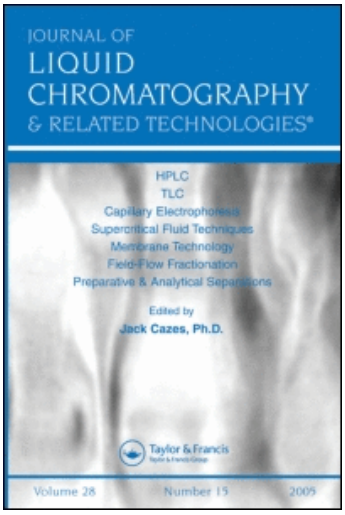


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# Rapid Analysis of Dithiaalkane Diols by HPLC

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# Rapid Analysis of Dithiaalkane Diols by HPLC

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## ABSTRACT

A reversed-phase high performance liquid chromatography (HPLC) method has been developed for simultaneous detection of dithiaalkane-diols, having the common formula $[(HO-CH_2-CH_2-S)_2(CH_2)_n]$ in a homologues series where $n = 1-10$. Both isocratic and gradient methods were developed, and the latter was found to be suitable for both qualitative and quantitative analysis. The method is simple, specific, and requires only small quantities of chemicals and reagents.

*Key Words:* Dithiaalkane diol; HPLC; Rapid analysis.

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## INTRODUCTION

Dithiaalkane diols are important intermediates and degradation products of chemical warfare agents such as sesquimustard (Q) and sulfur mustard (HD), the strong organic sulfur vesicants. Retrospective analysis of samples contaminated with chemical warfare agents, their precursors and degradation products, is an important component of verification in support of chemical weapon convention (CWC). Various methods are reported in literature for the analysis of chemical warfare agents (CWAs), their precursors, and also their degradation products,[1] but only one has been presented as an analytical procedure capable of detecting and quantifying only the simple diols.[2] Although most of the methods present a complete scheme for the analysis of CWAs, their precursors and also their degradation products, no HPLC method[2] has been reported for the analysis of higher homologues of diols of sulfur mustard. The method reported by Raghuveeran et al.[2] is not capable for the simultaneous detection of dithiaalkane diols having the common formula $[(HO-CH_2-CH_2-S)_2(CH_2)_n]$ in a homologues series with $n = 1-10$; because of higher molecular weights, these diols have insufficient volatility for direct GC analysis. In a few cases, derivatives have been used to improve the chromatographic property of less volatile agents,[3] however, derivatization can be a major source of error in quantitative chromatographic analysis. Common problems are extraneous materials extracted from the matrix either suppressing derivatization or reacting with the derivatizing agent, producing a complex background. Many derivatizations of polar analytes require concentrations of aqueous solution to be evaporated to dryness. Not only can this be the time limiting factor in the analysis, it can also be a major source of error. Analyte isolation and clean up must be appropriate to the derivatization method used. There is, therefore, a real need for an analytical procedure capable of detecting and quantifying the individual diols in various matrices, in order to assess their potential threat to human and animal health. In this paper, we describe a reversed-phase high performance liquid chromatographic (HPLC) method for the qualitative and quantitative determination of the individual diols. The method is rapid, simple, and specific for the detection of dithiaalkane diols.

## EXPERIMENTAL

### Instrumentation and Materials

The HPLC instrumentation consists of a Waters 600E pump, a Rheodyne injector with 5 μL loop, and Waters 486 tunable UV detector. A Waters

Symmetry C18 ($4.6 \times 150\,mm^2$, $5\,\mu m$) column was used for analysis. The detector was tuned at 200 nm. HPLC grade methanol was procured from S.D fine chemicals, India. Water was triple distilled from an all glass distillation apparatus. The aqueous methanol mobile phases were prepared by filtering the solvents through $0.45\,\mu$ membrane filters. The series of dithiaalkane diols [$HO-CH_2-CH_2-S-(CH_2)_n-S-CH_2-CH_2-OH$, $n = 1-6$, 8, and 10] were prepared in our laboratory, as per procedures available in literature.[4,5]

## HPLC Method Development

### Simultaneous Detection of Dithiaalkane Diols

Isocratic elutions were performed using mobile phases A (Water : MeOH, 80 : 20 v/v) and B (Water : MeOH, 50 : 50 v/v). The latter on a gradient run was carried out with elution program 0–100% of B in A in 40 min (linear); 100–0% of B in A in 10 min (linear). Mobile phases were prepared 12 hr before and degassed in an ultrasonicator immediately before the experiment.

