Application of Ion-Pairing to Separation of Selected Sulfonamides by Partition Chromatography

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Abstract □ Methods are presented for the separation and determination of individual sulfonamides in mixtures. These procedures are based on the ion-pair formation of the sulfonamides with the tetrabutylammonium ion, followed by separation on partition chromatographic columns. One procedure separates the trisulfapyrimidines (sulfamethazine, sulfamerazine, and sulfadiazine) and a second procedure separates sulfamethizole, sulfadiazine, and phenazopyridine hydrochloride. The separated sulfonamides are then determined by UV spectrophotometry and phenazopyridine hydrochloride by visible spectrophotometry. Ten commercial and five synthetic preparations were assayed by the procedures. Recoveries from the synthetic preparations ranged from 99 to 101%.

Keyphrases □ Sulfonamide mixtures—application of ion-pairing to separation by partition chromatography, commercial samples □ Ion-pairs—used in analysis of mixtures of sulfonamides, partition chromatography, commercial samples □ Tetrabutylammonium ion—ion-pair formation with sulfonamides, separation by partition chromatography, commercial samples □ Partition chromatography—use of ion-pairs for separation of sulfonamide mixtures, commercial samples

Sulfonamide combinations are found in a number of pharmaceutical preparations. Quantitation of the individual sulfonamides in these mixtures presents a difficult analytical problem. In the USP XVIII and NF XIII procedures (1, 2), the sulfonamides are separated by paper chromatography and determined colorimetrically by the Bratton–Marshall reaction. These procedures are time consuming and require an experienced analyst to obtain consistent results.

Other procedures used for the separation of sulfonamides include TLC (3, 4), partition chromatography (5), and GLC (6). Banes and Riggleman (7) summarized problems encountered in most of these techniques. They presented a procedure for the trisulfapyrimidines in which total sulfonamides are determined colorimetrically by the Bratton–Marshall procedure. Sulfadiazine is quantitated by its specific reaction with thiobarbituric acid, and sulfamethazine is separated by column partition chromatography and determined by UV spectroscopy.

The use of partition chromatography to separate sulfonamides has been hindered by the low solubility of the sulfonamides in many water-immiscible solvents. Solubility of the sulfonamides in chloroform and methylene chloride can be greatly increased by formation of ion-pairs with the tetrabutylammonium ion. Several workers (8–10) discussed the use of ion-pairs with quaternary ammonium ions in the extraction of acidic compounds. Ion-pair extraction in conjunction with partition chromatography was presented previously by Doyle and Levine (11, 12).

In the present report, methods are given for the analysis of two drug mixtures which are commonly encountered in this laboratory. One procedure (Method I) separates sulfamethazine, sulfamerazine, and sulfadiazine. Another procedure (Method II) separates sulfamethizole, sulfadiazine, and phenazopyridine. In both procedures, the sulfonamides are eluted from the partition column as ion-pairs with tetrabutylammonium ions. Separated sulfonamides are determined by UV spectrophotometry, and phenazopyridine hydrochloride is determined by visible spectrophotometry. Ten commercial and five synthetic drug preparations were assayed.

EXPERIMENTAL

Apparatus—A recording UV–visible spectrophotometer with 1-cm. cells was used.

Solvents—Chloroform, ether, hexane, and methylene chloride (all reagent grade) were used. Wash each solvent twice with equal volumes of water. Prepare 50% chloroform in hexane by mixing 500 ml. of chloroform with 500 ml. of hexane, and filter through filter paper.

Ten Percent Tetrabutylammonium Hydroxide in Water[1] (Reagent A)—Pipet 3.0 ml. of the solution into a flask and add about 20 ml. of water. Titrate the solution with 0.1 *N* HCl, using methyl red indicator. One milliliter of 0.1 *N* acid = 25.95 mg. of tetrabutylammonium hydroxide.

Sodium Hydroxide–Tetrabutylammonium Hydroxide Solution (Reagent B)—Pipet (with a 10-ml. measuring pipet) an aliquot of Reagent A, equivalent to 800 mg. of tetrabutylammonium hydroxide, into a 25-ml. graduated cylinder. Add 10.0 ml. of 0.2 *N* NaOH to the cylinder from a pipet. Dilute to 20.0 ml. with water and mix.

Standard Preparation—USP reference standards or NF grade standards of sulfadiazine, sulfamerazine, sulfamethazine, sulfamethizole, and phenazopyridine hydrochloride were used. For each sulfonamide to be assayed, prepare a standard solution of 1 mg./ml. in 1% ammonia in methanol; pipet exactly 1.0 ml. of this solution into a 100-ml. volumetric flask and dilute to volume with 0.12 *N* HCl. Prepare a phenazopyridine hydrochloride standard solution containing 5 mcg./ml. in 1% ammonia in ethanol.

