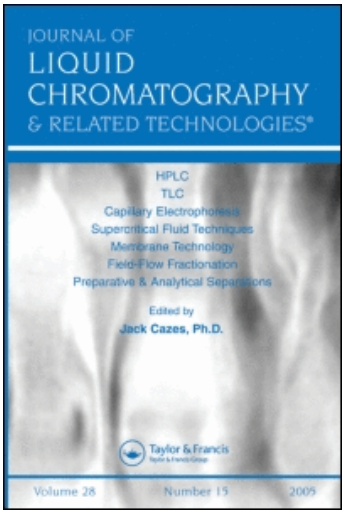

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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C. D. Raghuveeran[a]; R. C. Malhotra[a]; R. S. Dangi[a]
[a] Defence Research and Development Establishment, Gwalior, India

To cite this Article Raghuveeran, C. D. , Malhotra, R. C. and Dangi, R. S.(1993) 'Reversed-Phase High-Performance Liquid Chromatography of Sulphur Mustard in Water', Journal of Liquid Chromatography & Related Technologies, 16: 7, 1615 — 1624

To link to this Article: DOI: 10.1080/10826079308020977

URL: http://dx.doi.org/10.1080/10826079308020977

JOURNAL OF LIQUID CHROMATOGRAPHY, 16(7), 1615-1624 (1993)

REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF SULPHUR MUSTARD IN WATER

C. D. RAGHUVEERAN, R. C. MALHOTRA, AND R. S. DANGI
Defence Research and Development Establishment
Tansen Road, Gwalior - 474 002, India

ABSTRACT

A reversed-phase high-performance liquid chromatography method for the detection and quantitation of sulphur mustard (HD) in water is described with detection at 200 nm. The detection based on the solubility of HD in water revealed that extremely low quantities of HD (4 to 5 mg/L) only are soluble.

INTRODUCTION

Sulphur mustard [HD, 1,1'-thiobis(2-chloro ethane] is a powerful vesicant and a potent chemical warfare agent. It had been used in the first world war and was employed more recently in the Iran-Iraq war. Various analytical methods like thin layer chromatography (TLC), gas chromatogrphy (GC) and GC-Mass Spectrometry (GC-MS) are availble for the detection and

quantitation of HD. The last two analytical techniques are the most commonly used because of their sensitivity. But they involve tedious and time consuming sample work-up steps resulting in sample losses. For purposes of enforcement amd verification of the compliance of the proposed chemical disarmament convention, it will always be necessary to monitor the effluents from production units alleged to be producing HD; it will be aqueous solutions which will have to be monitored and none of the existing methods can be expediently used for the purpose. Reversed-phase HPLC with UV detection is the best suited for the detection of HD in aqueous samples which can be directly injected into the chromatograph and detected without any sample extraction and derivatisation steps.

HPLC methods are available for the analysis of certain analogues of HD. These are due to Bossle et al. (1) and Hallowell et al. (2). Both groups reported reversed-phase HPLC procedures for the detection of 2-chloroethyl ethyl sulfide (CEES) and 2-hydroxy ethyl ethyl sulfide (HEES). Bossle et al. (1) reported a derivatization procedure in which the HD analogues were converted to phenyl sulfonyl sulfylimine derivatives with chloramine-B and detected at 254 nm. Hallowell et al. (2) studied the hydrolysis of CEES to HEES and found UV detection at 200 nm preferable over electrochemical detection with platinum electrodes. But to this date there is no report of a HPLC procedure for the detection of HD with or without derivatisation. Derivatization of HD is difficult; e.g., chlormine-T, the classical reagent for derivatizing HD gives rise to a crystalline product but the reaction time is long and the yield is poor because of which the sensitivity is also low. Thus detection of HD without any derivatization appears to be the method of choice as far as HPLC is concerned.

We report here a reversed-phase HPLC method for the detection and quantitation of HD in water in which aqueous samples containing HD are directly injected into the column (a guard column is necessary). Effluent waters from bench top

synthesis of HD have also been analysed with this method. Our experience shows that water is still the medium of choice for the analysis of HD in water and aqueous effluents in spite of the minor handicap of its half life of ca. 4 minutes, which only calls for speedy analysis.

