APPLICATION OF HPLC FOR THE ANALYSIS OF MALATHION AND S-MUSTARD MIXTURE

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ABSTRACT

A method was developed for the chromatographic separation and quantification for a mixture of Malathion (as organophosphorus nerve agent), and S-mustard (as blister agent). The method used a high performance liquid chromatography (HPLC) with reversed phase C-18 column, and UV detection at 220 nm. The compounds were separated using isocratic elution acetonitrile-water (50%) at a flow rate of 0.7 mL/min in a period of 5 min. The retention times were (1.939) min for S-mustard and (3.063) min for Malathion. The calibaration curves were linear (R^2 = 0.999) in the studied concentration range (200 to 1000 µg/mL). Sensitivity of the method for quantification of Malathion was found to be higher than that of S-mustard by four times. In addition, the accuracy was found to be (~ 97%) for both.

KEY WORDS

Malathion, S-mustard , HPLC analysis,

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INTRODUCTION

Detection of chemical warfare agents (CWAs) in times of war or in acts of terrorism requires rapid and reliable methods. In recent years there has been a growing interest in the analysis of CWAs as well as their precursors, manufacturing byproducts and degradation products. This is mainly because of the coming into force of the Chemical Weapons Convention (CWC), which prohibits the development, production, stockpiling and use of CWAs [1]. Analysis of CWAs and related compounds may play a key role in the verification of the treaty; the destruction of existing stockpiled CWAs has to be carefully followed by a special analytical procedures.

The most significant chemical warfare agents in terms of military capacity and past use are the nerve and blister agents [2].

The common chemical warfare agents have varying degrees of volatility and dose, both a vapor hazard as well as a liquid contact hazard. This physical characteristic has made the analysis of chemical warfare agents amenable to the analytical techniques commonly employed for most environmental analyses, namely gas chromatography (GC) and liquid chromatography (LC) with a variety of detectors including mass spectrometry (MS) [3-5].

High pressure liquid chromatography (HPLC) can be employed not only for separation but also for identification, of different compounds by comparing the retention time of the unknown compound to that of a known one (standard) under the same operating conditions. However, HPLC technique can be used as qualitative and quantitative method of identification of organic compounds through retention time and peak area respectively [6].

EXPERIMENTAL WORK

Chemicals.

S-mustard (HD) was prepared in laboratory by Clark's method [7] and then it was purified by vacuum distillation. The obtained product has about 95% purity. Malathion 95% was obtained from (Nasr Company for intermediate chemicals). Ethanol 95% (UCCMA), acetonitrile HPLC grade (Aldrich), deionized water HPLC grade (Fluka).

INSTRUMENTATION

HPLC Agilent 1100 series equipped with auto sampler. HPLC column (150 mm × 2.1 mm ID) reversed phase Zorbax SB C-18 column with guard column. HPLC detector: UV-Vis diode array detector (DAD). All system parameters can be automatically controlled through HP 3DLC Chemistation software.

Operating Conditions

Mobile Phase**:**	Acetonitrile (50%) – Water (50%)**.**
Flow rate:	0.7 mL / min.
Elution:	Isocratic
Injection volume:	10 µl.
Detector:	UV Detector at 220 nm.
The stop time:	5 min.
Column temperature:	35 ºC

Stock And Standard Solutions

A stock solution of (8.12 mg/mL) S-mustard in ethanol 95% was obtained by dissolving 406 mg of S-mustard in 50 mL of ethanol. This solution was used as external standard for determination the retention time of S-mustard. The chromatogram of S-mustard is shown in Fig. (1).

A stock solution of (10 mg/mL) Malathion was prepared by dissolving 100 mg of Malathion in 10 mL of ethanol 95%. By accurate dilution 1 mg/mL solution of Malathion in ethanol was prepared. This solution was used as external standard for determination of the retention time of Malathion. The chromatogram of Malathion is shown in Fig. (2).

A stock solution of (2 mg/mL) S-mustard in ethanol was obtained by dissolving 20 mg of S-mustard in 10 mL of ethanol .

A stock solution of (2 mg/mL) Malathion in ethanol was obtained by dissolving 20 mg of Malathion in 10 mL of ethanol.

