Sarin Transport across Excised Human Skin II: Effect of Solvent Pretreatment on Permeability

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Abstract □ The effect of pretreating callus membranes with dimethyl sulfoxide, dimethylacetamide, dimethylformamide, formamide, dioxane, or methyl orthoformate on sarin permeability was studied at three temperatures to determine the activation energy for transport. In addition, the effect of membrane pretreatment on permeability through callus membranes from either a sarin–carboxypolymethylene gel or a sarin–polymethyl methacrylate gel was studied.

Keyphrases □ Sarin—transport across excised human skin, effect of pretreating callus membranes with dimethyl sulfoxide, dimethylacetamide, dimethylformamide, formamide, dioxane, methyl orthoformate □ Cholinesterase inhibitors—sarin, transport across excised human skin, effect of pretreatment on permeability □ Transepidermal transport—sarin, excised human skin, effect of pretreatment

The presence of water in the skin or diffusing media increases percutaneous absorption (1–7). Much work also has been done on the effects of solvents other than water; dimethyl sulfoxide (8–20), dimethylacetamide (17, 21), and dimethylformamide (17, 21) influence the penetration rate of various chemicals. Some of this work was reviewed recently (22).

Penetration rates of sarin through excised human callus tissue from dilute solution and concentrated gels under both anhydrous and hydrous conditions were reported previously (23). The purpose of this study was to determine the effect of solvent pretreatment of callus membranes with dimethyl sulfoxide, dimethylacetamide, dimethylformamide, formamide, dioxane, and methyl orthoformate on sarin transport rates and on the transport activation energy.

Table I—Influence of Membrane Conditioning in Solution Phase Sarin Transport Systems

Membrane Conditioning Solvent	Average Transport Rate, mg/cm²/hr		Rate[a] Ratio	Temperature
	Unconditioned	Conditioned		
Dimethyl sulfoxide[b]	0.032	0.553	17.3	25°
Dimethylformamide[b]	0.017	0.218	12.8	30°
Dimethylacetamide[b]	0.019	0.192	10.1	25°
Formamide[b]	0.008	0.810	101.3	25°
Dioxane[c]	0.010	0.034	3.4	25°
Methyl orthoformate[c]	0.017	0.003	0.18	25°

[a] Rate ratio = [rate (conditioned)/rate (unconditioned)]. [b] Membrane conditioned and *n*-heptane saturated with given solvent. [c] Membrane conditioned but no solvent in *n*-heptane.

EXPERIMENTAL

Permeability Cell—The design of the permeability cell depended on whether the study involved diffusion from a dilute sarin solution or from a concentrated sarin gel. Both cells were described previously (23).

Membrane Preparation—Callus membranes were microtomed to a thickness of 100 μm and delipidized in anhydrous ether for 24 hr as reported earlier (23).

The membranes were placed in the diffusion cell membrane holder, using a small amount of silicone grease as an edge sealer. All membranes were examined microscopically after positioning in the membrane holder, which was immersed in the particular membrane conditioning solvent for 24 hr.

Permeability Cell Preparation for Solution Phase Studies—The slide containing the membrane was positioned between the two chambers of the permeability cell. Then 50 ml of $\sim 7.8 \times 10^{-3}$ *M* sarin in *n*-heptane,

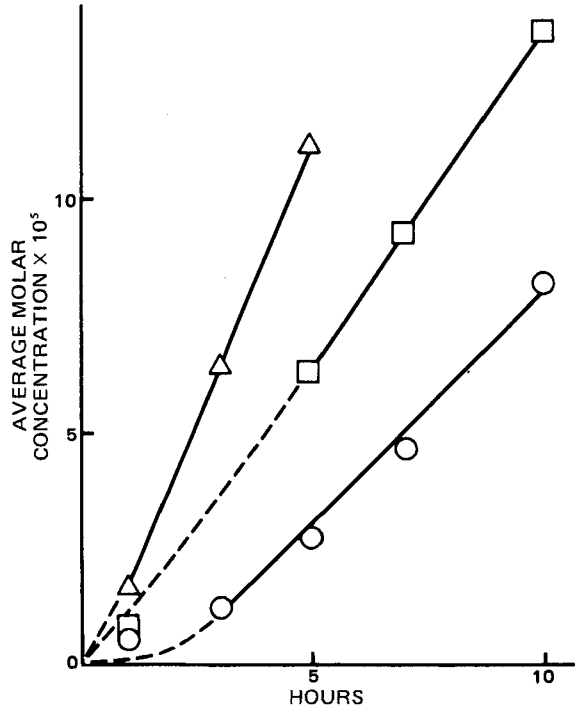

Figure 1—*Average sarin transport across dimethyl sulfoxide-conditioned callus membranes. Key:* ○, *25°;* □, *35°; and* △, *45°.*