Sample Preparations—*Tablets*—Weigh a portion of finely ground sample containing about 100 mg. of each sulfonamide and transfer with methanol to a 100-ml. volumetric flask containing 1 ml. of ammonium hydroxide. Shake occasionally during 15 min., dilute to volume with methanol, and mix.

Suspensions—With a "to contain" pipet, transfer a quantity of sample containing about 100 mg. of each sulfonamide to a 100-ml. volumetric flask containing 1 ml. of ammonium hydroxide. Add about 60 ml. of methanol and shake occasionally during 15 min., dilute to volume with methanol, and mix.

Method I: Sulfamethazine, Sulfamerazine, and Sulfadiazine—Mix 5.0 g. of diatomaceous earth[2] with 3.0 ml. of Reagent B, transfer to a chromatographic column[3] in two equal portions, and tamp after each portion. Pipet 3.00 ml. of the sample solution into a 100-ml. beaker and evaporate to dryness on a steam bath, using a gentle current of air. Pipet 1.0 ml. of Reagent A into the beaker and dissolve the residue. Add 2.0 g. of diatomaceous earth, mix until uniform, quantitatively transfer to the column, and tamp. Wipe the beaker, tamping rod, and spatula with a small piece of glass wool, add it to the column, and tamp.

[1] Cat. No. 10651, Eastman Kodak Co., Rochester, NY 14650
[2] Acid-washed Celite 545, Johns-Manville Corp., New York, N. Y.
[3] Cat. No. 420300, 250 × 22-mm. i.d., Kontes Glass Co., Vineland, N. J.

Table I—Effect of Varying Sodium Hydroxide Concentration on Column Chromatographic Elution of Sulfamethazine by Various Solvents[a]

Solvent	Percent Eluted with 100 ml. of Solvent				
	1 *N* NaOH	0.5 *N* NaOH	0.25 *N* NaOH	0.1 *N* NaOH	0.05 *N* NaOH
Ether	5	5	<1	<1	<1
10% Chloroform in hexane	—[b]	—	—	—	—
30% Chloroform in hexane	30	30	10	5	2
50% Chloroform in hexane	100	100	95	80	80
Chloroform	100	100	95	90	85
Methylene chloride	100	100	100	100	90

[a] One milligram of sulfamethazine was mixed with 2 g. of diatomaceous earth and 1 ml. of 4% tetrabutylammonium hydroxide in the respective sodium hydroxide solutions and packed in the column. [b] Dashes indicate no detectable drug in 100 ml. of eluate.

Table II—Effect of Varying Sodium Hydroxide Concentration on Column Chromatographic Elution of Sulfadiazine by Various Solvents[a]

Solvent	Percent Eluted with 100 ml. of Solvent				
	1 *N* NaOH	0.5 *N* NaOH	0.25 *N* NaOH	0.1 *N* NaOH	0.05 *N* NaOH
Ether	—[b]	—	—	—	—
10% Chloroform in hexane	—	—	—	—	—
30% Chloroform in hexane	—	—	—	—	—
50% Chloroform in hexane	85	65	35	30	25
Chloroform	100	100	100	100	100
Methylene chloride	100	100	100	100	100

[a] One milligram of sulfadiazine was mixed with 2 g. of diatomaceous earth and 1 ml. of 4% tetrabutylammonium hydroxide in the respective sodium hydroxide solutions and packed in the column. [b] Dashes indicate no detectable drug in 100 ml. of eluate.

Wash the column with 50 ml. of 50% chloroform in hexane and discard the eluate. Place a 400-ml. beaker under the column and elute with an additional 250 ml. of 50% chloroform in hexane. This fraction contains sulfamethazine. Replace the receiver with a 600-ml. beaker and elute the column with 400 ml. of 50% chloroform in hexane. This fraction contains sulfamerazine.

Place a 250-ml. beaker under the column and elute with 100 ml. of methylene chloride. This fraction contains sulfadiazine.

