Multiple Sulfa Compounds: High-Pressure Liquid Chromatographic Assay and Mobile Phase Correlation

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Abstract □ A mixture of sulfacetamide, sulfathiazole, and sulfabenzamide was used to develop a rapid high-pressure liquid chromatographic assay. In addition, this study provided a means to develop concepts relating solvent molar polarization parameters and retention times. A linear correlation between molar polarization and retention time was observed and will permit reasonably rapid predictions about the dependent variable.

Keyphrases □ Sulfacetamide—high-pressure liquid chromatographic analysis in mixtures with sulfathiazole and sulfabenzamide □ Sulfathiazole—high-pressure liquid chromatographic analysis in mixtures with sulfacetamide and sulfabenzamide □ Sulfabenzamide—high-pressure liquid chromatographic analysis in mixtures with sulfacetamide and sulfathiazole □ High-pressure liquid chromatography—analyses, sulfacetamide, sulfathiazole, and sulfabenzamide in mixtures □ Antibacterials—sulfacetamide, sulfathiazole, and sulfabenzamide, high-pressure liquid chromatographic analyses of mixtures

The fundamental concepts of adsorption and partition chromatography are sufficiently well developed that their application or extension to high-pressure liquid chromatography (HPLC) are, in general, quite straightforward (1). Perhaps the most difficult challenge in this area lies in the ability of the scientist to provide information of a predictive nature. Several researchers tried rational approaches to the use of liquid chromatography (1). Perhaps more to the point would be a better understanding of the behavior of chromatographic mobile phase systems based on relationships providing predictive insight for the selection of mobile phase systems.

A concept based on readily calculable values of polarizability, directly obtainable from the physical properties of the solvent system, is developed and discussed. This approach is similar to that of Snyder (2), but it is unique in that it does not relate to an elutropic series of solvents.

EXPERIMENTAL

Analytical Development—The primary purpose was to develop an analytical procedure for the rapid determination of three sulfa compounds: sulfacetamide, sulfabenzamide, and sulfathiazole. A convenient commercially available substrate, 10-μm silica gel[1], was filled into a 2-mm stainless steel tube, 50 cm long, using the usual neutral density filling techniques (3).

In the initial selection process for the mobile phase, trimethylpentane–chloroform (8:1) was used since it provided good UV transparency of the 254-nm detector[2]. The polar phase of the system, acetonitrile–isopropyl alcohol (1:1), was added in small increments until there was no further improvement in the resolution of the sulfa compounds on this silica gel column with the operating conditions. A small quantity of acetic acid (0.8%) was added *via* the polar phase to minimize the amount of tailing of such polar compounds. This addition permitted the mobile phase to produce clearly Gaussian shaped lines (Fig. 1). The final composition of this mobile phase was: trimethylpentane, 80%; chloroform, 9.8%; isopropyl alcohol, 4.7%; acetonitrile, 4.7%; and acetic acid, 0.8%.

The usual operating conditions chosen for such a determination were 1.75 ml/min for the flow rate, 80° for the operating temperature, and 1500 psi as the pressure. The use of a high-pressure injection port[3] for sample introduction permitted about 4 μg of each compound to be introduced into the system *via* a 10-μl injection slot without special sample preparation. Table I details the precision results obtained. The data collected from the commercial instruments during analysis were delivered to a fixed-purpose calculating integrator[4] where the digital integration and the determination of resolution (4) were performed. This HPLC unit was capable of both high temperature operation and solvent programming.

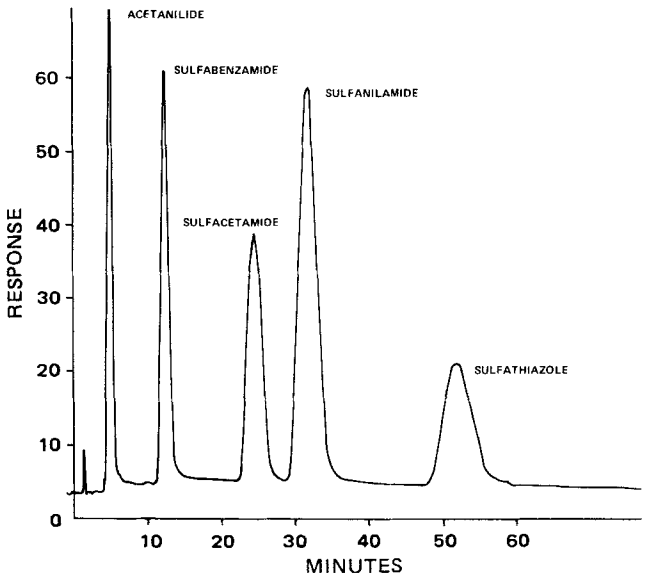

Figure 1—*Chromatogram of mixed sulfa compounds. Operating conditions were: 2.50-ml/mm flow rate, 1210 psi, 78.5°, 0.1 × 2 aufs, and* $\Sigma(X_i P\mu) = 9.9$.