Samples of dithiaalkane diols were prepared by dissolving 0.5–1 mg of compound in 1 mL of HPLC grade methanol. Each sample of $5\,\mu L$ was injected separately. A mixture sample was prepared by mixing $10\,\mu L$ of each sample solution and then injected into the HPLC.

## Estimation of Thiodiglycol and 1,2-*bis*(2-Hydroxy Ethyl Thio)Ethane in Soil

In another experiment, approximately 50 mg of sulfur mustard (HD) and 50 mg of sesquimustard (Q) each were spiked in 1 gm of soil samples (pH 7.61, moisture content 14%) separately, taken in two 50 mL glass tubes. The soil was pulverized before spiking. After spiking, the samples were kept in stoppered glass tubes at 30°C for 21 days. A blank soil sample was also kept for the same duration. After 21 days, all soil samples were extracted with 10 mL of dichloromethane followed by centrifugation. The supernatants were collected and evaporated to dryness followed by reconstitution with $250\,\mu L$ of triple distilled water. Calibration curves for thiodiglycol and 1,2-*bis*(2-hydroxy ethyl thio)ethane, the degradation products of sulfur mustard (HD) and sesquimustard (Q), respectively, were drawn by using the above HPLC method (Figs. 1 and 2).

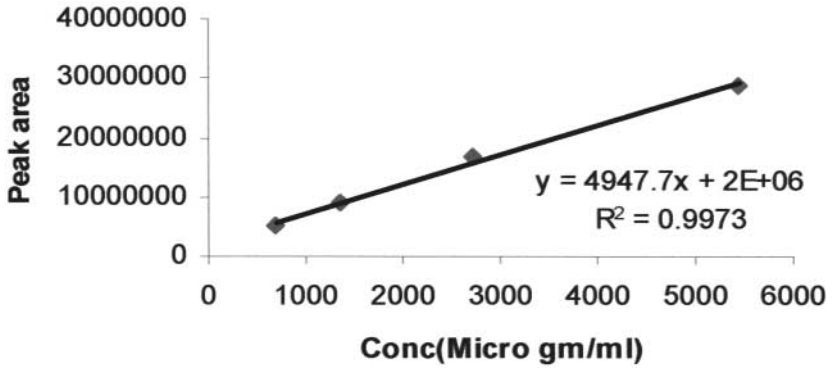



***Figure 1.*** Calibration curve for thiodiglycol, degradation product of sulfur mustard (HD).



## RESULTS AND DISSCUSSION

Sulfur mustard (HD) ($S(CH_2CH_2Cl)_2$) and its higher homologues of general formula $[(Cl-CH_2-CH_2-S)_2\ (CH_2)_n]$ where $n = 0.1-6$, have been found to be the decided vesicant (blistering action) to humans.[4] 1,2-di(2-chloro ethylthio) ethane (sesquimustard-Q), the third member of the series, has vesicant power approximately five times that of sulfur mustard (HD).[5] The corresponding dihydroxy compounds having the general formula $[(X-CH_2-CH_2-S)_2(CH_2)_n]$ where X = OH, are the precursors for producing