A mixture of S-mustard and Malathion was prepared by mixing 5 mL of 2 mg/mL S-mustard solution in ethanol with 5 mL of 2 mg/mL Malathion solution in ethanol. The obtained mixture is of 1 mg/mL concentration for S-mustard and Malathion. This mixture was used for preparation the calibration standards. The chromatogram of the mixture of S-mustard and Malathion is shown in Fig. (3).

Calibration Curves

For quantitative analysis of S-mustard and Malathion in the mixture, five standards were prepared as follows:
Different volumes (0.2, 0.4, 0.6, 0.8 and 1 mL) of the 1 mg/mL mixture solution were delivered into 2 mL caped vials and completed to 1 mL with ethanol. The obtained calibration standards were containing concentrations of (200, 400, 600, 800 and 1000 µg/mL). Each calibration standard was analyzed by HPLC, and the peak area of S-mustard and Malathion was plotted for each standard against concentration. Two calibration curves are obtained, the first for S-mustard Fig. (4) and the second for Malathion Fig. (5).

Sensitivity And Accuracy Of The Method

Sensitivity of this method is considered as the value of the slope of the calibration curve which is shown in Fig. (4) for S-mustard, and the value of the slope of calibration curve which is shown in Fig. (5) for Malathion.

For determination of the accuracy of this method, a mixture of S-mustard and Malathion of concentration 500 µg/mL was prepared and quantitatively analyzed by HPLC at the same operating conditions, and then the accuracy was calculated[6].

RESULTS AND DISCUSSION

Chromatogram

The chromatogram shown in Fig. (3) contains two main peaks which are separated with good resolution. The first peak located at retention time 1.939 min. which represents the S-mustard and the second peak located at retention time 3.063 min. which represent the Malathion. These qualitative identifications were done by comparing retention time with that of a standard under the same operating conditions as shown in Fig. (1,2). The peak located at retention time 1.547 min represents the impurities of Malathion. The peak located at retention time 0.886 min represents thiodiglycol which is the hydrolysis product of sulphur mustard. The peak located at retention time 0.565 min represents the ethanol which is the used solvent. S-mustard and Malathion were separated with good resolution as it is shown in Fig. (3).

Calibration Curves

For quantitative analysis of the mixture of S-mustard and Malathion, two calibration curves were constructed as described before, the first for S-mustard, Fig. (4) and the other for Malathion, Fig. (5). The two calibration curves have good linearity (R^2 = 0.999) over the studied concentration range (200-1000) µg/mL.

Sensitivity And Accuracy

The comparison between the slope values on the concentration range (200-1000) µg/mL for the two calibration curves show that, the sensitivity of quantification of Malathion by this analytical method is higher than that of S-mustard by four times.

The accuracy was determined by quantitative evaluation of a known concentration, of S-mustard and Malathion mixture. The mixture of S-mustard and Malathion has the concentration of 500 µg/mL for both. The measured peak areas were (956) for S-mustard and (4556) for Malathion. By using the constructed calibration curves, the obtained concentrations were (514.34 µg/mL) for S-mustard and (515.85) for Malathion which give accuracy (~ 97%).

CONCLUSION

An HPLC method was developed for the analysis of S-mustard and Malathion. The constructed calibration curve using this method was linear (R^2 = 0.999) over the studied concentration range (200 and 1000 µg/mL). The sensitivity of quantification of

Malathion by this method is higher than that of S-mustard by four times. The accuracy of this method was found to be (~ 97%) for both.

