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SPECTROPHOTOMETRIC AND GAS CHROMATOGRAPHIC METHODS FOR DETECTION AND ASSAY OF MUSTARD GAS

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ABSTRACT

A colorimetric method for detection and assay of mustard gas in urine after addition of thymolphthalein is reported. The detection limit was 80 ng/ml of urine. Mustard gas could also be detected in urine after extraction with gas chromatography using FID detector. The detection limit was 10 ng/ml.

In addition, thiodiglycol, a metabolite of mustard gas, could be converted to mustard with concentrated HCl at 100°C and detected with gas chromatography.

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INTRODUCTION

Mustard gas, 2,2 -dichlorodiethyl sulfide was used for the first time in World War I by the German military forces during the battle of Ypres on July 2, 1917. Although the use of chemical warfare agents is prohibited by the Geneva protocal of 1925, nevertheless Iraqi forces have constantly used mustard and other chemical warfare agents against Iranian civilians and soldiers alike. The usage of chemical warfare agents by Iraqi forces has been confirmed by a team of experts of the United Nations in March, 1984.[1] In order to support the medical observations of patients which were contaminated with mustard gas, an investigation was carried out to identify mustard gas or its main metabolite, thiodiglycol (2,2 -thiodiethanol)[2,3] in the urine.

There exists no sensitive procedure to detect thiodiglycol in biological fluids. Extraction of thiodiglycol from water or urine is rather difficult. Therefore, thiodiglycol was converted into mustard gas by means of concentrated HCl at 100°.

Although gas chromatography-mass spectrometry is the method of choice for the identification of mustard gas, several other analytical procedures have been reported. Colorimetry,[4,5,6] thin layer chromatography,[7-9] gas liquid chromatography,[10-13] and high performance liquid chromatography[14] have been used for detection and assay of mustard gas.

This paper reports detection and assay of mustard gas in the urine by colorimetry and gas chromatographic methods.

EXPERIMENTAL

A: Colorimetric Method

All reagents were of analytical grade. Sodium hydroxide solution (C= 0.1 M) was prepared by dissolving 4.0g sodium hydroxide in water and accurate dilution to 1000ml. A 2×10^{-2} M thymolphthalein was prepared by dissolving 430mg thymolphthalein in 36ml ethanol

followed by the addition of 13ml 0.1 M NaOH and dilution to 50ml.

Preparation of Calibration Curve for Mustard in a Solvent:

Different volumes (0.1-1ml of the 5×10^{-5} M and 0.2-2ml of the 5×10^{-4} M) of mustard solution were delivered into 25ml test tubes followed by 3ml of 0.02 M thymolphthalein and 5ml of pure ethanol. The mixture was heated in a water bath at 80°C for 30 minutes, cooled and acidified with one or two drops of glacial acetic acid. The solutions were transfered into 10ml volumetric flasks and diluted to the mark with ethanol. The blank used contained the same amount of thymolphthalein (water may be used as blank since thymolphthalein is colorless in the acid medium). The absorbance of the resulting solution is measured at 444nm wavelength. The result is summarized in Table I and Figures 1 and 2 (The calculation is based on the least square method).

Preparation of Calibration Curve of Mustard in Urine

Different volumes (0.1-1.2ml) of 5×10^{-4} M mustard solution were added to 3ml of the urine in 25ml test tubes. After adjustment of pH to 12-13 with sodium hydroxide (C=0.1 M) addition of 3ml of 0.02 M thymolphthalein and 5ml ethanol, the mixture was heated in a water bath at 80°C for 30 minutes, cooled and then acidified with one or two drops of glacial acetic acid. The solutions were transfered into 10ml measuring flasks and completed to the mark with ethanol. The blank used contained urine, the same amount of all

Table I. Variation of absorbance with mustard concentration.

ml. of 5×10^{-5} M of Mustard	Absorbance
0.1	0.013
0.2	0.023
0.4	0.038
0.6	0.052
0.8	0.071
1	0.102
ml. of 5×10^{-4} M of Mustard	**Absorbance**
0.2	0.181
0.4	0.368
0.8	0.735
1.2	1.098
1.6	1.439
2	1.843

