be responsible for the 1% discrepancy obtained for methaqualone in the mixtures. This would result in a significant error in calculating the diphenhydramine hydrochloride recoveries. Although samples equivalent to 75 mg of methaqualone were taken for the mixture, the procedure works equally well with mixtures containing 50 mg of methaqualone. The larger samples were used in the hope of being able to estimate accurately the antihistamine.

The visual and potentiometric end-points for methaqualone base are less distinct than are those for the CH_3COO^- species that is created from the hydrochloride by the addition of mercuric acetate. This indicates that methaqualone is a weaker base than the acetate anion. Furthermore, the presence of diphenhydramine hydrochloride further reduces the sharpness of the end-point with methaqualone base.

Because the pKb of methaqualone was not available from the literature for comparison, it was determined by a previously reported method (4). Figure 1 represents plots of pH *versus* milliliters of acetone for three concentrations of methaqualone. The pH values were measured at exactly half-neutralization. Figure 2 represents the three pH values obtained by extrapolation of the lines in Fig. 1 to 0 ml of acetone. Extrapolation to infinite dilution of the best straight line in Fig. 2 gives a resulting pH of 3.56. Since this is equal to pKa, the pKb of methaqualone was found to be 10.44.

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Improved Method for Salicylazosulfapyridine Analysis and Partial Characterization of Impurities in Commercial Salicylazosulfapyridine

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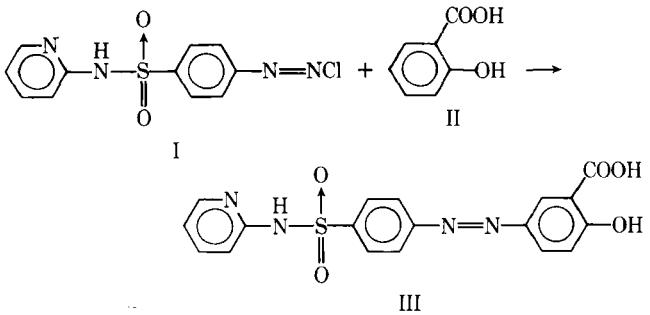
Abstract □ A column chromatographic system was developed that quantitatively separates salicylazosulfapyridine from impurities that contribute to the usual colorimetric method. The chromatographic–colorimetric method can be used to assay specifically for salicylazosulfapyridine as a raw material and in the final dosage form. Respective samples of two chromatographic mobile impurities were isolated, collected, and assayed by various means. Based on the data collected, partial characterizations of the chemical structure of these impurities were proposed. A third impurity, which was chromatographically immobile, was also studied and characterized as possibly being polymeric in nature.

Keyphrases □ Salicylazosulfapyridine bulk and tablets—chromatographic–colorimetric analysis, partial characterization of three impurities □ TLC—separation, salicylazosulfapyridine and three impurities □ Colorimetry—analysis, salicylazosulfapyridine and three impurities after TLC separation

The commercial synthesis (1) of salicylazosulfapyridine (III) involves the reaction between the diazonium salt of sulfapyridine (I) and *o*-hydroxybenzoic acid (II) (Scheme I).

The presence of the hydroxy *ortho–para*-director and the carboxy *meta*-director causes salicylazosulfapyridine to be the primary product. However, minor by-products which could interfere with the usual analytical method (2) are also expected to be present.

To determine if interfering impurities might be present, TLC analyses were performed on experimental salicylazosulfapyridine and commercial tablets, and it was possible to resolve three yellow-colored impurities in both types of samples. One impurity was essentially immobile (as indicated by the brown spot at the origin) and is speculated to be polymeric, resulting from the formation of a benzyne



Scheme I

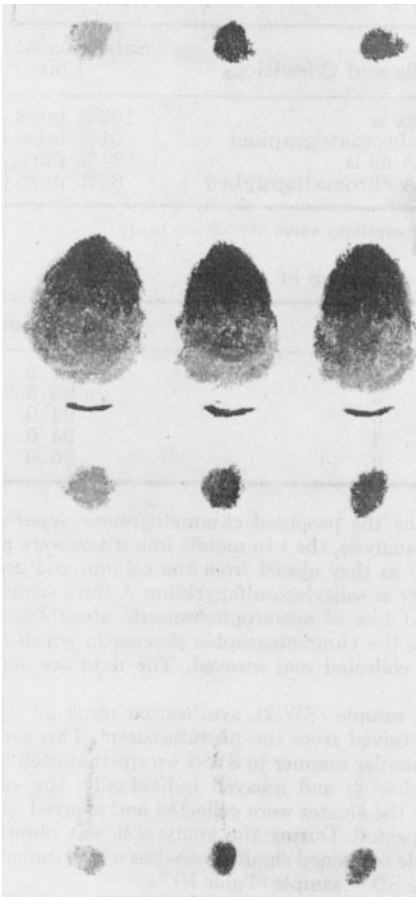


Figure 1—*Typical thin-layer chromatogram. Key: 1, salicylazosulfapyridine; 2, salicylazosulfapyridine tablets (recent lot); and 3, salicylazosulfapyridine (a lot several years older).*

intermediate (IV). The second impurity was positioned behind the salicylazosulfapyridine, and the data indicate that it is an isomer of the major component. The third impurity was positioned well ahead of salicylazosulfapyridine and was tentatively characterized by mass spectroscopy as an undiazotized sulfamide (V).