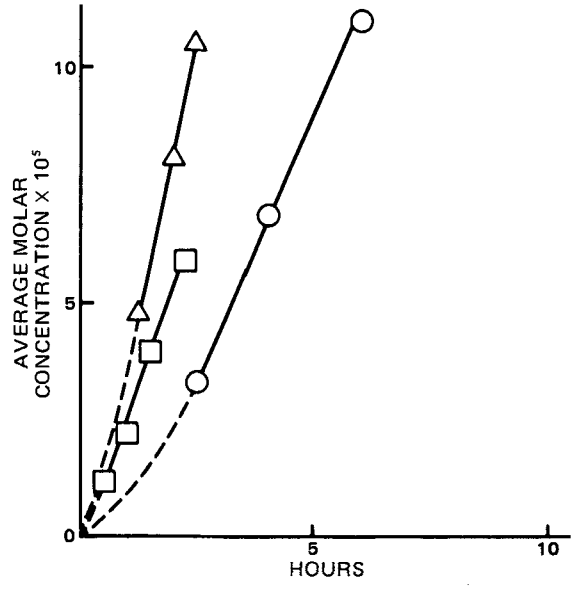

Figure 2—*Average sarin transport across formamide-conditioned callus membranes. Key:* ○, *25°;* □, *35°; and* △, *45°.*

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Table II—Influence of Membrane Conditioning and Gel Formation on Sarin Transport

Membrane Conditioning Solvent	Average Transport Rate, mg/cm²/hr: Polymethyl Methacrylate Gel, Unconditioned Solution Phase	Polymethyl Methacrylate Gel, Conditioned Gel Phase	Rate[a] Ratio	Carboxypolymethylene Gel, Unconditioned Solution Phase	Carboxypolymethylene Gel, Conditioned Gel Phase	Rate[a] Ratio
Dimethyl sulfoxide	0.005	5.47	911	0.008	18.2	2223
Dimethylformamide	0.085	24.0	282	0.004	6.64	1660
Dimethylacetamide	0.002	2.6	1300	0.008	8.60	1075
Formamide	0.003	3.34	1113	0.021	2.72	129.5
Dioxane	0.014	0.287	20.5	0.023	0.132	5.7
Methyl orthoformate	0.025	0.148	5.9	0.106	0.114	1.1

[a] Rate ratio = [rate (conditioned, gel)/rate (unconditioned, solution)].

Table III—Influence of Membrane Conditioning on Sarin Transport in Gel System

Membrane Conditioning Solvent	Solvent Dielectric Constant	Transport Rate, Polymethyl Methacrylate, mg/cm²/hr	Rate[a] Ratio	Transport Rate, Carboxypolymethylene, mg/cm²/hr	Rate[a] Ratio
None	—	0.407[b]	—	0.547[b]	—
Dimethyl sulfoxide	45	5.47	13	18.23	33
Dimethylformamide	27	24.0	59	6.64	12
Dimethylacetamide	38	2.6	6	8.60	15
Formamide	109	3.34	8	2.72	5
Dioxane	2.2	0.287	0.71	0.132	0.24
Methyl orthoformate	3	0.148	0.36	0.114	0.21

[a] Rate ratio = [rate (conditioned, gel)/rate (unconditioned, gel)]. [b] Reference 23.

at the appropriate temperature, was placed in the donor chamber with the simultaneous addition of an equal volume of *n*-heptane at the same temperature in the receiver chamber. The *n*-heptane in each chamber was saturated with the appropriate immiscible polar solvent at the temperature of the experiment but did not contain any dioxane or methyl orthoformate in the permeability comparison experiments. In the activation energy studies, the solvent composition was the same, except that 50 ml of dioxane or methyl orthoformate was added to 950 ml of *n*-heptane.

The permeability cells were maintained in a constant-temperature bath with gentle shaking in the plane of the membrane. The transport rate was followed by withdrawing 1-ml samples from the receiver chamber at suitable times and analyzing them colorimetrically for sarin.