MATERIALS AND METHODS

Instrumentation

The HPLC instrumentation consisted of a Shimadzu LC6A pump, a Rheodyne model 7125 injector fitted with a 10ul loop, a Shimadzu SPD 6AV UV-Visible spectrophotometric detector. The stationary phase was Polygosil C-18 (5u) packed in a 25 cm x 4 mm ID stainless steel column, both procured from M/S Machery Nagel,Germany. The column was packed in the upward displacement mode using a Shimadzu LC 5A pump at a pressure of 420 kg/cm2 and a Micromeritics Model 705 stirred column packer with a mixture of dioxane-toluene-cyclopentanol as the slurrying solvent and methanol as the packing solvent. The column had an efficiency of 35000 theoretical plates/m. A RCS-C18 cartridge column (5u; 10cm x 5mm I.D., from Waters Associates, U.S.A), had been used for preliminary work including the analysis of effluent waters from bench top synthesis of HD.

Chemicals

HPLC grade methanol and dichloromethane were procured from E.Merck, India. Water was quadruple distilled from an all glass distillation apparatus. The aqueous methanol mobile phase was prepared by filtering the solvents individually over 0.45 u membrane filters (cellulose acetate and PTFE). HD, 99% pure was prepared as per procedures available in the literature (3).

The spectral data for HD are : m/e 158, 111, 109. IR (cm^{-1}) : aliphatic, (-H) 2960; CH_2S, CH_2Cl: 1400; CH_2Cl, CH_2S: 1290, 1275; CCl: 700; proton NMR ($CDCl_3$) 3.650, 2.2´proton : 2,923, 1.1´ proton.

Chromatography

The mobile phase for the Polygosil C18 column was 63% methanol and for the RCS-C18 cartridge, 75% methanol. The flow rate for both solvents was set at 1.5ml/min. The mobile phase was thermostated at 37 ± 0.02^{o} C by circulating water from a Julabo constant temperature water bath (M/S Siskin, India) along with an Optilab 5814 Thermostir Eluent System of M/S Tecator, Sweden. The eluent from the reservoir was passed through an online deagasser (Erma, Japan). These precautions were necessary for obtaining a steady base line at 200 nm and highest sensitivity, viz., 0.004 au for the detection of HD in the 4 to 10 ng range.

Standards for the calibration of HD in the 10 to 100 ug/10 ml were prepared in 30% methanol by diluting suitable aliquots of a HD stock solution, 10 mg/ml in methanol. 10ul of the standard was injected.

Studies on the detection of HD in water were performed with films of 10 to 200 ug left at the bottom of 10 ml graduated, stoppered test tubes by evaporation from a dichloromethane solution of HD. The film of HD was overlaid gently with 10 ml water without disturbing the film and the aqueous phase (10ul) injected within 25 seconds. Experiments to simulate the generation of a saturated solution of HD were performed with 7.62 mg of HD (neat, 6ul) in 10 ml water. The contents were vigorously mixed and centrifuged for separation of HD and water as two phases. 10 ul of the aqueous phase was injected.

The effluent waters from the bench top synthesis of HD were analysed after diluting 0.1 ml of the effluent to 10 ml with 30% methanol. 10ul of the diluted effluent was injected.

RESULTS AND DISCUSSION

The HPLC procedure described here was designed for the sensitve detection and quantitation of HD in its native state in aqueous media. Fig.1 shows the chromatogram of HD in 30% methanol. The detector response for HD in this solvent was optimum. The calibration plot for HD is linear in the 10 to 100 ng range (Fig.2, upper trace). Fig.3 shows the chromatogram of HD, 40ug/10ml water by the gentle overlay procedure.

