SUMMARY

A nonaqueous method of analysis utilizing chloroform as solvent has been developed for four compounds and their dosage forms. The results obtained are in excellent agreement with those obtained by the official or manufacturers' methods, but the nonaqueous procedure is much more rapid.

Phenol–chloroform mixture was employed with good results as solvent for the analysis of two of the organic medicinal agents investigated.

N,N-Dimethylformamide–chloroform mixture was successfully utilized as a solvent system for the nonaqueous titration of four of the crystalline salts. However, the solvent could be applied only to one dosage form.

A method based on precipitation of the amine as the tetraphenylborate and then estimating the tetraphenylborate by nonaqueous titrimetry was developed. This procedure does not have as wide an application as does direct nonaqueous titrimetry, but good analytical data were obtained for five of the dosage forms. This method would appear to be susceptible to excipient interference.

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Keyphrases

Antiparkinsonism agents, synthetic
Titrimetry, nonaqueous-quantitative analysis
Tetraphenylborate salt formation
Perchloric acid in dioxane titrant
Mercuric acetate T.S. and mercuric acetate plus crystal violet T.S. indicators
Methyl red and methyl red plus crystal violet T.S. indicators
Tropaeolin 00—indicator
Colorimetric end point determination
Potentiometric end point determination

Gas Chromatographic Determination of Trisulfapyrimidines in Tablets

By JOHN W. TURCZAN

A procedure for the quantitative determination of individual sulfapyrimidines in mixtures has been developed. Analyses of known mixtures show an accuracy of 1–2 percent. The method is based on acid hydrolysis of the trisulfapyrimidines to sulfanilic acid and their respective heterocyclic amines, followed by the separation and determination of the amines by gas chromatography.

THE SEPARATION and quantitative determination of the three sulfapyrimidines, which are frequently dispensed together, is a difficult problem. In the usual methods of analysis the aromatic primary amino group or the acidic hydrogen (1) is determined. These procedures are therefore not specific and cannot determine individual sulfapyrimidines in a multi-sulfapyrimidine mixture. A paper chromatographic procedure (2) has been developed which identifies and quantitates mixtures of sulfapyrimidines and other sulfonamides.

An alternative method proposed here is based on gas chromatography after an acid hydrolysis (3, 4) of the sulfapyrimidines to sulfanilic acid and their respective heterocyclic amines. A study was made to determine optimum conditions for the hydrolysis. The following procedure was then used to analyze known mixtures and commercial tablets.

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EXPERIMENTAL

Apparatus—A Barber-Colman 5000 gas chromatograph with a hydrogen flame detector was used. The column, 6 ft. by 4 mm., was packed with 5% SE-30 and 5% Carbowax 20M on Chromosorb W (60–80 mesh; hexamethyldisilazane-treated). The column was conditioned overnight at 180° with a nitrogen flow of 140 ml./min. The operating conditions were: column temperature, 150°; flash heater temperature, 260°; cell temperature, 260°; carrier gas, prepurified nitrogen; flow rate of carrier gas (outlet), 120 ml./min.; pressure of hydrogen, 16 p.s.i.; air, 40 p.s.i.

The instrument was equipped with a 1-mv. recorder and all determinations were made with an electrometer setting of 300 and an attenuation of 2×; at this setting 1.2 mcg. of 2-amino pyrimidines will give a recorder response of about 70% of full-scale deflection.

Standard Solutions—*Internal Standard*—Four milligrams per milliliter, 2-amino-4,6-dimethylpyridine or 2-amino-4-methylpyridine in water. If procedure given below is to be used, take 5-ml. aliquot (yielding concentration of 0.2 mg./ml. when diluted) for standard and sample solution.

Standard Solution—A mixture containing 0.2 mg./ml. of 2-aminopyrimidine, 0.2 mg./ml. of 2-amino-4-methylpyrimidine, 0.2 mg./ml. of 2-amino-4,6-dimethylpyrimidine, and 0.2 mg./ml. of internal standard in water.

