

**Note**

**High-performance liquid chromatography analysis of 2-chloroethyl ethylsulfide and its decomposition by-products by derivatization**

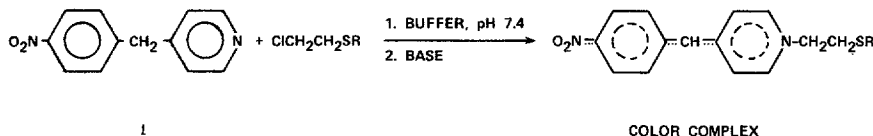
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The ability to detect, separate and quantitate trace amounts of sulfur mustards and their decomposition by-products in aqueous solution is of relevance and potential use in environmental studies as well as in general analytical methodology.

Sulfur mustards currently are analyzed by colorimetry using 4-(*p*-nitrobenzyl)pyridine (I)<sup>1</sup> according to the following reaction:

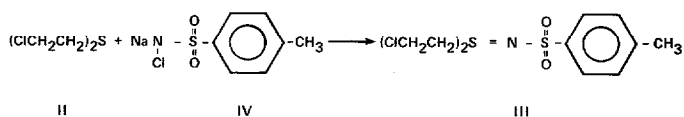


This is a general test and gives positive results for practically all alkylating agents and false positives for some acylating agents. In addition, this test cannot be used for the decomposition by-products.

Gas chromatography (GC) has also been employed<sup>2</sup>. However, water samples cannot be analyzed directly by GC but must undergo a lengthy extraction and work-up procedure before analysis can be performed.

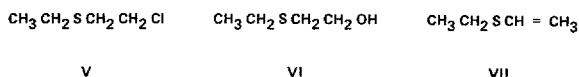
The sulfur mustards and their by-products do not absorb or fluoresce in the ultraviolet (UV) or visible spectral range. Thus, they are not amenable by themselves to high-performance liquid chromatography (HPLC) analysis using a UV or fluorescent detector.

The arenesulfonylsulfilimines were discovered shortly after World War I by Mann and Pope<sup>3</sup>. It was shown then that bis-(2-chloroethyl)sulfide (mustard gas) (II) was swiftly converted in aqueous solution to crystalline and innocuous S-bis-(2-chloroethyl)-N-tolylsulfonylsulfilimine (III) by treatment with sodium toluenesulfochloramide (chloramine-T) (IV) according to the following reaction:

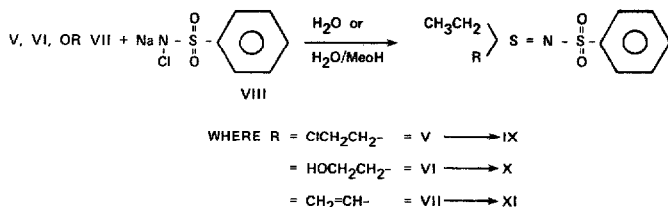


Since then, the reaction of alkylsulfides with salts of various N-chloroarene-sulfonylsulfonamides, *e.g.*, chloramine-B or -T, has been widely used as a facile means of preparing crystalline and innocuous derivatives of alkyl sulfides for characterization purposes.

This paper describes a precolumn enhancement reaction technique for the reversed-phase HPLC analysis of 2-chloroethyl ethylsulfide (V), a sulfur mustard type



compound, and its major decomposition by-products, ethyl 2-hydroxyethylsulfide (VI), and ethyl vinylsulfide (VII). These compounds were derivatized with sodium benzenesulfochloramide (chloramine-B) (VIII), which contains a strong UV chromophore, on a microscale in aqueous alcohol to form novel UV absorbing phenylsulfonylsulfilmines separately and in a combined mixture as indicated in the following equation:



Compounds IX, X, and XI were separated by reversed-phase HPLC and quantitated by UV detector response.

## MATERIALS\*

### Instrumentation

High-performance liquid chromatography HPLC analyses were carried out using a Waters Assoc. high pressure liquid chromatograph consisting of two Model 6000A pumps, a U6K injector, a Model 440 UV detector, a 730A data module, and a 720A systems controller. Separation was carried out using a Waters Assoc. Radial-PAK C<sub>18</sub> (10 μm) column.

### Chemicals

Water used for HPLC was distilled and deionized (10–14 megohm cm). Acetonitrile and methanol were HPLC grade (Burdick & Jackson, Muskegon, MI, U.S.A.). Compounds V and VII were obtained from Fairfield (Blythewood, SC, U.S.A.). Compound VI was obtained from Aldrich (Milwaukee, WI, U.S.A.). Compound VIII was obtained from Eastman Kodak (Rochester, NY, U.S.A.), and purified. All chemicals used gave analytical data consistent with their chemical structure.

\* The use of trade names in this document does not constitute an official endorsement or approval of the use of such commercial hardware or software.

## METHODS

*Preparation of the IX, X and XI standards for HPLC*

Quantities of each of the three sulfilimines were prepared for use as standards to determine optimum chromatographic conditions and effectiveness of analytical derivatization. The sulfide (1.0 mole) and VIII (1.1 mole) were stirred together in 10 ml of 30% cold methanol for 1 h. A white crystalline precipitate appeared almost immediately in all cases. The precipitate was filtered off, washed with a small amount of water, dried, and washed with ether. The precipitate was then recrystallized from ethanol. Spectral data were in agreement with the assigned structures.

*S-Ethyl-S-chloroethyl-N-phenylsulfonylsulfilimine (IX)*. The recrystallized product was obtained by the general procedure outlined above: yield 89%; m.p. 109–110°C. (Found: C, 42.9; H, 5.1; Cl, 12.8; N, 5.1; and S, 22.9. Calc. for  $C_{10}H_{14}ClNS_2O_2$ : C, 42.9; H, 5.0; Cl, 12.7; N, 5.0; and S, 22.9.)