Evaporate each fraction on a steam bath with an air stream. Dissolve the residues in 2 ml. of methanol, add 40 ml. of 0.12 *N* HCl, mix, transfer to separate 100-ml. volumetric flasks, and dilute to volume with 0.12 *N* HCl. Pipet a 15-ml. aliquot of each solution into separate 50-ml. volumetric flasks and dilute to volume with 0.12 *N* HCl. Record the UV spectrum from 400 to 220 nm., using 0.12 *N* HCl as the reference. Draw the baseline as a continuation of the curve between 400 and 360 nm., determine the corrected absorbance at the wavelength of maximum absorbance at about 242 nm., and calculate by comparison to standards.

Method II: Sulfadiazine, Sulfamethizole, and Phenazopyridine Hydrochloride—Mix 2.0 g. of diatomaceous earth with 1.0 ml. of Reagent B, transfer to the chromatographic column, and tamp. Pipet 3.00 ml. of sample solution into a 100-ml. beaker and evaporate to dryness on a steam bath, using a gentle current of air. Pipet 1.0 ml. of Reagent B into the beaker. Add 2.0 g. of diatomaceous earth, mix until uniform, quantitatively transfer to the column, and tamp. Wipe the beaker, tamping rod, and spatula with a small piece of glass wool, add it to the column, and tamp.

Place a 250-ml. beaker under the column. Wash the 100-ml. beaker with three 5-ml. portions of ether, adding each portion to the column. Elute the column with three 20-ml. portions of ether, allowing each to pass into the column before adding the next portion. Rinse the column tip with methanol. This fraction contains phenazopyridine. Replace the receiver with a second 250-ml. beaker and elute the column with 150 ml. of 50% chloroform in hexane. Rinse the column tip with methanol. This fraction contains sulfamethizole. Replace the receiver with a third 250-ml. beaker and elute the column with 100 ml. of methylene chloride. Rinse the tip with methanol. This fraction contains sulfadiazine.

For phenazopyridine hydrochloride, evaporate the first eluate on the steam bath with an air stream. Dissolve the residue in 40 ml. of ethanol, transfer the solution quantitatively to a 100-ml. volumetric flask containing 1 ml. of ammonium hydroxide, and dilute to volume with ethanol. Pipet a 15-ml. aliquot into a 50-ml. volumetric flask and dilute to volume with ethanol. Read the absorbance at 420 nm., using alcohol as the reference, and calculate by comparison to a standard.

For sulfamethizole and sulfadiazine, evaporate the respective eluates on a steam bath with an air stream. Dissolve the residues in 2 ml. of methanol, add 40 ml. of 0.12 *N* HCl, mix, transfer to separate 100-ml. volumetric flasks, and dilute to volume with 0.12 *N* HCl. Pipet a 15-ml. aliquot of each solution into separate 50-ml. volumetric flasks and dilute to volume with 0.12 *N* HCl. Record the UV spectrum from 400 to 220 nm., using 0.12 *N* HCl as the reference. Draw a baseline as a continuation of the curve between 400 and 360 nm. for sulfadiazine and between 360 and 340 nm. for sulfamethizole. Determine the corrected absorbance at the wavelength of maximum absorbance, about 242 nm. for sulfadiazine and about 268 nm. for sulfamethizole, and calculate by comparison to standards.

DISCUSSION

The extraction of the ion-pairs formed by tetrabutylammonium ions and sulfonamides from partition columns is controlled by the nature of the organic mobile solvent. Solvents with acidic protons, such as chloroform and methylene chloride, interact with the ion-pair to increase the extracting power of the organic phase. The effect of the concentration of such compounds on the partitioning of ion-pairs was studied by Higuchi and coworkers (13–15). They postulated a solvent cage of approximately two to five molecules of chloroform around the extracted ion-pair.

In the present study, the sulfonamides were readily extracted by chloroform and methylene chloride as ion-pairs with tetrabutylammonium ions from a basic partition column. The elution and separation of the ion-pairs from the partition column are influenced by the concentration of sodium hydroxide and tetrabutylammonium hydroxide in the column and the concentration of the chloroform or methylene chloride in the eluting solvent.

As the tetrabutylammonium hydroxide concentration is increased, the sulfonamides elute more rapidly. A tetrabutylammonium hydroxide concentration of about 4% was found to give the best elution times and separations for the sulfonamides studied.