The knowledge acquired from the TLC work was eventually applied to the development of a liquid–solid chromatographic system to separate salicylazosulfapyridine from the impurities for subsequent quantitative analyses.

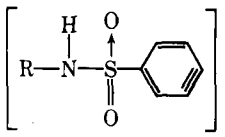
EXPERIMENTAL

Equipment—A spectrophotometer[1], glass columns (2.54 × 25.4 cm, 1 × 10 in.) with stopcocks, and filter paper[2] were used.

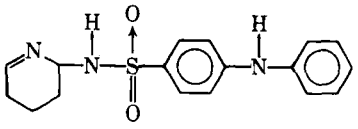
Materials—The following were used: acetone, analytical reagent; benzene, analytical reagent; methyl ethyl ketone, analytical reagent; pyridine, analytical reagent; Ottawa sand; glass wool; silicic acid[3], 100–200 mesh; 0.1 *N* sodium hydroxide; and 0.1 *N* acetic acid.

Column Preparation—Place a glass wool pledget in the bottom of the glass column and add 1.5–2 g of sand. Transfer a slurry containing 22 g of silicic acid and 50 ml of acetone into the column. Allow the acetone to drain from the column and then pass benzene

[1] Beckman DBG.
[2] Whatman No. 40.
[3] Mallinckrodt Chemical Co.



IV



V

through the column until all of the acetone has eluted. Repeat the procedure using acetone to displace the benzene. Maintain a level of solvent (about 5 mm) above the surface of the partition column. Place a piece of appropriately cut filter paper on top of the adsorbent.

Sample Preparation—Weigh approximately 200 mg (±0.1 mg) of salicylazosulfapyridine into a 10.0-ml volumetric flask. Add about 6 ml of pyridine to dissolve the sample and then dilute to volume with pyridine.

Analysis—Place 1.0 ml of the sample solution onto the top of the column and develop using a solvent system of methyl ethyl ketone–acetone–water (16:16:1) at a flow rate of 1–2 ml/min. The first material to elute is yellow and is not collected. The second band is salicylazosulfapyridine and is characterized by an orange color. Collect this material in a 100-ml beaker, taking care not to include material from the yellow band following the major component. Approximately 50–70 ml of eluate is collected.

Remove the solvent by evaporation on a steam bath. Dissolve the sample in a minimum amount of 0.1 *N* sodium hydroxide, quantitatively transfer into a 10.0-ml volumetric flask, and dilute to volume with 0.1 *N* sodium hydroxide. Pipet 5.0 ml of the sample into a 1-liter flask and add about 750 ml of water. Pipet 20.0 ml 0.1 *N* acetic acid into the flask and dilute to volume with water. Determine the absorbance at 359 nm using water as a blank and calculate the percent purity using Eq. 1:

$$\% \text{ purity} = \frac{\text{absorbance} \times 2000}{a\ (\text{ml mg}^{-1}\ \text{cm}^{-1}) \times b\ (\text{cm}) \times \dfrac{\text{wt of sample (mg)}}{10\ \text{ml}}} \times 100 \quad \text{(Eq. 1)}$$

Absorptivity *(a)* was found to be 63.0 from a sample of salicylazosulfapyridine purified by repeated extractions with ether. The resulting material was free of impurities when analyzed by TLC. The literature value of 65.8 was used to allow direct comparison of the data to previously published data.

TLC Analyses—A TLC system was initially developed to monitor the relative purity of bench size and subsequent production size lots of salicylazosulfapyridine. The analysis was performed using silica gel-coated plates and a solvent system of methyl ethyl ketone–acetone–water (4:1:1). The sample was dissolved in pyridine, and about 2 μl of solution was spotted on the plate. The plate was air dried to allow most of the pyridine to evaporate, and the plate was then developed by ascending chromatography (Fig. 1).

Isolation of Impurities—Several attempts were made to use preparatory TLC to isolate the impurities for subsequent identification analyses; none was successful. Consequently, various approaches were investigated including liquid–solid chromatography. When using silicic acid as the adsorbent and methyl ethyl ketone–acetone–water (16:16:1) as the solvent system, resolution equivalent to that achieved by TLC was obtained. The column was prepared by gently tamping a total of 200 g of silicic acid into a glass chromatographic column. A filter paper disk was placed on top of the silicic acid and the column was washed with acetone, followed by benzene, and finally with acetone prior to use.

Approximately 250 mg of sample was dissolved in a minimum amount of pyridine and quantitatively placed on the column and chromatographed using the methyl ethyl ketone–acetone–water system. As in the TLC analysis, one brown immobile material and two yellow mobile impurities were resolved from salicylazosulfapyridine. All three mobile components were collected into individual containers as they eluted from the column.