Permeability Cell Preparation for Sarin Gel Studies—The preparation of the carboxypolymethylene[1] and polymethyl methacrylate gels and cell were described earlier (23). Fifty milliliters of *n*-heptane saturated with dimethyl sulfoxide, dimethylformamide, dimethylacetamide, or formamide was added immediately to the receiver chamber to start the experiment. No dioxane or methyl orthoformate was placed in the *n*-heptane. The permeability cell was maintained at 30° in a water bath with gentle shaking in the plane of the membrane. The transport rate was followed as in the solution phase studies.

Colorimetric Assay—The analytical procedure employed was a modification of the Schoenmann method (24) and was described earlier (23).

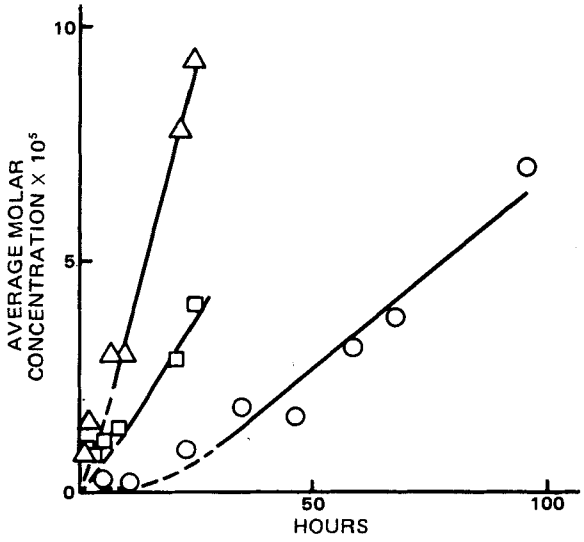

Figure 3—*Average sarin transport across dimethylformamide-conditioned callus membranes. Key:* ○, *25°;* □, *35°; and* △, *45°.*

RESULTS AND DISCUSSION

Solution Phase Permeability Comparison Studies—The initial sarin concentration in all experiments was $\sim 8 \times 10^{-3}$ *M*. Average sarin transport rates, in milligrams per square centimeter per hour, during the quasi-steady state through untreated membranes and the same solvent-conditioned membranes, along with a calculated membrane conditioning ratio, [rate (conditioned)/rate (unconditioned)] are shown in

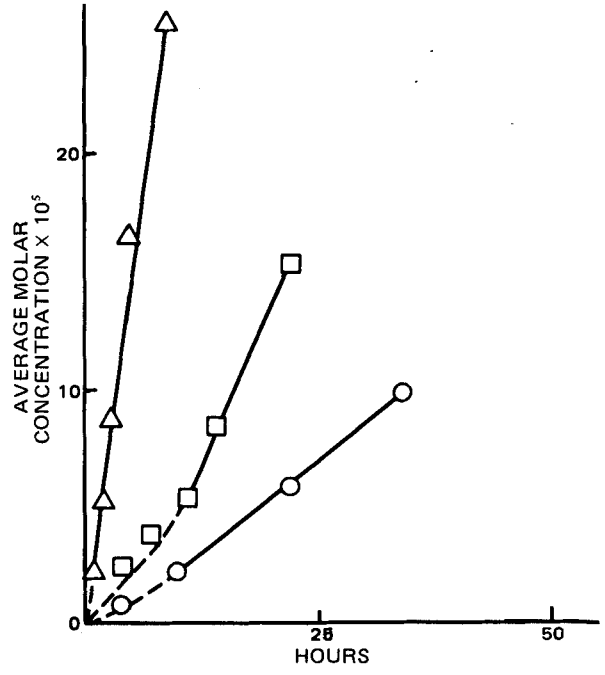

Figure 4—*Average sarin transport across dioxane-conditioned callus membranes. Key:* ○, *25°;* □, *35°; and* △, *45°.*

[1] Carbopol 934, B. F. Goodrich Chemical Co., Cleveland, OH 44100.

Table IV—Permeability Coefficients and Activation Energies for Solvent-Treated Membranes

Membrane Conditioning Solvent	Temperature	Permeability Coefficient, $k \times 10^4$/hr	Activation Energy, kcal/mole
Dimethyl sulfoxide	24.9°	13.00	8.2
	35.0°	18.65	
	44.9°	31.09	
Dioxane	25.0°	4.13	21.2
	34.9°	11.72	
	44.9°	39.00	
Methyl orthoformate	25.0°	2.75	22.3
	34.9°	10.05	
	44.8°	28.79	
Dimethylformamide	26.0°	1.07	14.4
	35.1°	1.83	
	45.0°	4.54	
Formamide	25.0°	28.29	6.2
	35.0°	37.51	
	44.5°	54.16	

Table I. Pretreating the membrane with dimethyl sulfoxide, dimethylformamide, dimethylacetamide, or formamide markedly increased the transport rate; dioxane caused only a slight increase, and methyl orthoformate actually decreased the rate.