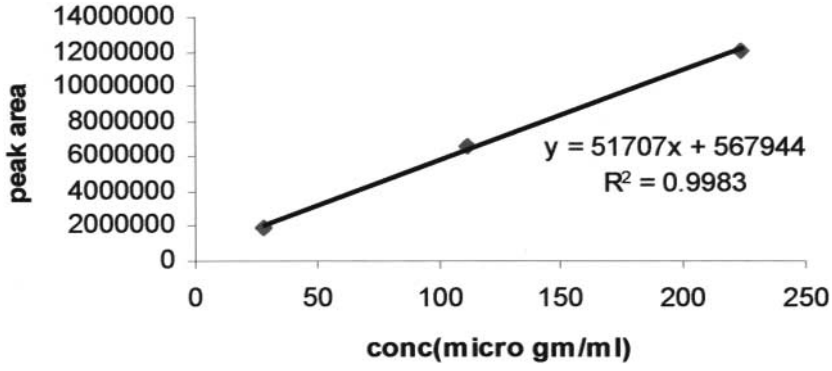



***Figure 2.*** Calibration curve for 1,2-*bis*(2-hydroxy ethyl thio)ethane, degradation product of sesquimustard (Q).

the above-mentioned potential vesicants.[5] These long chain diols, e.g., 3,6-dithia-1,8-octanediol and thiodiglycol results from the hydrolysis of sesqui-mustard (Q) and sulfur mustard (HD), respectively, in environmental and biological matrices.[3]

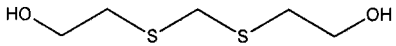
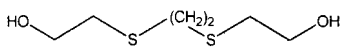
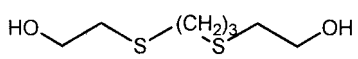
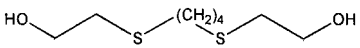
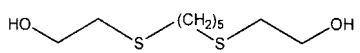
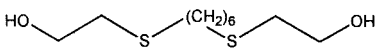
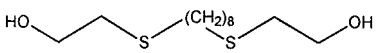
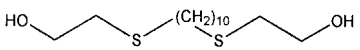
## Simultaneous Detection of Dithiaalkane Diols

Retention times of different dithiaalkane diols are given in Table 1 for different elution methods. From here, the codes given in Table 1 will be used for different compounds instead of their names for subsequent discussion. It was observed that the retention time of dithiaalkane diols increases with an increase in the number of $-CH_2$-units in the compound. Significant increments of retention time was observed only after the compounds contained three $CH_2$ units. Better resolution between compound 1, 2, and 3 were achieved when elution was carried out with mobile phase A. This happened due to the solubility of dithiaalkane diols decreased in the mobile phase containing a lower fraction of MeOH. In mobile phase A, MeOH content is just 20% by volume. Hence, it's solvation capacity for dithiaalkane diols was lower than mobile phase B (MeOH 50% by volume). Due to the poor solvation capacity of A, resolution between compounds 1, 2, and 3 were achieved, while compounds 7, 8, and 9 were not eluted from the column, even after 120 min of run. When mobile phase B was tried all compounds were eluted within 40 min of elution time, but the resolution between compounds 1, 2, and 3 were lost. To overcome such difficulty, the gradient run was developed involving both A and B. In this method, the solvation capacity of mobile phase was gradually increased by linearly increasing the contribution of B in the mobile phase. By this method resolution between compounds 1, 2, and 3 was protected, while all compounds were eluted within 50 min of run. Peak to peak retention time match was observed for individual components and components in the mixture (Fig. 3). A drift in baseline was observed as the run progressed and normalized again after re-equilibration. This could be attributed due to the increase in MeOH fractions in the mobile phase during the progress of the gradient, as MeOH absorbs significantly at 200 nm. Absorbance of mobile phase increased when MeOH composition increased, which was transformed as a drift in baseline.

## Estimation of Thiodiglycol and 1,2-*bis*(2-Hydroxy Ethyl Thio)Ethane in Soil

The pH of the soil was 7.61 with 14% moisture content. Retention times of thiodiglycol and 1,2-*bis*(2-hydroxy ethyl thio)ethane were 1.854 and

***Table 1.*** Retention time of dithiaalkane diols by different elution methods.

| Samples dithioalkane diols | Sample name | Sample code | Retention time (min) | | |
|---|---|---|---|---|---|
| | | | Isocratic A | Isocratic B | Gradient |
|  | *bis*(2-Hydroxy ethyl thio)methane | 1 | 3.997 | 2.961 | 4.157 |
|  | 1,2-*bis*(2-Hydroxy ethyl thio)ethane | 2 | 4.629 | 3.024 | 4.728 |
|  | 1,3-*bis*(2-Hydroxy ethyl thio)propane | 3 | 6.292 | 3.204 | 6.381 |
|  | 1,4-*bis*(2-Hydroxy ethyl thio)butane | 4 | 10.016 | 3.574 | 9.952 |
|  | 1,5-*bis*(2-Hydroxy ethyl thio)pentane | 5 | 19.465 | 4.264 | 14.391 |
|  | 1,6-*bis*(2-Hydroxy ethyl thio)hexane | 6 | Not eluted | 5.558 | 21.451 |
|  | 1,7-*bis*(2-Hydroxy ethyl thio)octane | 7 | Not eluted | 12.547 | 35.280 |
|  | 1,10-*bis*(2-Hydroxy ethyl thio)decane | 8 | Not eluted | 36.609 | 49.269 |

