Analysis of p-Aminosalicylic Acid, Its Salts and Dosage Forms, by Nonaqueous Titration

JAMES HUNT and MARTIN I. BLAKE

Abstract ☐ p-Aminosalicylic acid, its salts, and its dosage forms are determined by visual or potentiometric titration with sodium methoxide in benzene–methanol using dimethylformamide as the titration solvent. p-Aminosalicylic acid and its decomposition product m-aminophenol may be differentiated with this titration system. Salts of p-aminosalicylic acid are converted to the acid form by ion-exchange chromatography prior to titration. The procedure is applied to several dosage forms.

Keyphrases ☐ p-Aminosalicylic acid and salts—analysis ☐ Dosage forms, p-aminosalicylic acid and salts—analysis ☐ Column chromatography—separation ☐ Potentiometric titration—analysis

The USP XVII method (1) for the determination of p-aminosalicylic acid (PAS), its salts and dosage forms, involves the diazotization reaction and is based on procedures developed by Tarnoky and Brews (2) and Pesez (3, 4). The use of an external indicator and the nonselectivity of the reaction have been noted by Chatten (5) as shortcomings of the procedure. m-Aminophenol (MAP), the major breakdown product of PAS, is also diazotized in the titration process; in the official monographs the MAP content is determined separately by colorimetric analysis.

A variety of analytical methods has been proposed for the determination of PAS and its salts. These have been extensively reviewed by Lach and Cohen (6).

A number of nonaqueous titrimetric procedures have appeared in the literature. They have been reviewed by Kucharsky and Safarik (7) and are briefly noted here. Chatten (8) reported the visual titration of PAS with potassium hydroxide in methanol as titrant, acetone as the titration solvent, and thymol blue as indicator. For sodium p-aminosalicylate, methanol served as the titration medium and perchloric acid in dioxane as titrant. These methods were reported to be specific even in the presence of MAP. Butler and Ramsey (9) titrated PAS and its sodium salt potentiometrically with perchloric acid in glacial acetic acid. An acetic acid–carbon tetrachloride solvent mixture served as the titration medium. Stockton and Zuckerman (10) determined sodium p-aminosalicylate and its solutions by potentiometric titration with perchloric acid in propylene glycol and isopropyl alcohol (1:1), using the same solvent mixture as the titration medium. The decomposition products MAP and sodium bicarbonate did not interfere. Das and Palit (11) employed the same titrant and solvent system.

In the present study, PAS is titrated with sodium methoxide in benzene–methanol (10:1). The titration medium is dimethylformamide. The difference in the acidic properties of PAS and MAP permitted a dif-

Table I—Comparative Study of Proposed and Official Assays for p-Aminosalicylate Content in Dosage Forms

Dosage Form	Labeled Amount, g./Unit Dose	Label Claim Found, %	
		Proposed Method	Official Assay
***p*-Aminosalicylic Acid**			
Powder	—	99.86 ± 0.24[a]	100.04 ± 0.37
Sodium *p*-Aminosalicylate			
Powder	—	99.62 ± 0.29	100.52 ± 0.06
Capsule	0.50	100.71 ± 0.54	100.43 ± 0.26
Tablet	0.50	100.77 ± 0.86	99.82 ± 0.29
Tablet	0.69	98.70 ± 0.57	99.98 ± 0.26
Tablet	1.00	97.40 ± 0.32	100.50 ± 0.38
Calcium *p*-Aminosalicylate			
Powder	—	99.45 ± 0.17	99.40 ± 0.52
Tablet (chocolate coated)	0.50	100.43 ± 0.72	100.21 ± 0.18
Capsule	0.50	96.99 ± 0.42	100.58 ± 0.71
Solution	1.00 (5 ml.)	96.87 ± 0.89	102.18 ± 0.40

[a] Average deviation based on at least five determinations.

ferentiating titration of the two components. The salts of PAS are converted to the acid by ion-exchange chromatography prior to titration. The techniques are applied to several dosage forms.

EXPERIMENTAL

Apparatus—Titrations were performed visually or potentiometrically with a Fisher titrimeter, model 35, equipped with a sleeve-type calomel and platinum electrode system. A 50-ml. buret (1 cm. i.d.) was employed as a chromatographic column. It was plugged at the base with glass wool to support the resin column.

Reagents—(a) p-Aminosalicylic acid, m-aminophenol, and sodium and calcium p-aminosalicylate were the best quality available from commercial sources. The m-aminophenol was further purified by recrystallizing from hot water. (b) Tenth normal sodium methoxide in benzene–methanol (10:1) was prepared and standardized as described earlier (12). (c) Other chemicals and all solvents used in this study were reagent grade and were used without further purification. (d) Powder, capsule, and tablet dosage forms were supplied by Dorsey Laboratories, Lincoln, Neb. A solution of sodium p-aminosalicylate was obtained from Hines Veterans Administration Hospital, Hines, Ill., prepared as directed in their hospital pharmacy formulary.

