Separation of Sulfa Drugs by High Speed Liquid Chromatography▲

THEODORE C. KRAM

Abstract □ The high speed liquid chromatographic behavior of 21 sulfonamides was studied. Analysis of the effect of the ionic strength of the mobile phase upon retention times and of concomitant effects on peak widths resulted in a scheme that can be applied to the separation of many combinations of sulfonamides.

Keyphrases □ Sulfa drugs—separation, high speed liquid chromatography □ Sulfonamides—separation, high speed liquid chromatography □ High speed liquid chromatography—separation of sulfa drugs (sulfonamides) □ Dimethyl sulfoxide—as a solvent for sulfa drugs in high speed liquid chromatography

Recent developments in the design of high speed liquid chromatographs and accessories have comprised the most significant advance in separation technology since maturation of gas chromatography (1–5). The ability of gas chromatography to separate many closely related compounds has made it extremely useful; however, numerous important classes of compounds are excluded because they fail to meet requirements of volatility and thermal stability. High speed liquid chromatography eliminates these problems. Analyses may be conducted at room temperature; and since the carrier fluid, or mobile phase, is a liquid, the requirement of volatility is replaced by one of solubility. The extension of this technique to drug analysis was noted in two reports. Henry and Schmit (6) studied the separation of analgesic drug combinations using an anion-exchange resin column with an aqueous alkaline buffer whose ionic strength was varied. Variations in retention time were observed as a function of ionic strength. With the same chromatographic system, Henry[1] reported the separation of a trisulfapyrimidine mixture (sulfadiazine, sulfamerazine, and sulfamethazine). Compared with the USP procedure (7), this method is faster and simpler to manipulate.

This paper describes a study of the chromatographic behavior of a large group of sulfonamides. The use of variations in ionic strength was investigated to discover conditions that would be advantageous for the separation of members of this group, some of which occur together in dosage forms.

EXPERIMENTAL

Apparatus—A liquid chromatograph[2] equipped with a UV (254-nm.) photometer detector[3], both described in detail by Felton (3), is used. The column is stainless steel (1 m. long, 2.1 mm. i.d.), packed with spherical siliceous particles with a controlled porous surface[4]; this support is coated with a strong anion-exchange resin (8). The mobile phase is 0.01 *M* sodium borate (pH 9.2) containing sodium nitrate at varied concentrations (0.01, 0.04, 0.07, and 0.10 *M*). The system is operated at a pressure of 1200 psig. and a flow rate of 0.80 ml./min.

Sample Preparation—The sulfa drug is dissolved in dimethyl sulfoxide, reagent grade, to make a final concentration of 2 mg./ml.

RESULTS AND DISCUSSION

Twenty-one sulfa drugs meeting federal regulatory specifications for purity were studied. The utility of dimethyl sulfoxide as a solvent for sulfa drugs was previously established by this laboratory (9). The chromatographic behavior of these drugs may be expressed in terms of retention measurements. Retention times for the sulfas are given in Table I. A study of a number of compounds selected at random indicates that the retention times are reproducible within 1–2%. Relative retention values using the retention time for the sample solvent, dimethyl sulfoxide, as the reference are also included in Table I. An examination of the data indicates that, with