Mobile Phase Aspect of Separation—To extend the knowledge and utility of mobile phase systems, attention was focused on their colligative properties. Therefore, molar polarization contributions from each solvent were calculated (5) and summed together to yield a final value called total molar polarization, $P\mu$, for the solvent system (2, 5). A plot of $P\mu$ *versus* the reciprocal of the retention times of the current solutes is presented in Fig. 2.

RESULTS AND DISCUSSION

This HPLC method for the rapid analysis of sulfa compounds in a complex mixture permits the automation of data collection and reduction. Statistical analysis (Table I) reveals an acceptable 95% confidence limit. This method employs a unique, nonaqueous, multiple-component, mobile phase system. Its choice permits a wider range of operating conditions for chromatographic separations relative to other combinations of mobile phases and substrates (6). It will, because of its nonaqueous composition, permit longer silica gel column lifetimes (up to 6 months); furthermore, it is a simplified procedure for similar compounds.

However, to understand this mobile phase system better, it is important to consider the polarizability effects of such solvents and to examine how they might influence chromatographic solutes on supports such as silica gel. This adsorption–desorption phenomenon for such sulfa compounds from silica gel should be influenced by nondispersive, specific solvent interactions (4).

[1] LiChrosorb S160, E. M. Laboratories.
[2] Model 7000, Micromeritics Instrument Corp.
[3] Model 706L, Disc Instruments.
[4] Auto Lab IV, Spectra Physics Corp.

Table I—Precision Data for Sulfa Compounds *via* HPLC

Peak Areas[a], $S = 0.05 \times 2$				Peak Ratios		
Acetanilide (I), 0.10 mg/ml	Sulfabenzamide (II), 0.20 mg/ml	Sulfacetamide (III), 0.20 mg/ml	Sulfathiazole (IV), 0.20 mg/ml	$\left(\frac{II}{I}\right)$	$\left(\frac{III}{I}\right)$	$\left(\frac{IV}{I}\right)$
352,365	400,398	486,907	702,742	1.136	1.382	1.994
371,023	429,121	492,524	766,862	1.157	1.327	2.067
373,953	430,055	495,171	697,044	1.150	1.324	1.864
354,977	402,077	475,428	694,111	1.133	1.339	1.955
383,246	404,375	487,215	709,354	1.055	1.271	1.851
345,554	382,822	448,458	678,537	1.108	1.298	1.964
			Mean $\bar{x}$	1.123	1.324	1.949
			SD S	0.037	0.038	0.081
			95% *CL*	±0.039	±0.040	±0.085
				±3.5%	±3.0%	±4.4%

[a] Area counts.

For nonpolar molecules, such as pentane, the amount of molecular deformation resulting from a polarizing dc field is called the induced molar polarization: $P\alpha = \frac{4}{3}\pi N\alpha$, where N is Avogadro's number and the magnitude, α, reflects the amount of polarizability that the molecule experiences in that dc field. Furthermore, a dipole in a molecule experiences a similar, but induced, polarization. As a result, it moves itself relative to the dc field to minimize the action of that field. The condition that results in added polarization is referred to as permanent molar polarization and is expressed as $P\mu = \frac{4}{3}\pi N[\mu^2/3KT]$, where μ is the dipole moment and K is the Boltzman constant. The net observed polarization, $P = [P\alpha + P\mu]$, and its thermal coefficient yield the dipole moment. Rearranging terms, one can state that $P = \frac{4}{3}\pi N[\alpha + \mu^2/3KT]$ (7).

For relatively dilute solutions of dipolar molecules in nonpolar solvents, the Clausius–Mossotti equation is valid (8) and leads to the expression $P = [(\epsilon - 1)/(\epsilon + 2)]M/\rho$, where ϵ is the dielectric constant, M is the molecular weight, and ρ is the solvent density. To obtain the value for the total molar polarization, the value for $P\alpha$ must be computed. From Maxwell's relationship of $\epsilon = \eta^2$, where η is the refractive index, it follows that $P\alpha = [(\eta^2 - 1)/(\eta^2 + 2)]M/\rho$, and this expression is referred to as the Lorentz–Lorentz equation (8, 9).