Preparation of Column—A weak cation-exchange resin (Amberlite IRC-50) and a strong cation-exchange resin (Dowex 50W-X8) were used in this study. The resin columns were prepared as described earlier (13, 14).

General Assay Procedure—One-half to one milliequivalent of sodium or calcium p-aminosalicylate, accurately weighed, was dissolved in the smallest volume of dimethylformamide and the solution passed through the column containing the weak resin. Effluent was collected in a 100-ml. graduated cylinder. Additional dimethylformamide was added to the column until 75 ml. of ef-
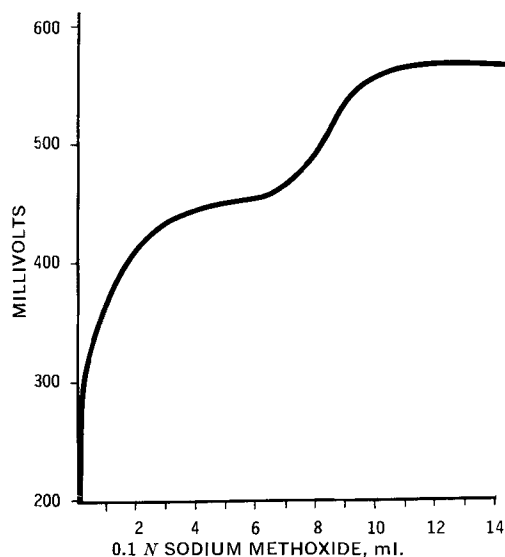
Figure 1—*Typical titration curve for MAP.*

fluent was collected. The effluent was titrated visually to the first permanent blue color using thymol blue as indicator (0.3% in methanol) or potentiometrically with the Fisher titrimeter. By the latter method, the end-point was determined from the inflection of the curve obtained by plotting volume of titrant *versus* millivolt readings.

Analysis of Dosage Forms—*Capsules and Tablets*—Twenty tablets were weighed and powdered, or 20 capsules were emptied as completely as possible and the contents weighed. A sample of the powder mass equivalent to between 0.5 and 1.0 meq. of *p*-aminosalicylate salt was dissolved in 25 ml. of dimethylformamide with the aid of magnetic stirring. The solution was treated as described under *General Assay Procedure.*

Chocolate-coated tablets of calcium *p*-aminosalicylate were soaked in distilled water until the coating dissolved. This required no longer than 5 min. The tablets were dried rapidly between filter paper and the dried tablets were assayed as described previously.

Solution—One milliliter of a solution, labeled to contain 1 g. of sodium *p*-aminosalicylate per 5 ml., was transferred by pipet to the column containing the weak resin. The column was eluted with dimethylformamide until a total of 75 ml. of effluent was collected. The effluent was titrated as described previously.

Official Assay Procedure—*p*-Aminosalicylic acid, the sodium and calcium salts, and their dosage forms were assayed as described in USP XVII (1).

Differentiating Titration of *p*-Aminosalicylic Acid and *m*-Aminophenol—Samples containing 1 meq. of MAP and a series of mixtures containing 1 meq. of PAS and varying amounts of MAP were titrated visually and potentiometrically in dimethylformamide with 0.1 N sodium methoxide as titrant.

Table II—Analysis of PAS and MAP Mixtures

Meq. Ratio of Components, PAS–MAP	Recovery, %	
	PAS	MAP
Visual Titration		
1.00:1.00	101.41 ± 0.98[a]	
1.00:0.50	99.66 ± 0.08	
1.00:0.33	97.55 ± 0.65	
1.00:0.25	99.97 ± 0.48	
1.00:0.20	99.47 ± 0.10	
Potentiometric Titration		
1.00:1.00	96.57 ± 0.42	99.21 ± 0.45
1.00:0.50	98.26 ± 0.37	99.74 ± 0.85
1.00:0.33	97.93 ± 0.22	101.11 ± 0.87
1.00:0.25	98.85 ± 0.54	103.84 ± 0.23
1.00:0.20	97.86 ± 0.34	102.35 ± 0.08