Procedure—*Preparation of Sample*—Weigh and finely powder not less than 20 trisulfapyrimidine tablets. Weigh accurately a portion of the powder equivalent to about 150 mg. of total sulfapyrimidines into a glass-stoppered 50-ml. centrifuge tube, add 4 ml. of concentrated sulfuric acid, stopper, mix, and place in an oven at 130° for 1.5 hr. Shake every 15 min. to insure complete contact of powder with hot acid. Cool by holding under running water and transfer quantitatively, using approximately 50 ml. of water, into a 100-ml. volumetric flask. Make alkaline to litmus by slowly adding approximately 8 ml. of 50% sodium hydroxide, maintaining solution at room temperature. Add 5.0 ml. (equivalent to 20 mg.) of internal standard and dilute to volume.

Chromatography and Calculations—Inject enough standard solution to insure 30–80% deflection (about 4–6 μl. if above procedure is followed). Measure the retention time (R.T.) of the standards from the solvent front to the apex of the peaks and calculate recorder response for each by multiplying retention time by the peak height (5–7). Using the recorder response for each standard and for the internal standard in the standard mixture, and their respective concentrations, determine the R value by Eq. 1. To obtain consistent results, try 3–4 preliminary injections. The R value should be constant within 3%; if it is not constant, make additional injections. When consistent responses are obtained, inject a sample to give approximately the same peak heights as the standard solution. Calculate the concentration of each sulfapyrimidine (using recorder responses) in its final solution by Eq. 2.

$$R = \frac{A_0 C_1}{A_1 C_0} \qquad \text{(Eq. 1)}$$

$$C_3 = \frac{A_3 C_2 A_1 C_0}{A_0 C_1 A_2} \qquad \text{(Eq. 2)}$$

where

A_0 = (R.T. × h) = recorder response for standard,
A_1 = recorder response of internal standard in standard mixture,
A_2 = recorder response of internal standard in sample mixture,
A_3 = recorder response of sample,
C_0 = concentration of standard,
C_1 = concentration of internal standard in standard mixture,
C_2 = concentration of internal standard in sample mixture,
C_3 = concentration of sulfapyrimidine in sample mixture.

Note: if the concentration of the internal standard in standard and sample is the same ($C_1 = C_2$), then Eq. 2 becomes:

TABLE I—KNOWN USP MIXTURE OF TRISULFAPYRIMIDINES

	Sulfadiazine			Sulfamerazine			Sulfamethazine		
Mixture	Added, mg.	Found, mg.	Recovery, %	Added, mg.	Found, mg.	Recovery, %	Added, mg.	Found, mg.	Recovery, %
1	50.1	49.47	98.7	49.9	49.65	99.5	51.5	52.02	101.0
2	48.8	47.73	97.8	50.6	50.04	98.9	51.8	51.54	99.5
3	48.9	49.19	100.6	52.6	51.76	98.4	49.4	48.86	98.9
4	55.3	54.69	98.9	50.2	49.55	98.7	49.7	50.35	101.9
5	52.7	51.86	98.4	49.1	48.51	98.8	48.6	48.21	99.2

TABLE II—COMMERCIAL TABLET PREPARATION

	Sulfadiazine			Sulfamerazine			Sulfamethazine		
Sample	Declared, mg.	Found, mg.	Declared, %	Declared, mg.	Found, mg.	Declared, %	Declared, mg.	Found, mg.	Declared, %
1	167	165.0	98.8	167	166.5	99.7	167	165.3	99.0
2	161	159.1	98.8	161	164.1	101.9	161	163.4	101.5
3	167	168.8	101.1	167	170.1	102.2	Not present or declared		
4	163	159.9	98.1	163	165.1	101.3	163	165.4	101.5
5	167	163.3	97.8	167	165.2	98.2	167	165.5	99.1
6	161	158.3	98.3	161	161.8	100.5	161	158.6	98.5
7	163	158.4	97.2	163	160.7	98.6	Not present or declared		
8	167	161.8	96.9	167	165.0	98.8	167	166.3	99.6