To calculate the net total molar polarization of the solvent system, each

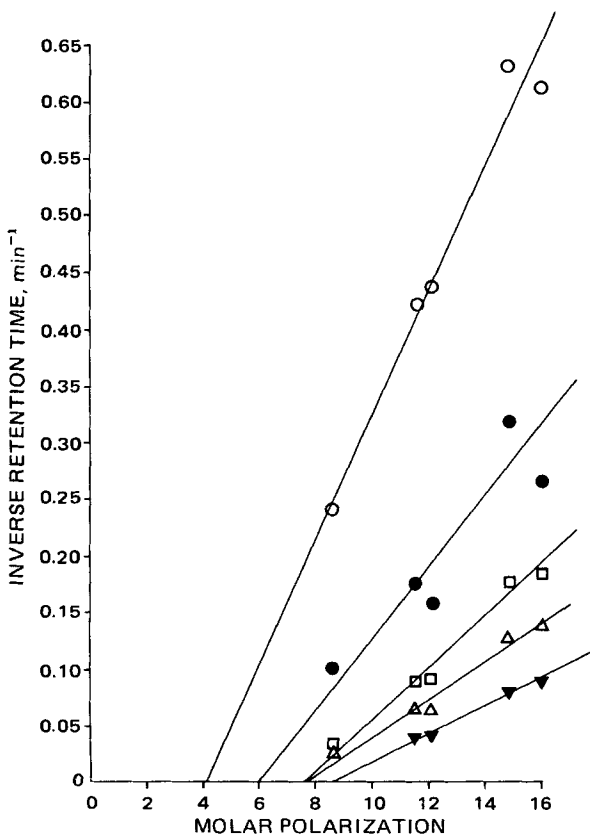

Figure 2—*Plot of inverse retention time* versus *molar polarization*, Pμ. *Key:* ○, *acetanilide;* ●, *sulfabenzamide;* □, *sulfacetamide;* △, *sulfanilamide; and* ▼, *sulfathiazole.*

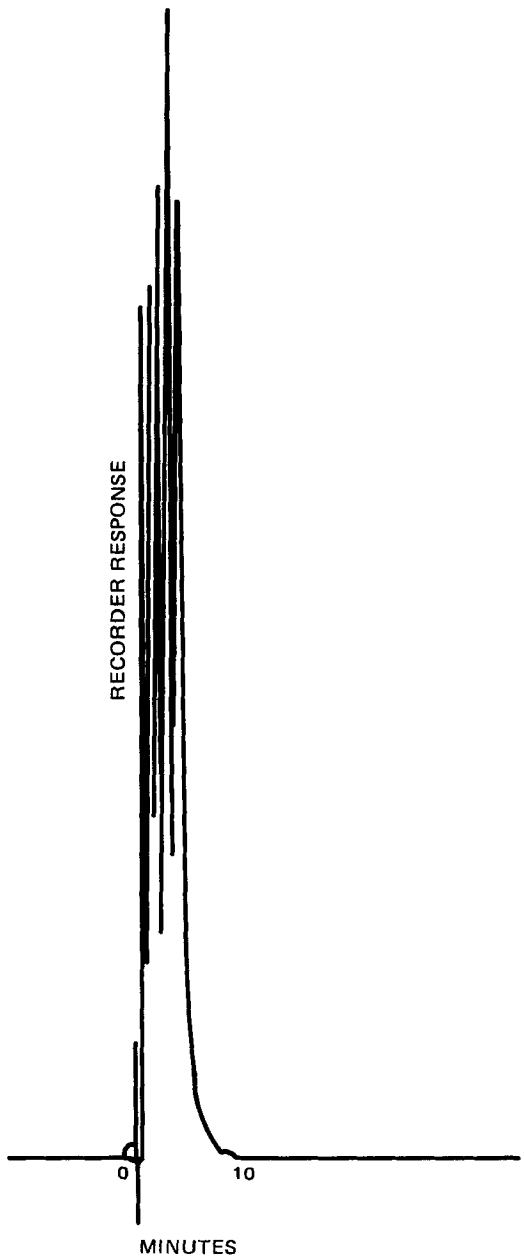

Figure 3—*Chromatogram of mixed sulfa compounds. Operating conditions were: 2.50-ml/mm flow rate, 1200 psi, 78.5°, 0.1 × 2 aufs, and* $\Sigma(X_i P\mu) = 2.0$.