Our main objective in this study was to estimate HD dissolved in aqueous samples and since the detection is based on the solubility of HD in water, our first task was to check the solubility of HD in water. The values reported in the literature for the aqueous solubility of HD in water varies from 0.7 to 1g/L. We observed that the aquoues solubility of HD was very much lower than the reported minimum value of 0.7g/L, determined originally by Hopkins (4) in 1920 using acidimetric and argentimetric methods of analysis of HD contained in the aqueous phase of a saturated solution in water. A simulation of this experiment in replicates was performed on a smaller scale for the detection by HPLC. 7.2 mg HD spiked in water (10 ml) was given a thorough shake followed by centrifuging for 2 to 3 minutes and the aqueous phase injected. Fig.4 shows the chromatogram. The peak areas for HD in the replicates were not reproducible, indicating that the aqueous phase in such systems contained indefinite quantities of HD dispersed in the bulk phase as emulsions but not in solution. The HD measured in these emulsions are not constant and hence cannot form the basis for the determination of aqueous solubilty of HD.

Our approach to determine the solubility of HD in water is based on the principle that we detect by HPLC only that portion of HD which goes into solution in water. Since with HPLC-UV detection it is possible to detect minimum 5 ng of HD, quantities of HD lower than the aqueous solubilty could be detected conveniently and directly, that is, without any other

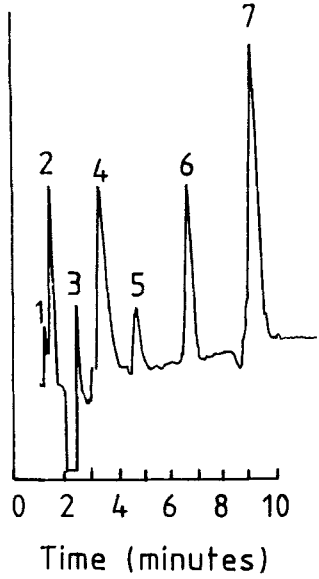


FIGURE 1. Chromatogram of HD in 30% methanol. column : Polygosil C18 (5u); mobile phase : methanol-water (63/37); flow rate, 1.5ml/min; detection: uv, 200nm; sensitivity, 0.004 au; peak identity: peak No.6, HD; others: due to injection solvent.

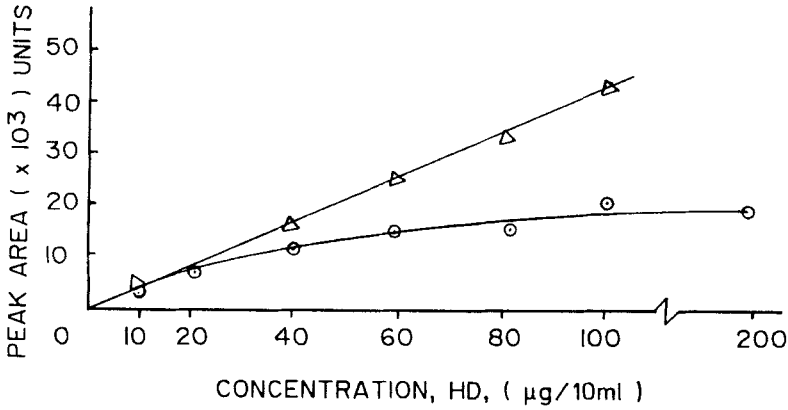
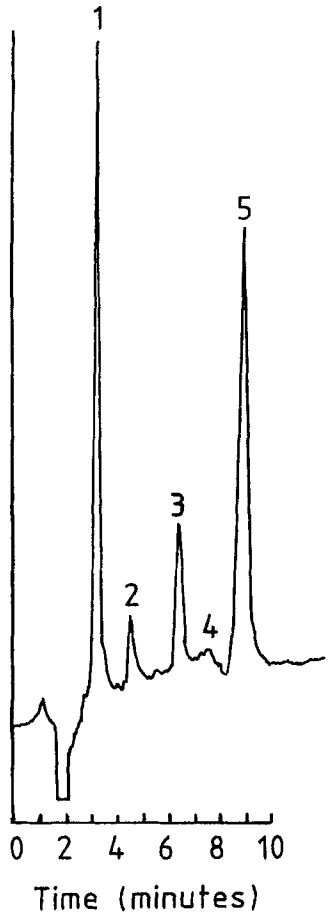


FIGURE 2. Calibration plot of HD in 30% methanol (Δ) and in water (o).