Gel Phase Permeability Comparison Studies—The sarin transport rate through the solvent-treated membranes used in the gel study was determined first using the anhydrous solution phase conditions described previously. The combined influence of membrane conditioning and increased sarin concentration on sarin transport is shown in Table II, along with a calculated ratio defined as [rate (conditioned, gel)/rate (unconditioned, solution)].

The influence of membrane conditioning can to some degree be separated from the concentration effects by comparing these sarin transport rates from the gels through solvent-conditioned membranes [rate (conditioned, gel)] to some previously reported transport rates (23) from the gels through unconditioned membranes [rate (unconditioned, gel)]. These data, along with a calculated rate ratio defined as [rate (conditioned, gel)/rate (unconditioned, gel)] are shown in Table III. This approach was necessary since it was not possible to study the rates through untreated membranes in the gel system followed by their determination through the same membranes after solvent treatment. Polymeric material, especially the polymethyl methacrylate, collected on the membranes and could not be removed, making their reuse impossible.

The transport rate change in the gel system was due not only to the effect of solvent conditioning of the membrane but also to the effect of the conditioning solvent on the polymer in the gel, which may affect the release rate from the gel. There appeared to be an optimum range for the dielectric constant for the conditioning solvent in each gel system. The best conditioner for the more oil-soluble polymethyl methacrylate gel was dimethylformamide with a dielectric constant of 27, whereas the best conditioner for the more hydrophilic carbomer gel was dimethyl sulfoxide with a dielectric constant of 45. In both cases, as the dielectric constant of the conditioning solvent shifted away in either direction from the optimum range, a decrease in the ratio [rate (conditioned, gel)/rate (unconditioned, gel)] was observed (Table III).

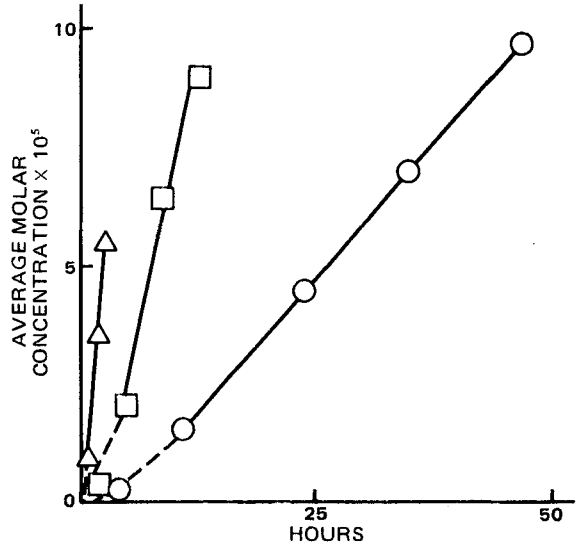

Figure 5—*Average sarin transport across methyl orthoformate-conditioned callus membranes. Key:* ○, *25°;* □, *35°; and* △, *45°.*

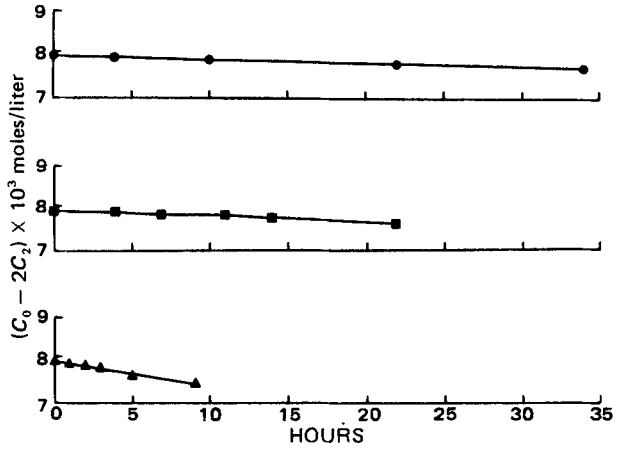

Figure 6—*First-order plots for sarin transport across dioxane-conditioned callus membranes. Key:* ●, *25°;* ■, *35°; and* ▲, *45°.*

Effect of Solvent Pretreatment on Transport Process Activation Energy—Different membranes were used at each temperature for each solvent treatment since repeated conditioning with dimethyl sulfoxide increased the subsequent permeability constants of the membranes.