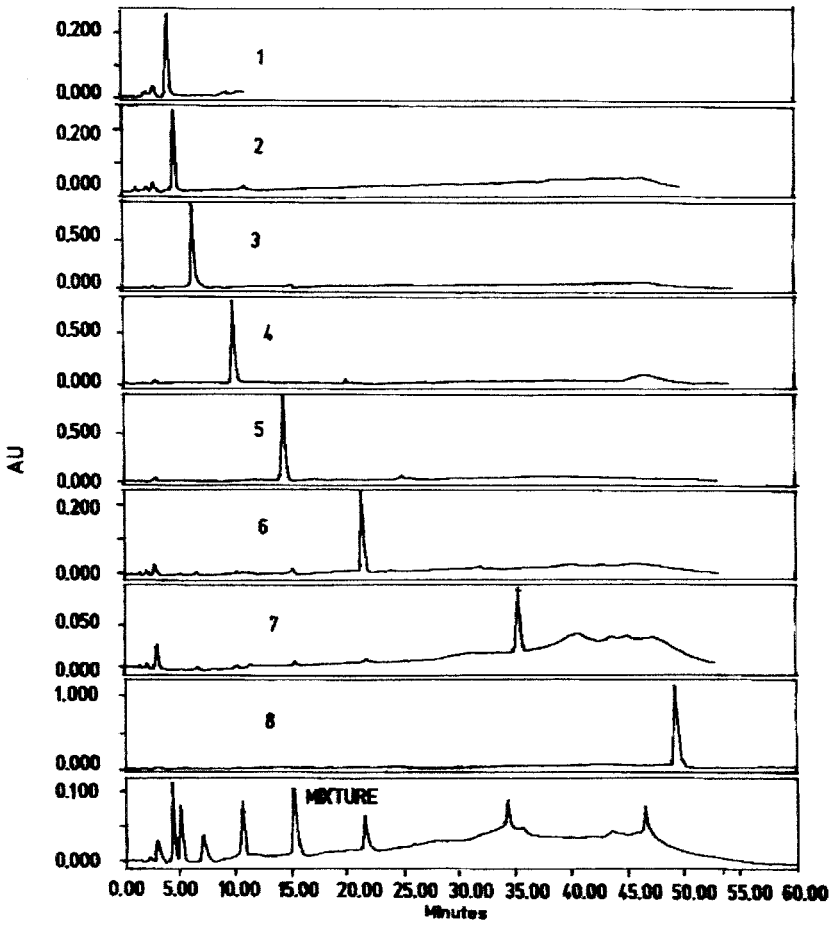



*Figure 3.* Chromatograms of dithiaalkane diols in the gradient elution method.

4.378 min, respectively, (Figs. 4 and 5). Thiodiglycol and 1,2-*bis*(2-hydroxy ethyl thio)ethane were detected in the soil samples spiked with sulfur mustard (HD) (Fig. 6) and (Q) (Fig. 7), respectively. The peaks were identified by comparison with standard samples (Figs. 4 and 5) and blank soil samples (Fig. 8). After quantitative estimation of thiodiglycol and 1,2-*bis*(2-hydroxy ethyl thio)ethane from soil samples by comparing the calibration curve, it was calculated that 0.3 mol% of HD and 0.03 mol% of Q were degraded to their respective diols in 21 days in those particular soil samples. The reason for very low degradation of sesquimustard and sulphur mustard may be attributed to their hydrophobicity. Solubilities of HD and Q in water are 800 mg/L