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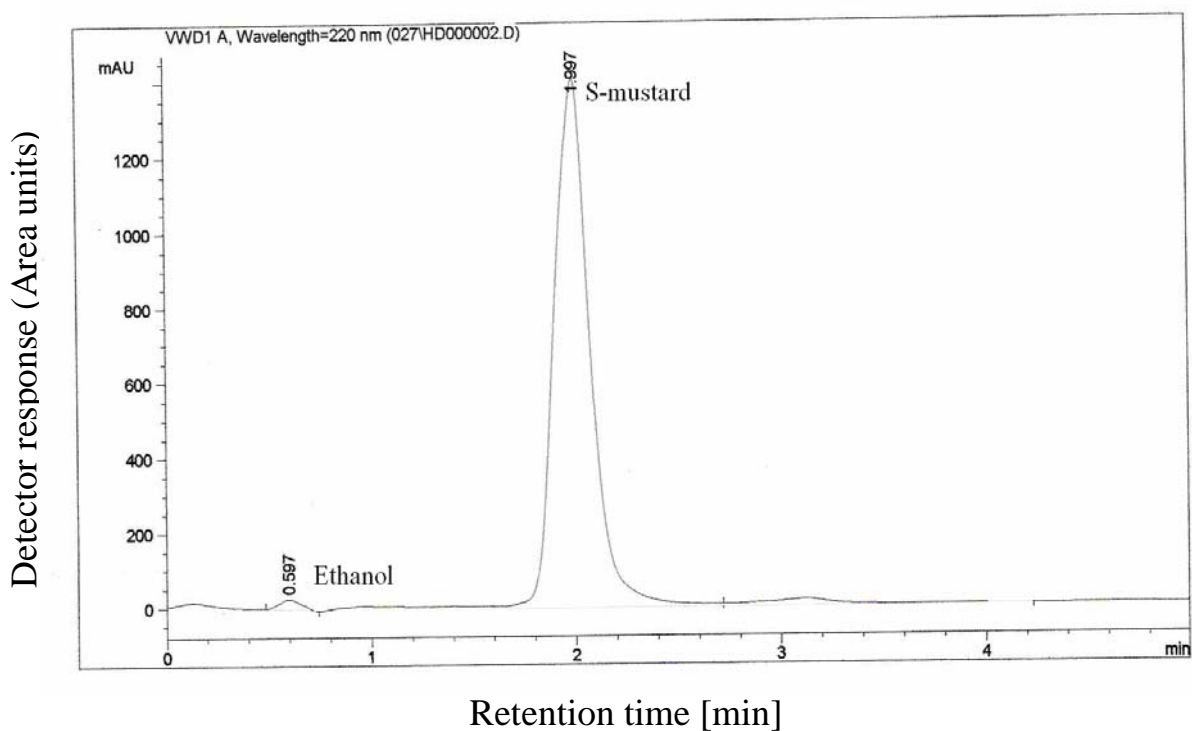[7] Sartory A., "Chemistry and Analysis of War Gases" Italy, 1940.
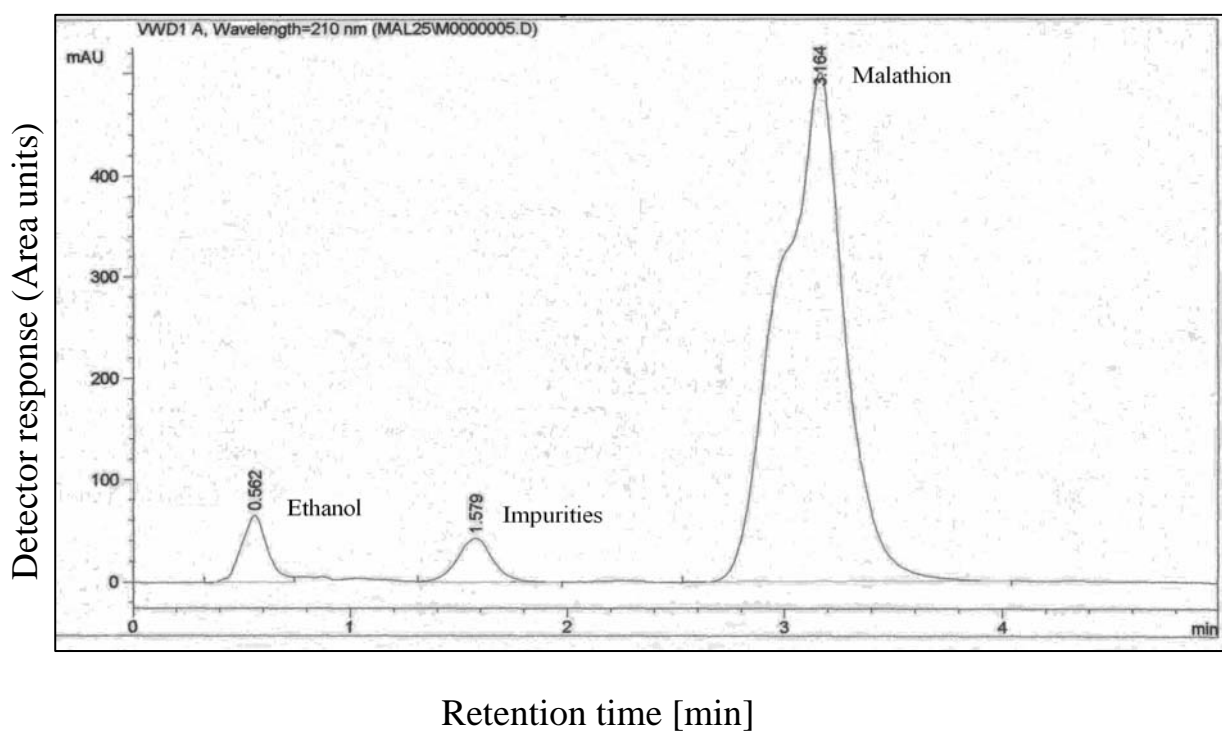
Fig.. (1) HPLC chromatogram of purified S-mustard