FIGIURE 3. Chromatogram of HD in water. Conditions as in Fig. 1. Peak identity: 1. dichloromethane. 2, 4 & 5, from water used in the experiment(see text).

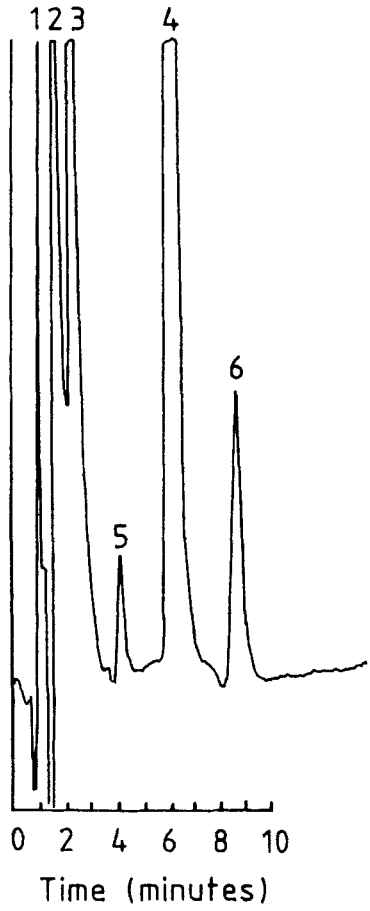


FIGURE 4. Chromatogram of HD, 7.2 mg in water (10 ml) after vigorous shaking followed by centrifuging (seetext). Conditions as in Fig. 1. Peak identity: 1. chloride (Cl^-). 2. thiodiglycol. 3. labile intermediate. 4. HD. 5 & 6, unknown, from water.

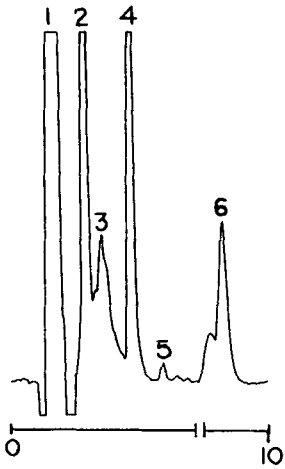


FIGURE 5. Chromatogram of HD in effluent water from bench top synthesis of HD. Conditions : column, Waters RCS-C18 (5u); mobile phase, methanol-water, (75/25); flow rate: 1.5ml/min; other conditions as in Fig.1. Peak identity - See text.

calibration. The detectability of HD in the range 10 to 40 ug (equivalent to 1 to 4 mg/L) is linear and from 40 to 200 ug/10ml is constant (Fig.2, lower trace). So 40 ug of HD in 10ml (equivalent to 4mg/L) is the true solubilty in water.

Fig.5 shows the monitoring of HD in aqueous effluents from bench scale synthesis of HD. The peak at 4.667 min is that of HD while those at 1.82 and 2.9 min were identified as chloride anion and thiodiglycol respectively. The peak at 3.5 min is due to some unknown labile substance, probably a product of hydrolysis of HD acting as an intermediate in the final hydrolysis of HD to thiodiglycol. The presence of these substances did not interfere with the detection of HD. The method repoted here for the detection of HD by reversed-phase HPLC with detection at UV, 200nm is simple and fast (which is a

prerequisite to detect HD before it hydrolyses to thiodiglycol). It can be used for detection of HD in aqueous samples. Further, it can also be applied for the determination of HD in biological samples using solid phase extraction methods to isolate HD. Work is currently in progress with this end in view.

ACKNOWLEDGEMENTS

We are grateful to Dr.R.V.Swamy, Director, Defence Research & Development Establishment, Gwalior and Dr. P.K. Ramachandran, Scientist Emeritus, DRDO for the interest evinced by them in this study.

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Received: April 18, 1992
Accepted: September 4, 1992