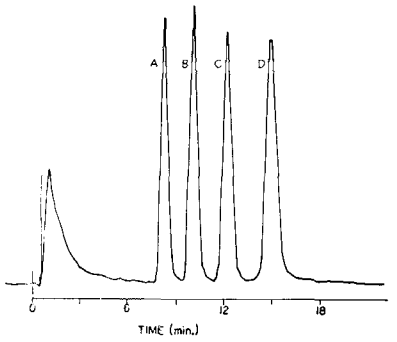

Fig. 1—Chromatogram of 3 sulfapyrimidines and internal standard mixtures. Key: A, 2-aminopyrimidine; B, 2-amino-4-methylpyrimidine; C, 2-amino-4,6-dimethylpyrimidine; and D, 2-amino-4,6-dimethylpyridine (internal standard); 1.2 mcg. of each injected.

$$C_3 = \frac{A_3 C_0 A_1}{A_0 A_2} \qquad \text{(Eq. 3)}$$

All calculations are made in terms of the final dilution. To calculate the concentration in the original sample, the sample size, the molecular weights, and the average weight per tablet must be taken into account; then Eq. 3 becomes:

$$C_3 = \frac{A_3 C_0 A_1}{A_0 A_2} \times \frac{\text{av. wt./tab.} \times \text{mol. wt. of sulfa drug}}{\text{wt. of spl.} \times \text{mol. wt. of hydrolysis product}}$$

$$= \text{mg. of sulfa drug/tablet} \qquad \text{(Eq. 4)}$$

DISCUSSION AND RESULTS

To determine the optimum conditions for hydrolysis, the experimental conditions were varied and completeness of hydrolysis was determined by UV spectrophotometry. The time of hydrolysis was kept at 1 hr. and the amount of sulfapyrimidine at 100 mg., while the concentration of hydrolyzing acid was varied from 20% to concentrated. Concentrated sulfuric acid (reagent grade) was found to be necessary for complete hydrolysis.

The time of hydrolysis was varied from 10 min.–180 min., while the amount of acid was kept constant at 2 ml. of concentrated sulfuric acid and the amount of sulfapyrimidine at 100 mg. The minimum time necessary for complete hydrolysis was found to be 1 hr.

An experiment in which the amount of sulfapyrimidine was varied from 50–220 mg., while the amount of concentrated acid was kept constant at 2 ml. and the time at 1 hr., demonstrated that no more than 190 mg. should be treated with 2 ml. of acid.

Individual 150-mg. portions of sulfadiazine, sulfamerazine, and sulfamethazine were hydrolyzed under the following conditions: 4 ml. of concentrated sulfuric acid, 90 min., at 130°. The resulting solutions were analyzed by both UV spectrophotometry and gas chromatography. Recoveries by gas chromatography correlate well with those by UV spectrophotometry. They ranged from 98.9–101.1% by UV and from 95.6–99.5% by GC for sulfadiazine; 98.1–101.5% by UV, and 97.8–99.1% by GC for sulfamerazine; 98.0–102.0% by UV, and 97.4–99.2% by GC for sulfamethazine.

Five synthetic mixtures containing equal parts of sulfadiazine, sulfamerazine, and sulfamethazine were analyzed by the proposed procedure (GLC) (Table I) with very good results. Analysis of eight commercial preparations (Table II) showed good agreement with declared values.

If the relative retention time (R.R.T.) of sulfadiazine, which yields 2 aminopyrimidine, is taken as 1.00, then the R.R.T. of sulfamerazine, which yields 2-amino-4-methylpyrimidine, is 1.24, that of sulfamethazine, which yields 2-amino-4,6-dimethylpyrimidine, is 1.52, and that of the internal standard, 2-amino-4,6-dimethylpyridine, is 1.89 (Fig. 1).

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Keyphrases

Trisulfapyrimidine tablets analysis
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Acid hydrolysis
UV determination of hydrolysis
GLC analysis