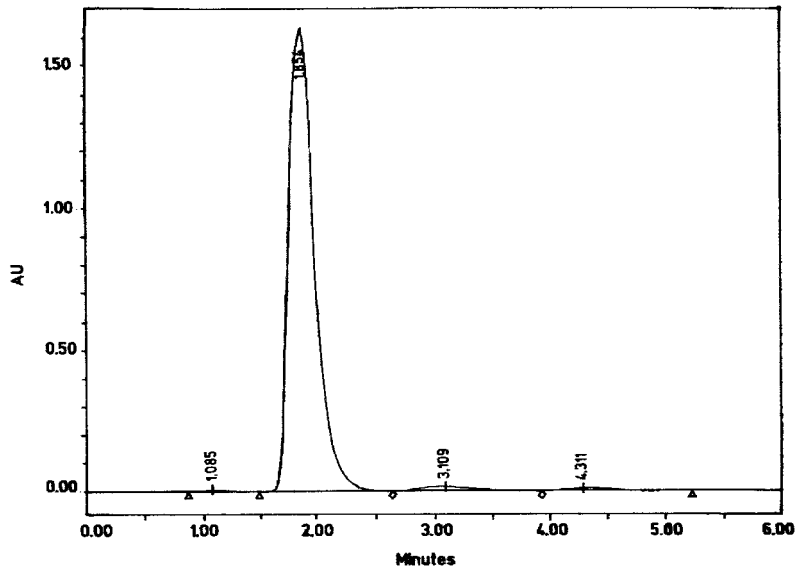



*Figure 4.* HPLC profile of thiodiglycol (retention time, 1.854).

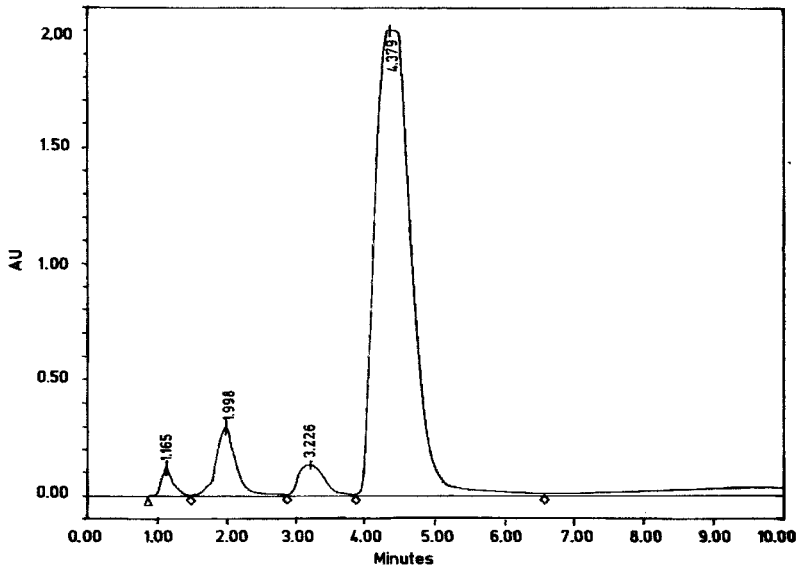



*Figure 5.* HPLC profile of 1,2-*bis*(2-hydroxy ethyl thio)ethane. (retention time, 4.379).

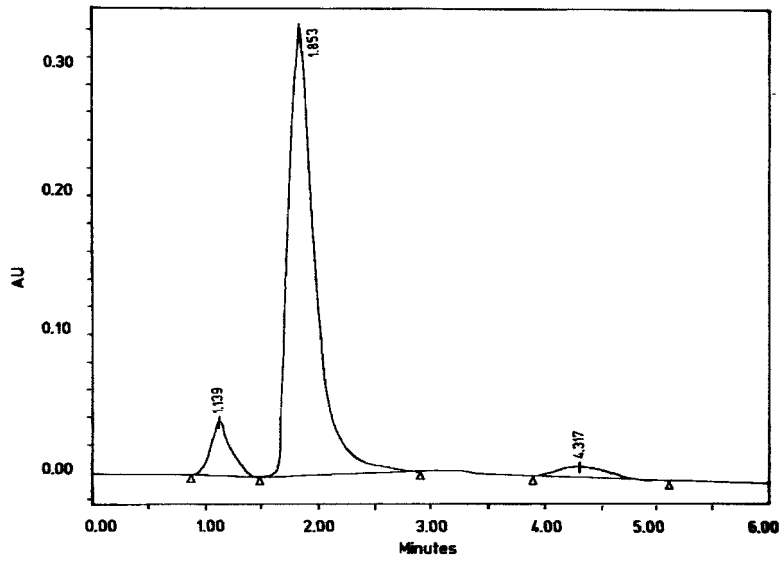



*Figure 6.* HPLC profile of sample obtained from soil spiked with sulphur mustard (HD).