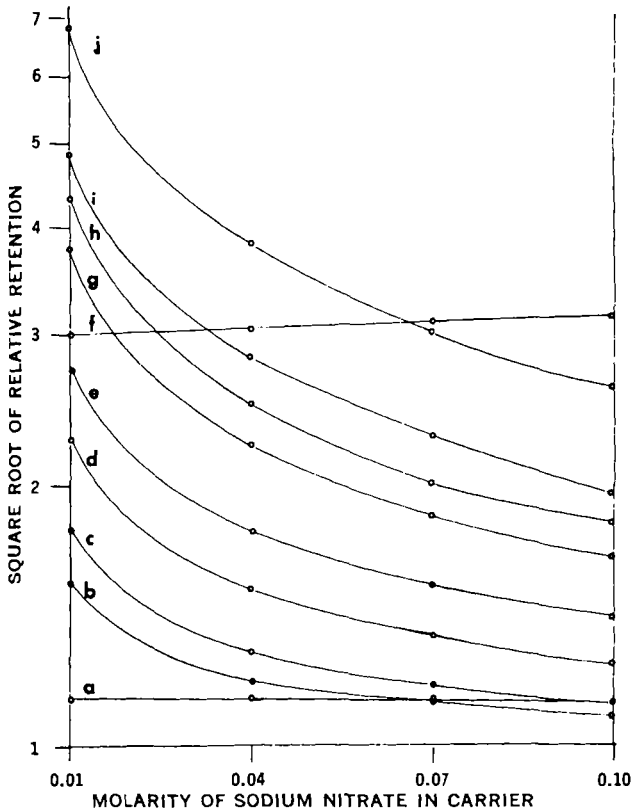

Figure 1—*Square root of relative retention time* versus *concentration of sodium nitrate in the mobile phase. Key: a, sulfaguanidine; b, sulfadiazine; c, sulfamerazine; d, sulfamethazine; e, sulfapyridine; f,* N¹*-acetyl sulfisoxazole; g, sulfisoxazole; h, sulfadimethoxine; i, sulfamethizole; and j, sulfathiazole.*

[1] R. A. Henry, E. I. du Pont de Nemours and Co., Wilmington, Del., private communication.

[2] du Pont model 820, E. I. du Pont de Nemours and Co., Wilmington, Del.

[3] du Pont model 410, E. I. du Pont de Nemours and Co., Wilmington, Del.

[4] Zipax, E. I. du Pont de Nemours and Co., Wilmington, Del.

Table I—Retention Time (Minutes)[a] of Sulfonamides for Various Concentrations of Sodium Nitrate

Compound	Retention Time			
	$NaNO_3$, 0.01 *M*	$NaNO_3$, 0.04 *M*	$NaNO_3$, 0.07 *M*	$NaNO_3$, 0.10 *M*
Sulfanilamide	2.13 (1.24)	2.14 (1.20)	2.10 (1.19)	2.11 (1.22)
Sulfaguanidine	2.18 (1.27)	2.26 (1.28)	2.22 (1.27)	2.17 (1.25)
Sulfacetamide	3.55 (2.06)	2.32 (1.31)	2.04 (1.17)	1.88 (1.09)
Sulfadiazine	4.10 (2.38)	2.54 (1.43)	2.21 (1.26)	2.04 (1.18)
Sulfamerazine	5.33 (3.10)	2.91 (1.64)	2.40 (1.37)	2.17 (1.25)
Sulfamethazine	9.23 (5.35)	4.06 (2.29)	3.14 (1.80)	2.69 (1.55)
Sulfamethoxypyridazine	9.23 (5.37)	4.14 (2.35)	3.12 (1.78)	2.69 (1.55)
Sulfisomidine	9.48 (5.51)	4.29 (2.42)	3.24 (1.85)	2.71 (1.56)
Sulfapyridine	12.6 (7.33)	5.52 (3.11)	4.07 (2.33)	3.40 (1.96)
Sulfamethoxazole	12.7 (7.40)	5.52 (3.02)	3.70 (2.11)	3.21 (1.85)
Sulfisoxazole, N^1-acetyl	15.3 (8.91)	16.5 (9.29)	16.6 (9.59)	17.0 (9.79)
Sulfisoxazole	22.2 (13.5)	8.84 (4.99)	5.86 (3.35)	4.69 (2.70)
Sulfisoxazole diolamine	23.1 (13.7)	8.67 (4.90)	5.80 (3.32)	4.67 (2.70)
Sulfadimethoxine	31.8 (18.4)	11.0 (6.18)	7.04 (4.03)	5.60 (3.23)
Sulfachlorpyridazine	33.0 (19.2)	12.1 (6.80)	7.42 (4.24)	5.76 (3.32)
Sulfamethizole	40.1 (23.3)	14.3 (8.07)	9.18 (5.25)	6.68 (3.85)
Sulfabenzamide	41.0 (23.8)	14.6 (8.23)	9.79 (5.60)	7.29 (4.20)
Sulfathiazole	76.0 (44.1)	26.0 (14.7)	15.8 (9.12)	11.8 (6.78)
Sulfaphenazole	79.0 (45.9)	27.0 (15.3)	16.9 (9.62)	12.2 (7.06)
Sulfaquinoxaline	—	54.9 (31.0)	33.5 (19.1)	24.1 (14.0)
Succinylsulfathiazole	—	97.4 (55.0)	35.4 (20.2)	18.5 (10.7)

[a] Retention time for sample solvent, dimethyl sulfoxide, given in parentheses.

some exceptions, the mobility of each sulfonamide increased with increases in the molarity of sodium nitrate in the mobile phase.

