcrystalline cellulose,[1] dextrin, gelatin, acacia, tragacanth, fumaric acid, sodium chloride, and potassium chloride.

Ordinarily the spectrophotometric measurement of so many components and the setting up of so many simultaneous equations is neither convenient nor accurate. In this case, however, it has been possible to choose conditions where certain of the absorptivities approach zero, while others are maximal. In addition one set of the chosen conditions has, by hydrolysis, reduced the number of components present. The calculations are thus greatly simplified.

The procedure is inherently simple and accurate. As a test of these factors, 5 different known mixtures were prepared and presented to a technician who, although experienced in spectrophotometry, had never analyzed mixtures by this procedure. The results obtained on the one sample which was analyzed from each mixture are given in Table II. Duplicate absorbance readings were made on each cell of sample, but no replication of samples was permitted. Results were calculated (from the averages of the absorbance readings) exactly as described above with no prior knowledge of sample composition assumed. The total error in 25 determinations was −0.004 Gm.; the average deviation was ±0.002 Gm.

This procedure has been in use for 6 months in routine analysis and quality control of products containing some but not all of the 5 compounds, as well as numerous excipients. It is rapid, complete analysis of one sample requiring about 10 min. of the operator's time when multiple samples are being run, with another 10 min. required for calculation. Its precision, based on repeated analysis of standards during this time, can be expressed as a coefficient of variation of 0.5%. When necessary, this value could easily be decreased further by employment of techniques of ultra high precision spectrophotometry (12).

[1] Marketed as Avicel by the American Viscose Corp.

REFERENCES

(1) Smith, G., *J. Assoc. Offic. Agr. Chemists*, **42**, 462 (1959).
(2) "Official and Tentative Methods of Analysis of the Association of Agricultural Chemists," 6th ed., Association of Agricultural Chemists, Washington, D. C., 1945, p. 675.
(3) Parke, T. V., Ribley, A. M., Kennedy, E. E., and Hilty, W. W., *Anal. Chem.*, **23**, 953(1951).
(4) Tinker, R. B., and McBay, A. J., *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 315(1954).
(5) Jones, M., and Thatcher, R. L., *Anal. Chem.*, **23**, 957 (1951).
(6) Winefordner, J. D., and Latz, H. W., *ibid.*, **35**, 1517 (1963).
(7) Hollis, D. P., *ibid.*, **35**, 1682(1963).
(8) Pernarowski, M., and Padval, V. A., *J. Pharm. Sci.*, **52**, 218(1963).
(9) Josephina, A. C., *Anales Fac. Farm. Bioquim. Univ. Nacl. Mayor San Marcos (Lima)*, **6**, 519(1955); through *Chem. Abstr.*, **53**, 13506*i*(1959).
(10) Bukowska, H., *Chem. Anal. (Warsaw)*, **4**, 105(1959); through *Chem. Abstr.*, **53**, 14419*g*(1959).
(11) Machek, G., *Sci. Pharm.*, **29**, 73(1961).
(12) Reilley, C. N., and Crawford, C. M., *Anal. Chem.*, **27**, 716(1955).

Effects of Ionizing Radiation on Two Gelatin Fractions III

Carbonyl Group Analyses and Electron Spin Resonance Studies

By LEONARD P. PRUSAK* and BARTLEY J. SCIARRONE

Two fractions, F-I and F-II, obtained by alcohol fractionation of commercial pigskin gelatin, and having molecular weight values of 173,000 and 86,000, respectively, showed the following relationship with respect to additional carbonyl group content resulting from low-level irradiation under a 3 Mev. Van de Graaff: 1 per cent solution > 5 per cent solution (gel) > film. Irradiated films (F-I and F-II) showed doublets with 25 gauss line separation in electron spin resonance studies. These, together with previously published data, indicate that the gelatin fractions undergo molecular weight changes through free radical mechanisms involving scission, crosslinking, and weak bond formation.

In previous papers (1, 2) the authors showed the isolation of 2 fractions, F-I and F-II, from commercial pigskin gelatin; the irradiation technique used with a 3 Mev. Van de Graaff accelerator; acid-base titration behavior; sedimentation velocity and intrinsic viscosity studies; and molecular weight determinations.

We now give further experimental data which indicate that irradiation of these gelatin fractions produces changes in structure through free radical mechanisms.

The currently accepted concept regarding radiation effects on organic materials is that chain scission is synonymous with molecular weight decrease, and crosslinking is a sign of molecular weight increase. Intermediate variations are explained on grounds that both scission and crosslinking occur. This thinking may be extended to the irradiation of complex polymers, assuming that conditions are employed which lead to these ultimate effects. It is doubtful, on the basis of experimental data available today, that gelatin is susceptible to predictable irradiation behavior.

Some factors which must be considered before rupture or linkage are proposed as explanations for shifts in molecular weight of irradiated gelatin include solvent–solute interaction, absorbed irradiation dose, presence or absence of oxygen, solute concentration, nature of solvent, thermal history, aging, irradiation temperature, etc.

Since the formation of free radicals in solids and liquids exposed to irradiation has been established, explanations of molecular weight shifts in irradiated gelatin require consideration of free radical formation. This, in turn, poses the question as to indirect effects of the solvent if irradiation is carried out on a fluid system. Experimental evidence indicates that irradiation of oxygenated protein solutions, such as gelatin, results in the formation of carbonyl functions (3, 4) and that these are traceable to intermediate free radicals. Radiolytic cleavage of the peptide chain yields an amide and an additional carbonyl group:

Received March 9, 1962, from the College of Pharmacy, Rutgers—The State University, Newark, N. J.
Accepted for publication January 27, 1966.
Abstracted in part from a dissertation submitted by L. P. Prusak to the Graduate School, Rutgers—The State University, Newark, N. J., in partial fulfillment of Doctor of Philosophy degree requirements.
The assistance of Mr. W. A. Yager and Mr. S. H. Glarum, Bell Telephone Laboratories, Murray Hill, N. J., in obtaining and analyzing spin resonance data is gratefully acknowledged.
The authors thank Dr. Charles Artandi, Research Director, Ethicon, Inc., Somerville, N. J., for valuable advice during the irradiation phase of these studies.
* Present address: Johnson & Johnson, New Brunswick, N. J.