Retention time [min]

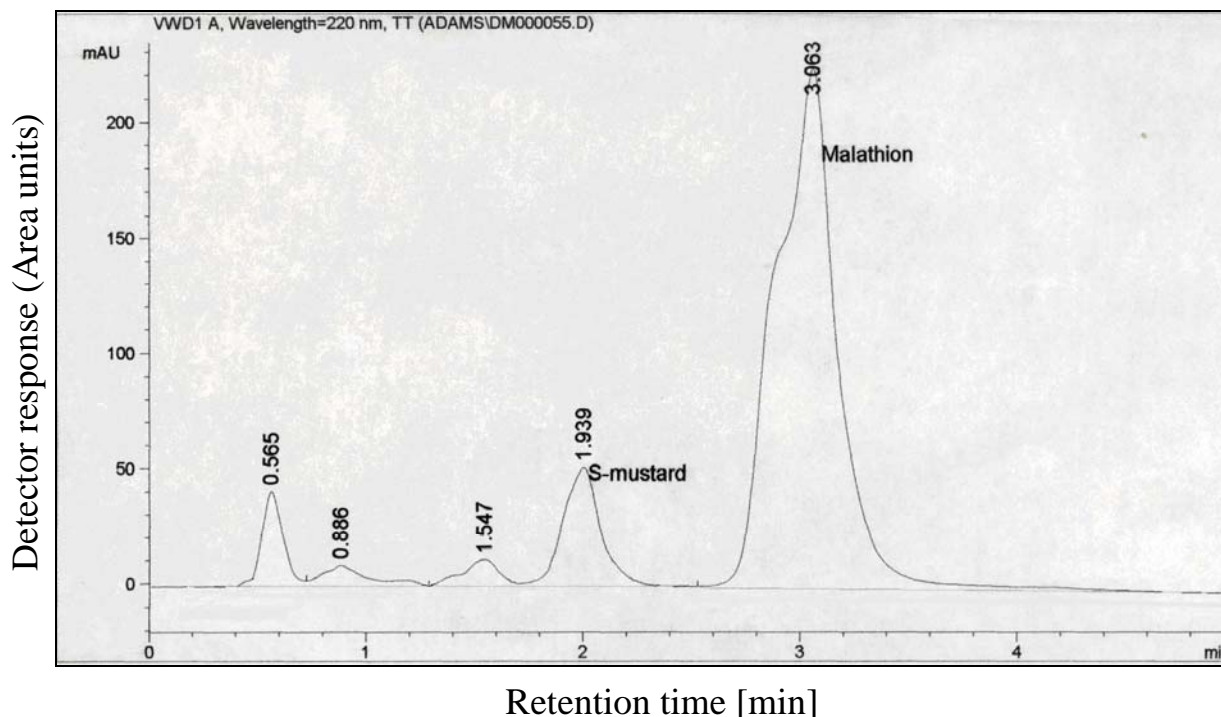Fig. (2) HPLC chromatogram of Malathion.