Plots of the average molar concentration on the receiver side *versus* time at the three temperatures studied are shown in Figs. 1–5. The slope in the quasi-steady states allows calculation of the transport rate. A plot of log $(C_0 - 2C_2)$ *versus* time is shown in Fig. 6 for the dioxane solvent system at the three temperatures. The permeability coefficient at each

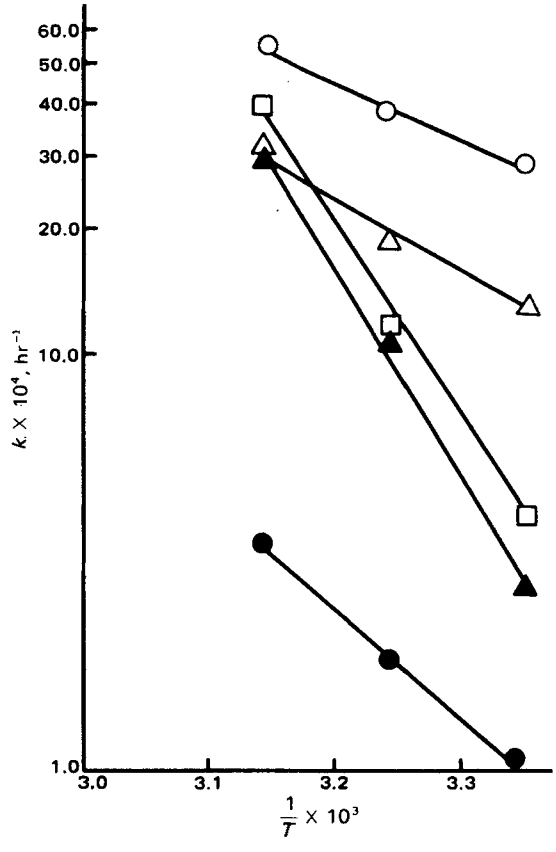

Figure 7—*Arrhenius plot of average permeability coefficients for sarin penetration through conditioned callus membranes at 25, 35, and 45°. Key:* ○, *formamide;* □, *dioxane;* △, *dimethyl sulfoxide;* ▲, *methyl orthoformate; and* ●, *dimethylformamide.*

temperature is calculated from the slope as described previously (23). The activation energy for the transport process for each solvent system is obtained from the slope of an Arrhenius plot of log permeability constant *versus* the reciprocal of the absolute temperature (Fig. 7). The permeability coefficients at each temperature and the activation energy for each system are shown in Table IV, with the expected inverse order between the rate ratio and the activation energy being observed.

REFERENCES

(1) T. Higuchi, *J. Soc. Cosmet. Chem.*, **11**, 85 (1960).

(2) J. E. Treherne, *J. Physiol.*, **133**, 171 (1956).

(3) W. B. Shelly and F. M. Melton, *J. Invest. Dermatol.*, **13**, 61 (1949).

(4) J. B. Shelmire, Jr., *Arch. Dermatol.*, **82**, 24 (1960).

(5) E. P. Laug, E. A. Vos, F. M. Kunze, and E. J. Umberger, *J. Pharmacol. Exp. Ther.*, **89**, 52 (1947).

(6) H. Leslie-Roberts, *Br. J. Dermatol.*, **40**, 325 (1928).

(7) D. E. Wurster and S. F. Kramer, *J. Pharm. Sci.*, **50**, 288 (1961).

(8) A. Horita and L. J. Weber, *Life Sci.*, **3**, 1389 (1964).

(9) S. W. Jacob, M. Bischel, and R. J. Herschler, *Curr. Ther. Res.*, **6**, 193 (1964).

(10) R. B. Stoughton and W. Fritsch, *Arch. Dermatol.*, **90**, 512 (1964).

(11) A. M. Kligman, *J. Am. Med. Assoc.*, **193**, 796 (1965).

(12) R. J. Feldmann and H. I. Maibach, *Arch. Dermatol.*, **94**, 649 (1966).

(13) H. L. McDermot, G. W. Murray, and R. M. Heggie, *Can. J. Physiol. Pharmacol.*, **43**, 845 (1965).