Table II—Total Molar Polarization of Typical Solvent Systems

	Solvent					
	Trimethylpentane	Chloroform	Acetonitrile	Isopropyl Alcohol	Acetic Acid	$\Sigma P\mu$
MW/ρ	165	80.18	52.86	76.91	57.20	—
ϵ	1.94	4.806	37.5	19.92	6.15	—
η	1.404	1.443	1.344	1.38	1.372	—
$P\mu$	−0.95	23.58	37.34	47.47	23.14	—
System						
A: % (v/v)	80	9.8	4.4	5.0	0.8	100%
X_i	0.627	0.158	0.074	0.123	0.018	1.000
$P\mu_i$	−0.596	3.726	2.763	5.839	0.417	12.15
B: % (v/v)	80	12.3	4.4	2.5	0.8	100%
X_i	0.641	0.203	0.075	0.063	0.018	1.000
$P\mu_i$	−0.609	4.787	2.801	2.991	0.417	11.61
C: % (v/v)	80	14.8	4.4	ϕ	0.8	100%
X_i	0.655	0.250	0.076	ϕ	0.019	1.000
$P\mu_i$	−0.622	5.895	2.838	ϕ	0.440	8.55
D: % (v/v)	80	12.0	2.2	5.0	0.8	100%
X_i	0.556	0.286	0.033	0.109	0.016	1.000
$P\mu_i$	−0.528	6.794	1.232	5.174	0.370	12.99
F: % (v/v)	76.6	9.0	4.4	9.5	0.7	100%
X_i	0.562	0.136	0.067	0.220	0.015	1.000
$P\mu_i$	−0.534	3.207	2.502	10.44	0.347	15.97
G: % (v/v)	69.8	20.0	4.4	5.0	0.8	100%
X_i	0.505	0.297	0.068	0.113	0.017	1.000
$P\mu_i$	0.480	7.003	2.539	5.364	0.393	14.82

solvent's contribution based on its mole fraction is added and, by rearranging terms, the following equation results:

$$P\mu = \sum_{1}^{N} [X_i(P - P\alpha)_i] \quad \text{(Eq. 1)}$$

Further substitution yields:

$$P\mu = \sum_{1}^{N} X_i \{[(\epsilon - 1)/(\epsilon + 2)] - [(\eta^2 - 1)(\eta^2 + 2)]\}M/\rho \quad \text{(Eq. 2)}$$

which readily yields $P\mu$ (total molar polarization) (Table II).

When the retention time, t_r, is examined and plotted *versus* $P\mu$, a linear relationship is evident. These two parameters are shown in Fig. 2; the second variable can be predicted easily by knowing the first when using such a plot. Alternatively, the values of $P\mu$ and t_r can be plotted rectalinearly with respect to each other, and a hyperbolic function is observed. With three data points and the use of numerical methods (10, 11), one can solve for the expression using Cramer's determinates. Such an approach yields the constants for this function. In addition, a program written in BASIC permits quick and convenient calculation of new values for either $P\mu$ or t_r by merely inserting several data points into the program without additional plotting. This approach permits the analyst to go from one set of chromatographic conditions to another very quickly and to predict either parameter accurately. This comparison is rather evident through a comparison of Figs. 1 and 3 where the changes in retention times were very simple using these concepts (see Table III for derived constants).

Table III—Hyperbolic Constants from HPLC Data

Compound	Derived Constants			
	A	*B*	*C*	*K*
Acetanilide	−1.05	−0.125	−0.14	0.55
Sulfabenzamide	−20.11	0.50	−0.19	6.20
Sulfanilamide	80.31	−5.77	−0.68	8.29
Sulfacetamide	144.7	−9.91	−0.48	2.27
Sulfathiazole	−21.59	+0.32	−0.12	12.34

REFERENCES

(1) P. Scott and P. Kureca, *Anal. Chem.*, **45**, 749 (1973).

(2) L. R. Snyder, "Principles of Adsorption Chromatography," Dekker, New York, N.Y., 1968, chap. 8.

(3) J. Kirkland, *J. Chromatogr. Sci.*, **8**, 618 (1970).

(4) L. R. Snyder and J. J. Kirkland, "Modern Liquid Chromatography," American Chemical Society, Washington, D.C., 1973, p. 97.

(5) "Conformational Analysis," E. L. Eliel, Ed., Wiley–Interscience, New York, N.Y., 1976, p. 156.

(6) J. Kirkland, "Modern Practice of Liquid Chromatography," Wiley–Interscience, New York, N.Y., 1970, chaps. 10 and 11.

(7) W. J. Moore, "Physical Chemistry," 2nd ed., Prentice-Hall, Englewood Cliffs, N.J., 1954, p. 314.

(8) *Ibid.*, p. 321.

(9) C. P. Smith, "Techniques of Organic Chemistry," 3rd ed., vol. I, part III, Interscience, New York, N.Y., 1960, p. 2599.

(10) E. C. Hohmann and F. J. Lockhart, *Chem. Tech.*, **2**, 614 (1972).

(11) E. Johnson, *Chem. Eng.*, Aug. 11, 1969.

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