Retention time [min]

Fig.. (3) HPLC chromatogram for a mixture of S-mustard and Malathion.
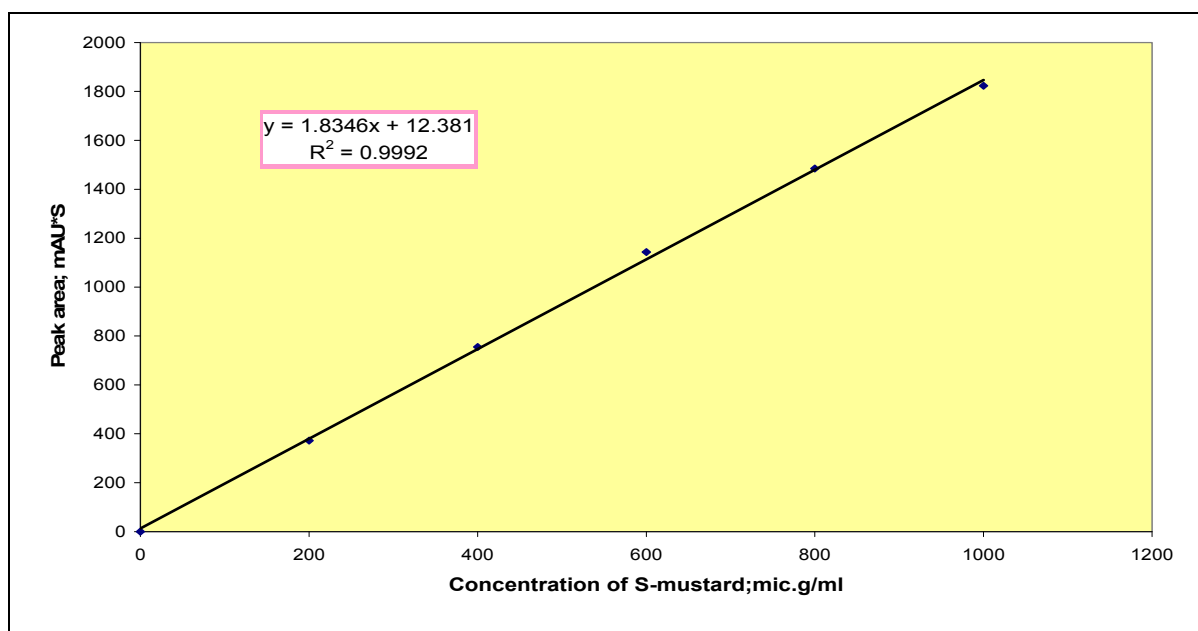

