several inocula prepared in this manner, prepare inoculated plates as described for the specific antibiotic assay. Fill each cylinder with the reference concentration of the antibiotic and then incubate the plates. After incubation, examine and measure the zones of inhibition produced on the plates. The volume of suspension that produces the optimum zones of inhibition with respect to both clarity and diameter determines the inoculum to be used for the assay. Table IV gives optimal zone sizes that might be expected for each assay.

For Turbidimetric Assays—Proceed as described for plate assays, but instead of agar, add varying amounts of the suspension to 100 ml. of the broth described for the assay in Table V, using the volume described under "Suggested Milliliters of Suspension . . ." as a guide. Using the several inocula prepared in this manner, proceed as described for the specific antibiotic assay, but run only the high and low concentrations of the standard response line. After incubation, read the absorbances of the appropriate tubes. Determine which inoculum produces the best response between the low and high antibiotic concentrations, and use this inoculum for the assay.

DETAILS OF ASSAY PROCEDURES

For those assays to which the uniform procedure does not apply, reference should be made to the appropriate section of the Code of Federal Regulations (3).

In Table IV, under the column headed "Medium," there are the subheadings "Base" and "Seed." Unless otherwise specified, 21 ml. of base agar and 4 ml. of seed agar should be used for each plate. For turbidimetric assays in Table V, 9 ml. of inoculated broth should be added to each tube containing 1 ml. of sample, unless otherwise specified.

The column marked "Initial Solution of Standard" indicates the method for dissolving the reference standard to be used for the assay. Unless a concentration of 1000 mcg. or units per milliliter is indicated, the solution should immediately be further diluted with the indicated buffer to obtain a stock solution of convenient concentration.

The entries under "Final Concentration for Standard Response Line" are self-explanatory. The italicized concentration in each series is the reference concentration. Official FDA working standards are supplied to laboratories receiving antibiotic certification services. For other laboratories, reference standards are supplied by the USP or NF for some antibiotics listed in those compendia

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Determination of Sodium *p*-Aminosalicylate in the Presence of *m*-Aminophenol

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Abstract □ The *p*-aminosalicylic acid content in mixtures containing *m*-aminophenol is determined by a modification of the official assay procedure. The *m*-aminophenol is removed by passing a solution of the mixture in dimethylformamide through a column containing a strong cation-exchange resin. The eluate is then treated according to the official method.

Keyphrases □ *p*-Aminosalicylic acid—determination in *m*-aminophenol mixtures, ion-exchange chromatography □ Ion-exchange chromatography—determination of *p*-aminosalicylic acid in *m*-aminophenol mixtures

The official procedure for determining *p*-aminosalicylic acid, its salts and dosage forms, is based on the diazotization reaction involving aromatic amines. In USP XVII, an external indicator was used; while in USP XVIII, the end-point is detected potentiometrically. *m*-Aminophenol, the major breakdown product of *p*-aminosalicylic acid, if present, is also diazotized and constitutes an interference in the official assay procedure. However, the *m*-aminophenol content is determined by spectrophotometric analysis.

A variety of techniques have been proposed for the determination of *p*-aminosalicylic acid and its salts. These were reviewed by Lach and Cohen (1) and were referred to in an earlier paper (2). In the latter paper, a nonaqueous differentiating titration procedure was proposed for analyzing mixtures of *p*-aminosalicylic acid and *m*-aminophenol. Titrations were conducted potentiometrically, with sodium methoxide as the titrant and dimethylformamide as the titration solvent. Titrations were also performed visually, using thymol blue as the indicator. The presence of *m*-aminophenol does not interfere with the end-point detection for *p*-aminosalicylic acid. In a subsequent paper (3), a procedure was described for the separation and determination of mixtures containing *p*-aminosalicylic acid and *m*-aminophenol. Separation was effected by use of a strong cation-exchange resin. *m*-Aminophenol was

retained by the column, while *p*-aminosalicylic acid appeared in the eluate. Both components were recovered and analyzed by nonaqueous titration.

In the present study, a modification of the official assay procedure is proposed for determining the *p*-aminosalicylic acid content in mixtures containing *m*-aminophenol. The *m*-aminophenol is removed by passing a solution of the mixture in dimethylformamide through a column containing a strong cation-exchange resin. The eluate is then treated by the standard diazotization procedure.

EXPERIMENTAL

Apparatus—All titrations were performed potentiometrically with a titrimeter[1] equipped with a sleeve-type calomel and platinum electrode system. A 50-ml. buret (1 cm. i.d.) served as the chromatographic column.

Reagents and Chemicals—A strong cation-exchange resin[2] (200–400 mesh), *m*-aminophenol, sodium *p*-aminosalicylate, and dimethylformamide were obtained from commercial sources and were the best quality available. The *m*-aminophenol was further purified by recrystallizing from hot water. All other chemicals and solvents employed in this study were reagent grade and were used without further purification. A sodium nitrite solution (0.1 *M*) was prepared and standardized according to USP XVIII (4).

Column Preparation—The cation-exchange resin column was prepared, regenerated, and conditioned to dimethylformamide as described earlier (5, 6). Prior to being used for a run, the column was washed with 25 ml. of dimethylformamide. The height of the column was 6.5 cm., and the flow rate for all separations was 0.5 ml./min.