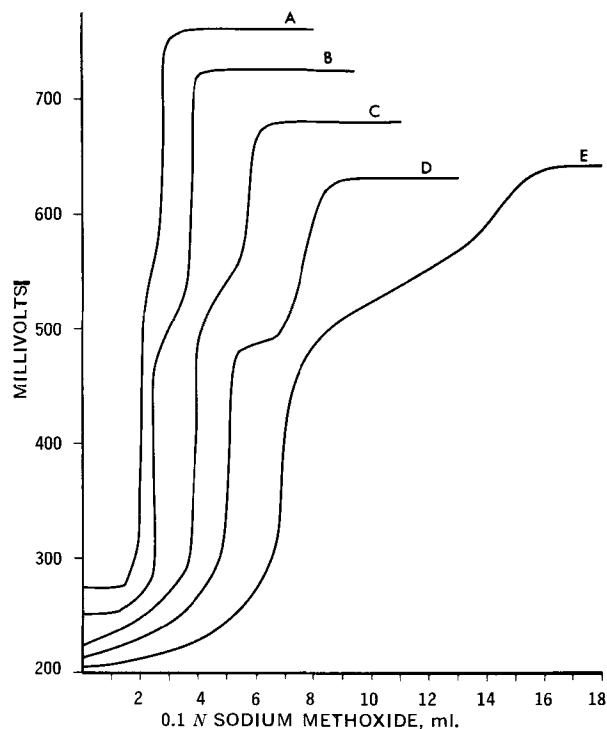[a] Average deviation based on at least five determinations.

Figure 2—*Differentiating titration of mixtures of PAS and MAP in varying meq. ratios: A, 1:0.20; B, 1:0.25; C, 1:0.33; D, 1:0.50; and E, 1:1.0.*

Chromatographic Separation of *p*-Aminosalicylic Acid and *m*-Aminophenol—A series of mixtures corresponding to those described in the previous section was passed through a chromatographic column containing a strong cation-exchange resin. Aliquots of the mixture were treated as described in the *General Assay Procedure*, except that a strong resin (Dowex 50W-X8) was used in place of the weak resin.

RESULTS AND DISCUSSION

A major drawback to the official assay procedure for PAS, its salts and dosage forms, is that the breakdown product MAP also responds to the assay. As a result, apparently quantitative recoveries may be obtained even though extensive decomposition may have taken place. Although the USP includes a limits test for MAP based on colorimetric analysis, it would be desirable to have a simple titrimetric procedure which would be specific for PAS. Most non-aqueous titration procedures proposed in the literature involve the titration of the amino group with perchloric acid in a suitable solvent system. Although Chatten (5,8) titrated the carboxyl group in PAS with potassium hydroxide in methanol, the sodium salt was determined with perchloric acid.

In the present study, PAS was titrated visually with sodium methoxide using thymol blue as the indicator. The end-point was readily detectable with one drop of titrant. The sodium and calcium salts and their dosage forms were titrated similarly after treatment with the weak cation-exchange resin (Amberlite IRC-50), which converted the salt to the free PAS. The results of the analyses are recorded in Table I. Comparative assays were performed by the official procedure. In almost every case the percent recovery by the official assay was essentially the same or higher than by the proposed method.

MAP was not titratable visually with sodium methoxide. Samples dissolved in dimethylformamide produced an indicator color change with the first drop of titrant. However, suitable inflections were obtained by potentiometric titration. A typical titration curve is shown in Fig. 1. The average percent recovery based on a series of 10 titrations was 99.22 ± 1.09%.

The effect of the presence of added MAP on the titration of PAS was tested. A series of mixtures of PAS and MAP, listed in Table II, was titrated visually with sodium methoxide. The presence

of MAP did not interfere with the visual end-point determination in the titration of PAS. This is probably attributable to the large difference in pKa values for these compounds: 3.25 for PAS (15) and 9.71 for MAP (16). When the mixtures were titrated potentiometrically, two inflections in the titration curve were obtained, indicating the feasibility of a differentiating titration procedure. The titration curves for the series of mixtures are shown in Fig. 2, and the analysis data are listed in Table II. In the titration curves the first inflection is attributable to PAS, while the second end-point is due to the MAP. Although the differentiating titration may not be useful for determining the MAP content when present in low concentrations (e.g., 10% or less), both the visual and the potentiometric titration procedures are specific for the PAS content.

The weak cation-exchange resin (Amberlite IRC-50) was found to be effective for converting the salts of PAS to the free acid. The PAS was eluted from the column with dimethylformamide and the eluate titrated with sodium methoxide. When mixtures of PAS (or its salt form) and MAP listed in Table II were passed through the resin column, both PAS and MAP appeared in the eluate. A differentiating titration yielded quantitative recoveries for both components. Apparently the PAS and MAP are too weakly basic to be retained by a carboxylic acid-type resin. When the strong cation-exchange resin (Dowex 50W-X8) was used in place of the weak resin, the MAP was retained by the column while the PAS passed through and was recovered quantitatively in the eluate. The percent recovery based on the potentiometric titration of seven samples was 100.22 ± 0.56%. A sulfonic acid-type resin (Dowex 50W-X8) is a sufficiently strong acid to extract the MAP from the mixture.