Tables I and II illustrate the effect of the sodium hydroxide concentration and the solvent composition on the elution rate for the

Table III—Analysis of Sulfadiazine, Sulfamethazine, and Sulfamerazine in Commercial and Synthetic Drug Preparations by Method I

Sample	Percent of Declared			Total Sulfonamides (Bratton-Marshall), %
	Sulfamethazine	Sulfamerazine	Sulfadiazine	
Tablets 1	98.6	97.5	96.8	98.1
Tablets 2	103.0	101.3	101.0	99.6
Tablets 3	102.7	99.3	101.3	101.2
Tablets 4	93.8	100.7	103.5	98.3
Suspension 5	104.0	102.5	110.4	104.4
Suspension 6	101.7	101.8	100.4	100.9
Suspension 7	—[a]	92.8	97.4	95.1
Synthetic 1	100.1	100.0	101.0	
Synthetic 2	98.8	101.0	99.4	
Synthetic 3	99.1	98.8	101.3	

[a] Dashes indicate that these components are not present in these mixtures.

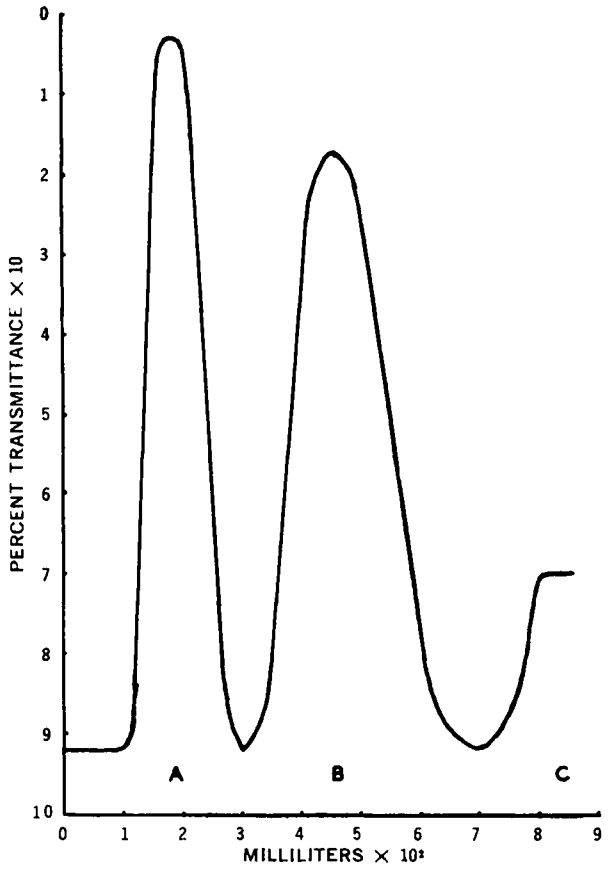

Figure 1—*Separation of sulfamethazine (A), sulfamerazine (B), and sulfadiazine (C) obtained by eluting the partition column with 50% chloroform–hexane.*

sulfamethazine and sulfadiazine ion-pairs. The increase in elution rate for the sodium hydroxide concentration range (0.05–0.2 *N*) is slight. However, the sodium hydroxide decreases the tailing of the eluting ion-pairs, resulting in a more complete separation.

In Method I, the sample is placed in a 10% tetrabutylammonium hydroxide layer. This enables the sulfonamides to reach the 0.1 *N* NaOH–tetrabutylammonium hydroxide separation layer in a narrower band and improves the separation. Figure 1 demonstrates the separation of the sulfamethazine, sulfamerazine, and sulfadiazine obtained by Method I. Also, methylene chloride is preferred to chloroform for the elution of sulfadiazine since there is less interference in the UV region.

RESULTS

Six commercial preparations of the trisulfapyrimidines and one sample containing sulfamerazine and sulfadiazine were assayed by Method I. Three commercial preparations of sulfamethizole, sulfadiazine, and phenazopyridine hydrochloride were assayed by Method II. Synthetic samples of these products were also analyzed. The results are reported in Tables III and IV. In addition, total sulfonamide content in the same commercial preparations was determined by the Bratton–Marshall procedure (7), with the results presented in Tables III and IV.

Table IV—Analysis of Sulfamethizole, Sulfadiazine, and Phenazopyridine Hydrochloride in Commercial and Synthetic Drug Preparations by Method II

Sample	Percent of Declared: Sulfamethizole	Sulfadiazine	Phenazopyridine Hydrochloride	Total Sulfonamides (Bratton–Marshall), %
Tablets 1	103.0	104.2	100.0	102.1
Tablets 2	99.6	99.9	96.8	98.4
Tablets 3	103.0	103.8	100.2	102.6
Synthetic 1	101.1	100.6	99.8	
Synthetic 2	98.7	100.8	98.8	

SUMMARY

This investigation of the separation of four sulfonamides by ion-pair partition chromatography showed that the proposed procedures were rapid and gave good recoveries (99–101%). The analytical technique might well be extrapolated to include related acidic compounds.

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