Table I—Effectiveness of Chromatographic Separation

SW-1 Sample and Conditions	Salicylazosulfapyridine Found[a], %
As is (not chromatographed)	85.2
Salicylazosulfapyridine band from column	77.1
Two mobile impurities from column	5.7
All eluates collected and assayed as a unit	84.1

[a] Lit. (2) absorptivity value of 65.8 was used.

Table II—Evaluation of Salicylazosulfapyridine from Improved Process

SW-2 Sample and Conditions	Salicylazosulfapyridine Found[a], %
As is (not chromatographed)	95.5
Salicylazosulfapyridine band from column	92.0
Impurity preceding salicylazosulfapyridine	0.3
Impurity following salicylazosulfapyridine	4.8

[a] Lit. (2) absorptivity value of 65.8 was used.

To determine the purity and to confirm the relative identity of the collected eluates, each was analyzed by TLC. The first yellow eluate collected corresponded to the material that chromatographed ahead of salicylazosulfapyridine and contained a trace amount of the major component. The second eluate, salicylazosulfapyridine, was free of any detectable impurities. The third eluate corresponded to the material that traveled slower than salicylazosulfapyridine and appeared to be a single component.

RESULTS AND DISCUSSION

Characterization of Impurities—Because of the chromatographic nature of the immobile brown sludge, it was speculated to be structurally different than the major component and the two yellow mobile impurities. During discussions with the manufacturer[4], it was suggested that the brown sludge resulted from the alkaline decomposition of diazotized sulfapyridine. Consequently, diazotized sulfapyridine was decomposed with alkali and the resulting material was found to have the same chromatographic characteristics as the immobile sludge in commercial salicylazosulfapyridine.

The two yellow mobile impurities were assayed spectrophotometrically and found to have significantly different spectra. The later eluting material has a spectrum with maxima at 357, 323, 265, and 237 nm. Qualitatively, the spectrum is the same as salicylazosulfapyridine. Because of the similarity of the spectra and the orienting effect of both the hydroxy and carboxylic groups, it is proposed that this component is a position isomer of salicylazosulfapyridine.

The spectrum of the component that elutes first shows maxima at 323 and 237 nm. The isolated material was examined by mass spectrometry and major peaks were found at 261, 169, 94, 67, 64, and 48 amu. The dissimilarity between spectra of the unknown and salicylazosulfapyridine and sulfapyridine indicates that this unknown moiety is quite different than salicylazosulfapyridine. If it is assumed that SO_2 is present in the molecule, the mass spectra data lend themselves to a component having the proposed structure of an undiazotized sulfamide (V).

Effect of Impurities on Analyses—To determine the amount of contribution from the impurities using the colorimetric method (2), a sample of an experimental lot (SW-1) of salicylazosulfapyridine was assayed. One aliquot of the sample was assayed directly without a chromatographic separation, and a second aliquot was assayed using the proposed chromatographic separation. During the second analysis, the two mobile impurities were also collected as a sample as they eluted from the column and assayed in the same manner as salicylazosulfapyridine. A third sample was run to determine if loss of spectrophotometric absorbing material occurred from the chromatographic process in which all of the eluates were collected and assayed. The data are summarized in Table I.

A second sample (SW-2), synthesized using an improved process, was received from the manufacturer[4]. This sample was assayed in a similar manner to SW-1 except that each mobile impurity was collected and assayed individually; the experiment in which all of the eluates were collected and assayed as a composite was not repeated. During the analysis it was observed that the SW-2 sample contained significantly less of the immobile impurity than did the SW-1 sample (Table II).

Table III—Evaluation of Samples from Other Sources

Sample and Conditions	Salicylazosulfapyridine Found[a], %
Tablets as is	102% label amount
Tablets chromatographed	91% label amount
Powder A as is	94% purity
Powder A chromatographed	82% purity

[a] Lit. (2) absorptivity value of 65.8 was used.

Table IV—Precision of Assay

Sample	Purity Found, %
1	94.6
2	93.5
3	94.0
4	94.0
5	96.0

Analysis of Samples from Alternative Sources—Up to this point, the work was primarily associated with material from one source. In an effort to compare the composition of this material[4], samples of salicylazosulfapyridine tablets[5] and a 1-year-old laboratory sample of salicylazosulfapyridine powder (A) supplied by a second manufacturer[6] were assayed with and without a chromatographic separation (Table III).

Determination of Precision—Using the proposed chromatographic–spectrophotometric method, five aliquots of salicylazosulfapyridine were assayed (Table IV).

A brief statistical analysis shows an average purity of 94.4% and a standard deviation of ±1%. The confidence interval using the Student t test (0.05) was 94.4 ± 2.6% (91.8–97%).

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[4] Crompton-Knowles.

[5] Azulfidine.

[6] Salsbury Laboratories.