*S-Ethyl-S-2-hydroxyethyl-N-phenylsulfonylsulfilimine (X)*. The recrystallized product was obtained by the general procedure outlined above except that water alone was used as the reaction solvent and diethyl ether: chloroform was used for recrystallization. The yield was 83%; m.p. 75–76.5°C. (Found C, 45.8; H, 5.8; N, 5.6; and S, 24.5. Calc. for  $C_{10}H_{15}NS_2O_3$ : C, 45.9; H, 5.8; N, 5.4; and S, 24.5.)

*S-Ethyl-S-vinyl-N-phenylsulfonylsulfilimine (XI)*. The recrystallized product was obtained by the general procedure outlined above: yield, 82%; m.p. 86.5–88°C. (Found C, 49.1; H, 5.2; N, 6.0; and S, 26.5. Calc. for  $C_{10}H_{13}NS_2O_2$ : C, 49.3; H, 5.4; N, 5.8; and S, 26.4.)

*Chromatographic procedure*

Analytical separations were performed under the following conditions: sample size, 20  $\mu$ l; flow-rate, 1.5 ml/min; column temperature, ambient; mobile phase, acetonitrile–water (30:70); UV detector, 254 nm.

Standard solutions of IX, X and XI were injected onto the column and their retention times determined. Calibration curves conforming to Beer's law were obtained by injecting known concentrations (1.0, 2.0, 4.0, 10.0 and 20.0  $\mu$ g/ml) of the sulfides as sulfilimine derivatives onto the column in triplicate and measuring the resulting peak areas.

*Analytical derivatization*

To one equivalent of each of the sulfides in one ml of methanol is added two equivalents of VIII. The mixture was heated with stirring at 60°C for 1 h. After cooling, 20- $\mu$ l samples were introduced onto the column through a continuous-flow loop injector. Peak areas were measured and computed with an on-line integrator (data module).

In this way, concentrations of V, VI and VII were prepared individually and in combined mixtures at 1.0, 2.0, 4.0, 10.0 and 20.0  $\mu$ g/ml for detection as the sulfilimines species.

## RESULTS AND DISCUSSION

From the literature<sup>4</sup>, arenesulfonylsulfilimines show a strong absorption peak

TABLE I  
UV SPECTRA OF IX, X AND XI IN ACETONITRILE

Compound	$\lambda_1$ (nm)	Log $\epsilon_1$	$\lambda_2$ (nm)	Log $\epsilon_2$	$\lambda_3$ (nm)	Log $\epsilon_3$	Log $\epsilon_{254}$
IX	224	3.9	265	2.7	272	2.6	2.7
X	225	3.9	265	2.7	272	2.6	2.7
XI	226	4.0	264	2.8	272	2.7	2.9

around 230 nm (log  $\epsilon$  4.0–5.0) and a weak absorption peak in the area of 270 nm (log  $\epsilon$  3.0–4.5). As seen in Table I, compounds IX, X and XI show strong absorption peaks at 224–226 nm as well as a weak absorption at 272 nm. Another weak peak also was observed for all three compounds at 265 nm. Also in Table I, the log  $\epsilon$  values at 254 nm for IX, X and XI are shown.

The qualitative capability for this technique is illustrated in Fig. 1 in which the complete separation of IX, X, and XI is achieved in 15 min. Retention times of IX, X and XI were 11.00, 3.32 and 6.72 min, respectively. Excess chloramine-B reagent is unretained on the column and does not interfere with the analysis.

Quantitation also was readily achieved by reversed-phase HPLC. The reactions were reproducible and detector response was linear for V, VI, and VII in concentrations of 1.0–20.0  $\mu\text{g/ml}$ . The overall efficiency of the derivatization reaction for the three sulfides was 85–99% by comparison with standardized materials. The detection limits for V, VI and VII were 10, 12 and 21 ng, respectively. The limits of detection

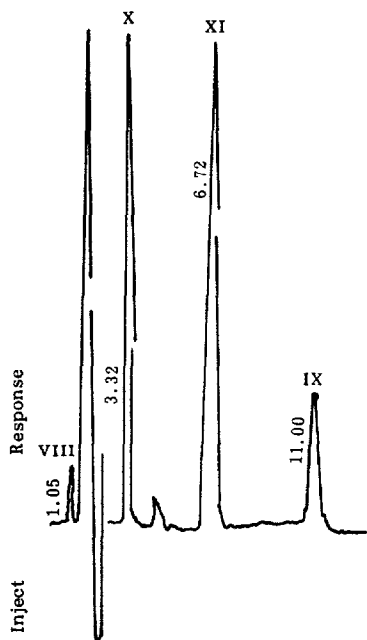


Fig. 1. Chromatogram of the separation of VIII, IX, X and XI.

are based on the method described by Hubaux and Vos<sup>5</sup> using a 95% confidence level with  $\alpha$  and  $\beta$  being equal to 5% (*i.e.*, one out of 20 data may fall outside two standard deviations of the fitted curve).

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#### REFERENCES

- 1 J. Epstein, R. W. Rosenthal and R. J. Ess, *Anal. Chem.*, 27 (1955) 1435.
- 2 T. L. Fisher, M. Jaskot and S. Sass, personal communication.
- 3 F. G. Mann and W. J. Pope, *J. Chem. Soc.*, (1922) 1052.
- 4 T. L. Gilchrist and C. J. Moody, *Chem. Rev.*, 77 (1977) 409.
- 5 A. Hubaux and G. Vos, *Anal. Chem.*, 42 (1970) 849.