In an attempt to systematize the data, a number of plots were made. The square root of the relative retention was plotted (Fig. 1) as a function of sodium nitrate concentration for some of the compounds. A family of curves appeared, indicating similar responses to variations in ionic strength, except in the case of acetyl sulfisoxazole and sulfaguanidine. As the data in Table I show, sulfanilamide also exhibits no response to ionic strength modifications. Acetyl sulfisoxazole and sulfaguanidine are not acids; sulfanilamide is a rather weak acid. These compounds would not be expected to give rise to anionic species or would do so only feebly. Thus, they would not be expected to respond to changes in ionic strength in processes involving anion-exchange phenomena or in any interactions involving anions. Accordingly, their retention behaviors should be reasonably constant as the salt concentration is varied, and this is indeed the case.

A plot of peak width (at 10% height) *versus* retention time (Fig. 2) is presented as an aid in selecting suitable conditions for separation of sulfa drugs.

As is well established in chromatography, band spreading increases with retention time. In the present case, the plot is linear (Fig. 2) and has a slope of 0.18 min./min. of retention time. Although all the points do not fall in line, their deviations from it are small enough so that the plot can be used to predict the approximate peak width for a given compound from knowledge of its retention time. With a 10% maximum valley between two peaks, resolution is generally sufficient for quantitative measurements based on peak height or triangulation procedures.

Table I and Fig. 2 can be used to determine the optimum sodium nitrate concentration to be used for a separation. For example, sulfadiazine (t_r = 2.6 min.) and sulfaguanidine (t_r = 2.3 min.) in 0.04 *M* sodium nitrate are not separable. However, by decreasing sodium nitrate molarity to 0.01 *M*, sulfadiazine [t_r = 4.1 min., bandwidth at 10% height (b.w. 10) = approximately 0.7 min.] can be separated from sulfaguanidine (t_r = 2.3 min., b.w. 10 = approximately 0.4 min.).

Similarly, optimum sodium nitrate levels may be predicted for the official trisulfapyrimidines. At 0.04 *M* sodium nitrate, sulfadiazine and sulfamerazine overlap, but at 0.01 *M* sodium nitrate, satisfactory separation can be predicted from the combined use of the table and graph, as is seen from data obtained for each compound: sulfadiazine (t_r = 4.1, b.w. 10 = approximately 0.7 min.), sulfamerazine (t_r = 5.3, b.w. 10 = approximately 0.9 min.), and sulfamethazine (t_r = 9.2, b.w. 10 = approximately 1.7 min.). The chromatogram shown in Fig. 3 does give the actual separation achieved and indicates some small overlap of sulfadiazine and sulfamerazine.

Whereas the analysis of sulfonamide combinations generally requires lower concentration levels of sodium nitrate in the mobile phase, the use of greater concentrations of sodium nitrate may be desirable where only single sulfas are encountered. For example, succinylsulfathiazole, which exhibited a retention time of 97 min. in 0.04 *M* sodium nitrate, eluted in 18.5 min. at 0.1 *M*. The retention time of sulfathiazole was 76 min. at 0.01 *M* but 11.6 min. at 0.1 *M*. Modification of ionic strength may also be considered as a possible means of eliminating interferences due to excipients.

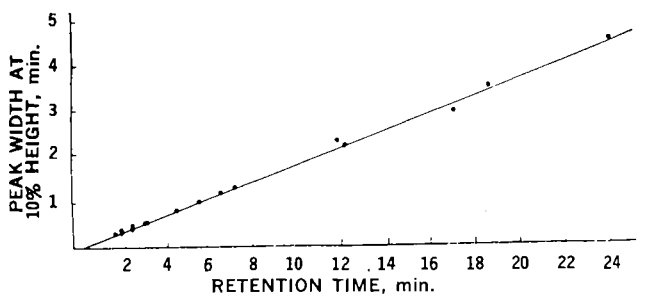

Figure 2—*Peak width of sulfonamides at 10% height* versus *retention time. Column = "SAX" (Strong Anion Exchange, du Pont). Mobile phase = 0.01* M *borax–0.1* M *$NaNO_3$. Pressure = 1200 psig.*