Assay Procedure—Stock solutions containing sodium *p*-aminosalicylate and *m*-aminophenol were prepared by dissolving appropriate amounts of the powders, accurately weighed, in 50 ml. of dimethylformamide. The composition of the stock solutions corresponded to the 10 mixtures listed in Table I. A 10-ml. aliquot of the stock solution was transferred by pipet to the resin column. When the solution disappeared below the top layer of the resin, additional dimethylformamide was added to the column until 50 ml. of eluate was collected in a 100-ml. calibrated glass-stoppered cylinder. Distilled water was added to the 100-ml. mark. The cylinder was stoppered and shaken thoroughly to assure the formation of a homogeneous solution. The contents of the cylinder were transferred quantitatively to a 200-ml. beaker. The solution was analyzed for aminosalicylate content by the diazotization procedure described for sodium aminosalicylate in USP XVIII (4), starting with: "..., and add 25 ml. of glacial acetic acid...." A blank run was conducted for each stock solution, and the necessary corrections were made to the actual runs. The blank determination usually consumed less than 0.30 ml. of sodium nitrite solution.

Aliquots of the stock solutions were also analyzed by the official procedure directly. The chromatographic separation step was omitted in these runs.

DISCUSSION

In aqueous solution, *p*-aminosalicylic acid and its salts break down rapidly and impart a dark-brown coloration to the solution, the intensity of the color increasing with time. The primary degradation product is *m*-aminophenol, which is unstable and is readily oxidized, forming a series of complex polymeric products which eventually precipitate from the solution.

As already noted, *m*-aminophenol undergoes the diazotization reaction and interferes with the official assay procedure. Ion-exchange chromatography has been explored as a possible technique for separating the *m*-aminophenol from the *p*-aminosalicylic acid. In an earlier paper (5), a weak cation-exchange resin[3] was used to convert sodium *p*-aminosalicylate to *p*-aminosalicylic acid, thus permitting the differentiating titration of *m*-aminophenol and *p*-aminosalicylic acid. Both compounds are apparently too weakly basic to be retained by a carboxylic acid-type resin. When a strong cation-exchange resin[2] was employed, the *m*-aminophenol was held back by the resin, and the *p*-aminosalicylic acid appeared in the eluate. This technique was utilized in the present study to remove *m*-aminophenol from mixtures containing both *m*-aminophenol and *p*-aminosalicylic acid. The eluate was then analyzed by the official procedure for *p*-aminosalicylic acid content.

[1] Fisher, model 35.
[2] Dowex 50-X8 (or equivalent).
[3] Amberlite IRC-50

Table I—Data for Analysis of Sodium *p*-Aminosalicylate in the Presence of *m*-Aminophenol

Sodium *p*-Aminosalicylic Acid Taken[a], mg.	*m*-Aminophenol Added, mg.	Sodium *p*-Aminosalicylic Acid Recovered[b], %
0.0	50.0	0.0
50.0	50.0	96.2
60.0	40.0	96.4
70.0	30.0	98.5
80.0	20.0	97.5
90.0	10.0	95.5
100.0	0.0	99.7
100.0	100.0	98.7
120.0	80.0	100.8
140.0	60.0	98.3
160.0	40.0	100.8
180.0	20.0	97.5

[a] Contained in a 10-ml. aliquot of stock solution. [b] Average of duplicate runs on each aliquot from same stock solution.

In preliminary studies, it was found that separation of *m*-aminophenol from *p*-aminosalicylic acid could only be achieved if the ion-exchange procedure was performed under nonaqueous conditions, as described in this paper. Although the diazotization mixture contained 50% dimethylformamide, the dimethylformamide apparently did not interfere in the diazotization reaction. This is reflected in the low blank readings (0.30 ml.). When 50% dimethylformamide was used as the eluting solution, separation of *m*-aminophenol from *p*-aminosalicylic acid was not successful. It appears that in an aqueous medium, dimethylformamide imparts sufficient basicity to the solution to effect displacement of the *m*-aminophenol from the resin sites. However, when the resin is conditioned to dimethylformamide and if it is the eluting solvent, the *m*-aminophenol is strongly held by the acidic sites on the resin and separation is possible. Table I shows analysis data for a series of 10 synthetic mixtures of sodium *p*-aminosalicylate and *m*-aminophenol. The data indicate that separation of the *p*-aminosalicylic acid from *m*-aminophenol was successful. Quantitative recoveries for *p*-aminosalicylic acid are reported.

When aliquots of the stock solutions were analyzed by the official procedure (not chromatographed), quantitative recovery for the total of *p*-aminosalicylic acid and *m*-aminophenol was obtained.

The proposed procedure was developed as an assay method for studying the kinetics of the decomposition of *p*-aminosalicylic acid and its salts in liquid dosage forms. It is also applicable as a general assay method for *p*-aminosalicylic acid and its salts, free and in dosage forms. The need for obtaining the amount of *m*-aminophenol present in order to determine the *p*-aminosalicylic acid content is obviated.

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