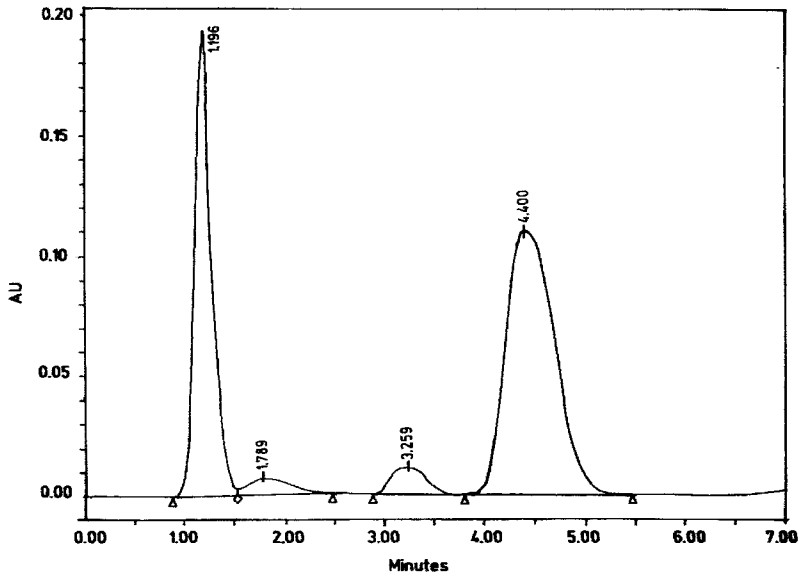



*Figure 7.* HPLC profile of sample obtained from soil spiked with sesquimustard (Q).

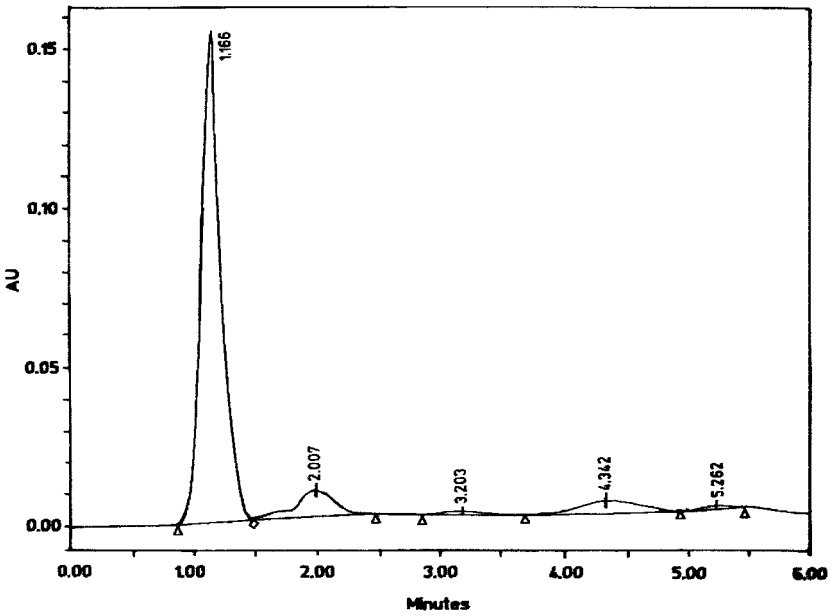



*Figure 8.* HPLC profile of blank soil sample.



and 25 mg/L, respectively.[6] As Q is more hydrophobic than HD, it degraded to a lesser extent in comparison to sulphur mustard.

## CONCLUSION

The proposed gradient HPLC method for simultaneous detection of dithiaalkane diols provides excellent resolution. Quantitative degradation studies of corresponding mustards can also be carried out using this HPLC method simultaneously, without going for derivatization.

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