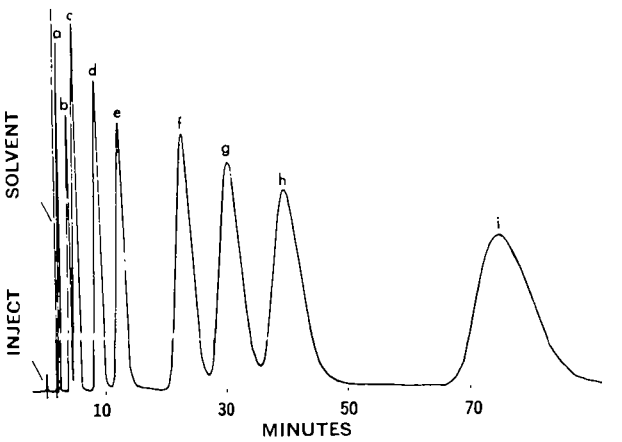

Figure 3—*Liquid chromatogram of: (a) sulfaguanidine, (b) sulfadiazine, (c) sulfamerazine, (d) sulfamethazine, (e) sulfapyridine, (f) sulfisoxazole, (g) sulfadimethoxine, (h) sulfamethizole, and (i) sulfathiazole. Mobile phase = 0.01* M *borax–0.01* M *$NaNO_3$. Column = "SAX" (Strong Anion Exchange, du Pont). Pressure = 1200 psig. Flow rate = 0.80 ml./min.*

One advantage of liquid chromatography results from the variety of parameters (selection of column, mobile phase, and temperature) that can be manipulated to produce a desired separation or accelerated elution. In a reverse phase system, the ionic strength of the mobile phase may also be adjusted. Advance knowledge of acid–base or ion-pairing properties of a number of compounds can simplify selection of the optimum ionic strength for their separation.

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Present address: Bureau of Narcotics and Dangerous Drugs, Special Testing and Research Laboratory, Washington, DC 20537

▲ Inquiries should be directed to: Helen L. Reynolds, Department of Health, Education, and Welfare, Public Health Service, Food and Drug Administration, Washington, DC 20204

TECHNICAL ARTICLES

Effect of Moisture on Tensile Strength of Bulk Solids I: Sodium Chloride and Effect of Particle Size

TERENCE EAVES* and TREVOR M. JONES▲

Abstract □ The effect of moisture on the tensile strength of packed beds of a variety of particle-size fractions of sodium chloride is reported. At a constant state of packing, the tensile strength of the coarse noncohesive fractions is shown to increase to a plateau as the moisture content rises. This is attributed to an increase in the number and dimension of liquid pendular bonds. For the finer, cohesive fractions, the effect of moisture is initially to cause an increase in tensile strength due possibly to a reduction in interparticle separation as well as pendular bridging. Above a critical moisture content, the tensile strength decreases due to a disruption of the inherent forces of cohesion and the failure to produce new pendular bonds at points of near contact.

Keyphrases □ Tensile strength, bulk solids—effect of moisture on variable particle-size sodium chloride □ Moisture—effect on tensile strength of variable particle-size sodium chloride, bulk solids □ Particle size of bulk solids—effect of moisture on tensile strength

The tensile strength of a powder bed plays an important role in the fundamental theory of shear cell testing (1) and also has been used as an empirical measure of the cohesive properties of the bed (2, 3).

Particle characteristics which influence tensile strength are size distribution (4–12), shape (13), closeness of packing (14, 15), and method of packing (16). In addition, tensile strength is affected by the age of the bed (2), the temperature (17), and the presence of moisture (18–24).

Investigations into the effects of moisture on tensile strength often have been based on ideal models of uniform spheres. Furthermore, there has been a tendency to attribute observed tensile strength changes

Table I—Preparation and Characterization of Size Fractions of Sodium Chloride

Size Fraction, μ	Mean Diameter, μ	Method of Preparation	Shape: Elongation Ratio	Shape: Description According to British Standard 2955 (1958)
250–353	302[a]	Sieving	1.06	Crystalline (cubic)
178–250	214[a]	Sieving	1.06	Crystalline (cubic)
75–178	127[a]	Sieving	1.19	Crystalline (cubic)
32–75	54[a]	Sieving	1.50	Angular
<32 μ (1)	14[b]	End-runner milling	1.17	Irregular
(2)	13[b]	End-runner milling	—	Irregular
(3)	13[b]	End-runner milling	—	Irregular
<32 μ	5[b]	Fluid energy milling	1.16	Irregular

[a] Mean arithmetic (sieve) diameter. [b] Mass median (Coulter) diameter.