In preliminary studies, ethylenediamine, acetone, isopropyl alcohol, methyl isobutyl ketone, acetonitrile, and dimethylformamide were examined as solvents for the differentiating titration of PAS and MAP. Dimethylformamide was found to produce the most reproducible and clearly defined end-points. The sleeve-type calomel and platinum electrode system produced good differentiating titration curves where the conventional sleeve-type and glass electrode system was unsuccessful in differentiating PAS and MAP.

SUMMARY

The proposed assay procedure has advantage over the official assay in that the PAS content is determined by direct visual titration. MAP, if present, does not interfere with the end-point detection. Quantitative separation of MAP from PAS may be achieved by passing the mixture through a column of strong cation-exchange resin (Dowex 50W-X8). The MAP is retained by the column while the PAS appears in the eluate. Mixtures of MAP and PAS may be differentiated by potentiometric titration. While this procedure is not suitable for determining low concentrations of MAP, as is required for the official dosage forms (1% or less), it is useful for studying the kinetics of the decomposition of PAS in dosage forms, particularly solutions.

REFERENCES

(1) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, pp. 36, 37, 586, 587, 882.
(2) A. Tarnoky and B. A. Brews, Biochem. J., 45, 508(1949).
(3) M. Pesez, Bull. Soc. Chim. Fr., 30, 918(1949).
(4) M. Pesez, Bull. Soc. Chim. Biol., 31, 1369(1949).
(5) L. G. Chatten, in "Titrimetric Methods," D. S. Jackson, Ed., Plenum Press, New York, N. Y., 1969, pp. 146–154.
(6) J. L. Lach and J. Cohen, Drug Stand., 28, 65(1960).
(7) J. Kucharsky and L. Safarik, "Titrations in Nonaqueous Solvents," Elsevier, New York, N. Y., 1965, p. 182.
(8) L. G. Chatten, J. Amer. Pharm. Ass., Sci. Ed., 45, 556 (1956).
(9) A. Q. Butler and J. C. Ramsey, ibid., 42, 338(1953).
(10) J. R. Stockton and R. Zuckerman, ibid., 43, 273(1954).
(11) M. N. Das and S. R. Palit, J. Ind. Chem. Soc., 31, 34(1954).
(12) M. I. Blake, J. Amer. Pharm. Ass., Sci. Ed., 46, 287(1957).
(13) M. C. Vincent and M. I. Blake, Drug Stand., 26, 206(1958).
(14) M. I. Blake and D. A. Nona, J. Pharm. Sci., 52, 945(1963).
(15) "The Merck Index," 8th ed., Merck and Co., Rahway, N. J., 1968, p. 62.
(16) S. Diembeck, E. Grauer, and H. Jager, Archiv Pharm., 301, 628(1968).

Improved Differential Spectrophotometric Determination of Rifamycins

CARMINE R. PASQUALUCCI*, ARISTIDE VIGEVANI, PIETRO RADAELLI, and GIAN G. GALLO

Abstract □ A differential spectrophotometric method for the determination of rifamycin B, rifamide, rifamycin SV, and rifampin, based on the oxidation by $NaNO_2$ of the hydroquinone moiety of the compounds, is described. The application of the method to the determination of rifamycin B in fermentation broths and of rifampin in capsules and in syrup is reported. Precision and accuracy data are given.

Keyphrases □ Rifamycins, dosage forms—differential determination □ Fermentation broths—rifamycin determination □ Hydrolytic oxidation—rifamycin determination □ Colorimetric analysis—spectrophotometry

Rifamycin B (I) (1) is a metabolite produced by S. mediterranei, while rifamide (II) (2), rifamycin SV (III) (3), and rifampin[1] (IV) (4) are the derived semi-synthetic antibiotics presently in therapeutic use.[2] All these rifamycins[3] possess a characteristic chromophoric group which permits their spectrophotometric determination (2, 5–7). Furthermore, based on the quinone–hydroquinone nature of the chromophore, a differential spectrophotometric method has been described for rifamycin B and SV (8). The subjects of the present

---

[1] The international nonproprietary name for this compound is rifampicin.
[2] Rifamide as Rifocin M; rifamycin SV as Rifocin; rifampin as Rifadin.
[3] Rifamycins LXIII (rifamycin LXII: G. C. Lancini, G. G. Gallo, G. Sartori, and P. Sensi, J. Antibiot. (Tokyo), 22, 369(1969).