Fig.(4) Calibration curve of S-mustard in ethanol obtained over the concentration range (200-1000) μg/mL using HPLC
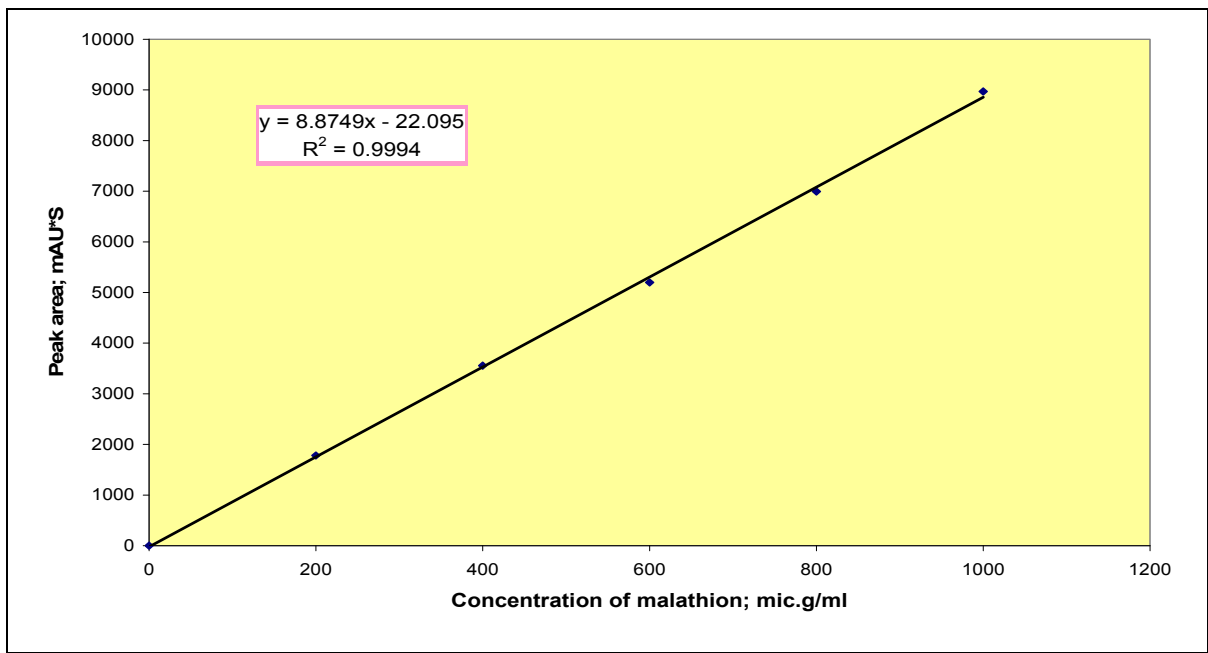
Fig.(5) Calibration curve for Malathion in ethanol obtained over the concentration range (200-1000) µg/mL using HPLC
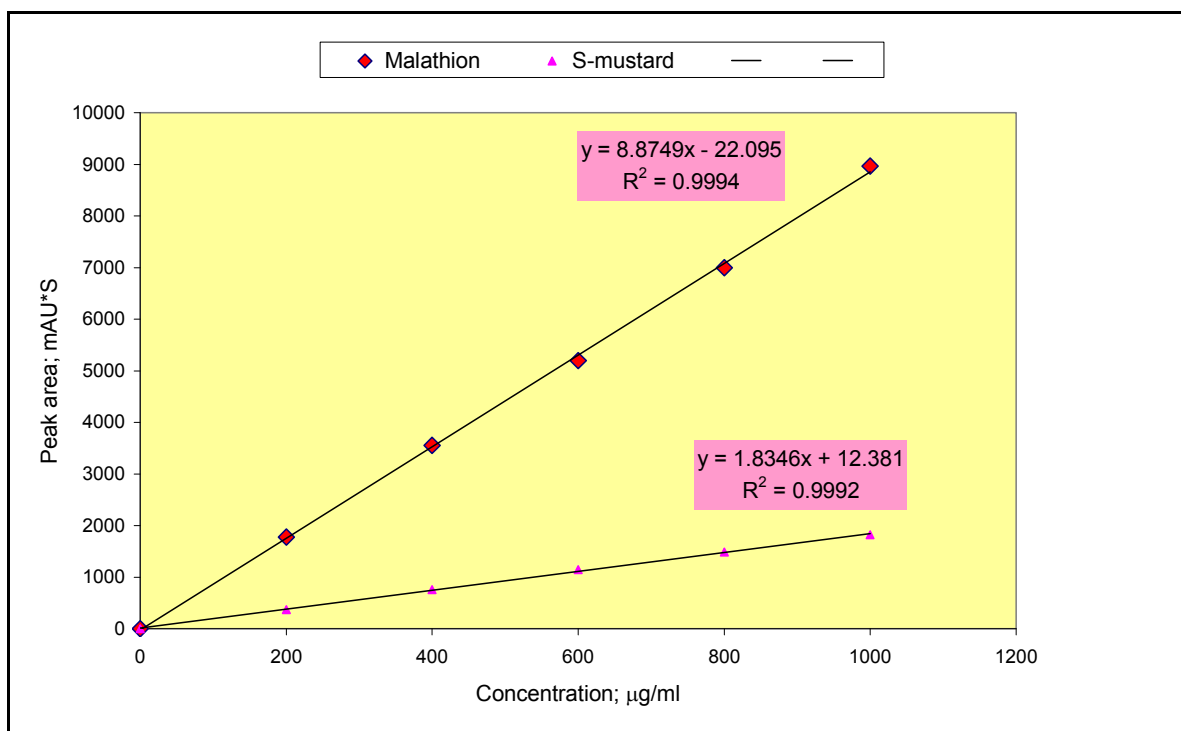

Fig.(6) Calibration curve for both Malathion and S-mustard in ethanol obtained over the concentration range (200-1000) µg/mL using HPLC.