(14) J. M. Stelzer, J. L. Colaizzi, and P. J. Wurdock, *J. Pharm. Sci.*, **57**, 1732 (1968).

(15) H. L. McDermot, A. J. Finkbeiner, W. J. Willis, and R. M. Heggie, *Can. J. Physiol. Pharmacol.*, **45**, 299 (1967).

(16) J. E. Wahlberg and E. Skog, *Acta Dermatol.*, **47**, 209 (1967).

(17) D. D. Munro and R. B. Stoughton, *Arch. Dermatol.*, **92**, 585 (1965).

(18) W.-W. Shen, A. G. Danti, and F. N. Bruscato, *J. Pharm. Sci.*, **65**, 1780 (1976).

(19) J. P. Astley and M. Levine, *ibid.*, **65**, 210 (1976).

(20) F. Marcus, J. L. Colaizzi, and H. Barry, *ibid.*, **59**, 1616 (1970).

(21) R. J. Scheuplein, Final Report (1965) to U.S. Army Chemical Research and Development Laboratories, Md., Contact DA 18-108-AMC-148 (A).

(22) B. Idson, *J. Pharm. Sci.*, **64**, 901 (1975).

(23) D. E. Wurster, J. A. Ostrenga, and L. E. Matheson, Jr., *ibid.*, **68**, 1406 (1979).

(24) B. Gehauf, J. Epstein, G. B. Wilson, B. Witten, S. Sass, V. E. Bauer, and W. H. C. Rueggeberg, *Anal. Chem.*, **29**, 278 (1957).

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High-Performance Liquid Chromatographic Determination of Azosemide in Commercial Animal Feed

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Abstract □ A high-performance liquid chromatographic (HPLC) method was developed for the assay of 2-chloro-5-(1*H*-tetrazol-5-yl)-4-[(2-thienylmethyl)amino]benzenesulfonamide (I) in animal feed diet mixtures. The sample is extracted with acetone, an aliquot of the extract is evaporated to dryness, and the residue is dissolved in chloroform. This solution can be subjected to either of two cleanup procedures. The first traps I on a sodium hydroxide–diatomaceous earth column. Compound I is then recovered using an acetic acid–ethyl acetate–chloroform eluent, which is removed by evaporation, followed by dissolution of the residue in aqueous sodium hydroxide. The second procedure involves the direct extraction of I from the chloroform solution. A linear relationship exists between the HPLC UV detector response at 254 nm and concentration up to 170 μg/ml. The sodium hydroxide extraction procedure resulted in an overall recovery (±*SD*) of 92.9 ± 1.6 and 97.4 ± 1.8% ($n = 5$) at levels of 200 and 6000 ppm, respectively. Overall recoveries (±*SD*) obtained by the diatomaceous earth column procedure for the 200- and 6000-ppm levels were 90.0 ± 2.0 and 95.9 ± 1.6% ($n = 5$), respectively.

Keyphrases □ Azosemide—analysis, high-performance liquid chromatography, commercial animal feed □ Diuretic agents—azosemide, high-performance liquid chromatographic analysis, commercial animal feed □ High-performance liquid chromatography—analysis, azosemide, commercial animal feed

Azosemide, 2-chloro-5-(1*H*-tetrazol-5-yl)-4-[(2-thienylmethyl)amino]benzenesulfonamide (I), is a new diuretic and saluretic agent (1). The drug has been formulated into tablets, injectable solutions, and lyophilizates in combination with other diuretics for the treatment of hydropic conditions (2, 3).

In long-term toxicological studies, drug administration to laboratory animals is usually done by incorporation into feed because of the inconvenience of individual manual dosing and the time involved in preparing individual dosages. Analytical methodology is required to determine that appropriate dosages are administered and that the drug is uniformly distributed and stable in the feed mix under the storage conditions used. Recent papers dealt with the determination of cyclobenzaprine, sulindac, halofenate, estradiol, and phenobarbital in animal feed mixtures for use in toxicity and/or carcinogenesis studies (4–6).

Reported quantitative analyses of sulfonamides in animal feed include spectrophotometric measurement of the diazotized sulfonamide after alumina column cleanup (7), GLC assay after purification by conventional extraction methods (8, 9), quantitative TLC (10), and spectrophotofluorometry and GLC after successive percolation through silica and alumina columns or successive base–acid extractions (11).

This paper presents a method for the assay of I in animal feed mix at 200- and 6000-ppm levels using high-perfor-

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