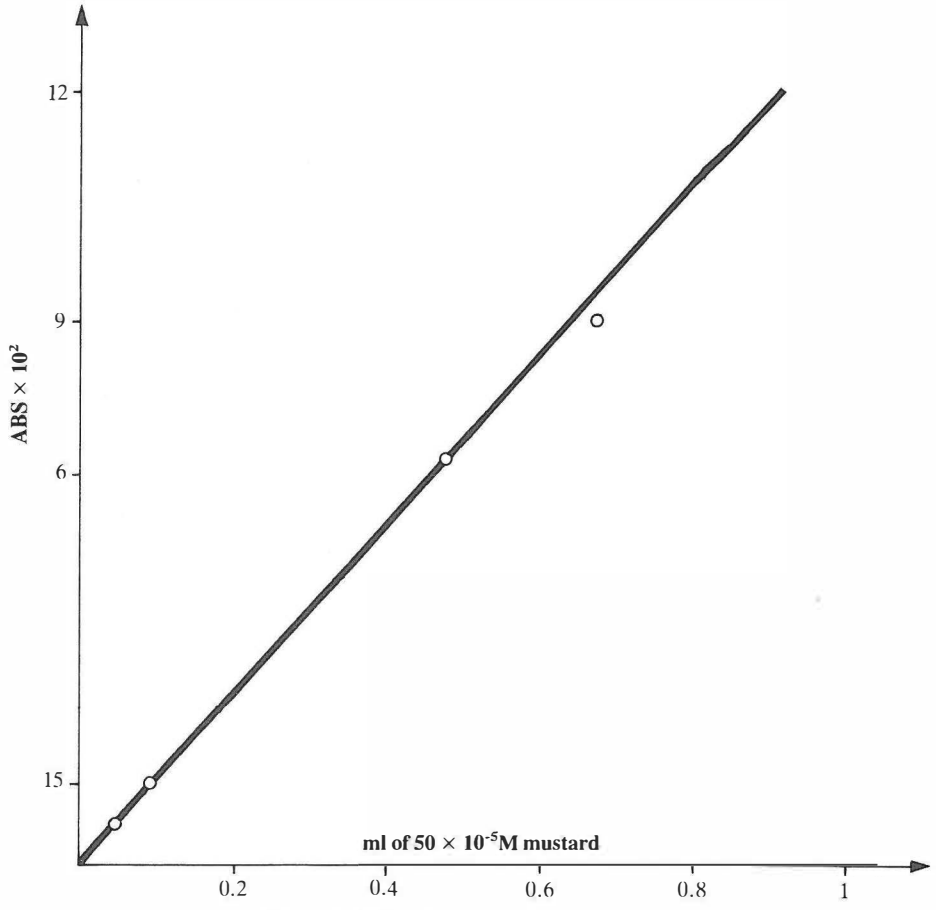


Figure 1. Calibration curve of mustard in ethanol.

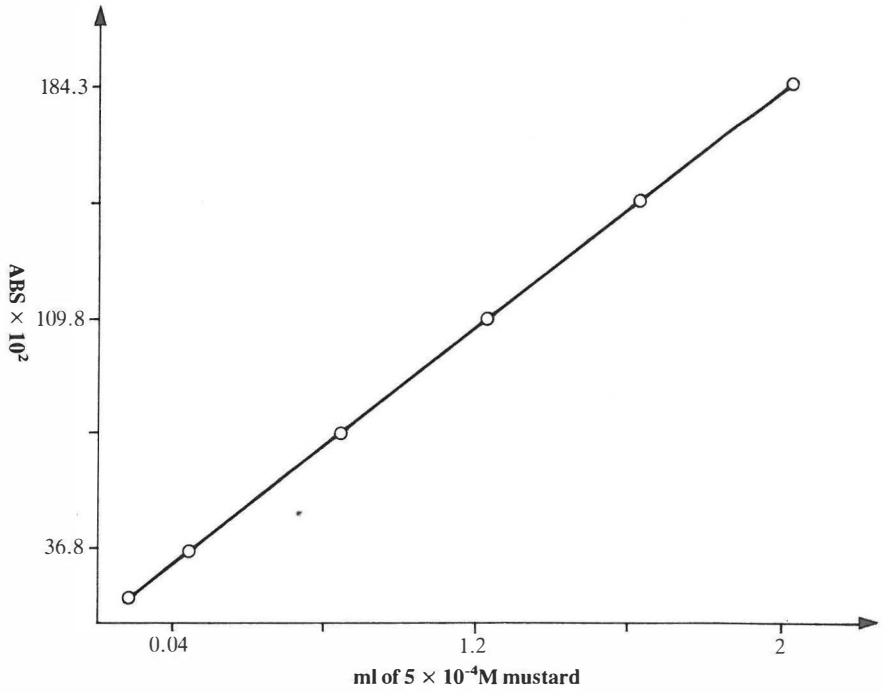


Figure 2. Calibration curve of mustard in ethanol.

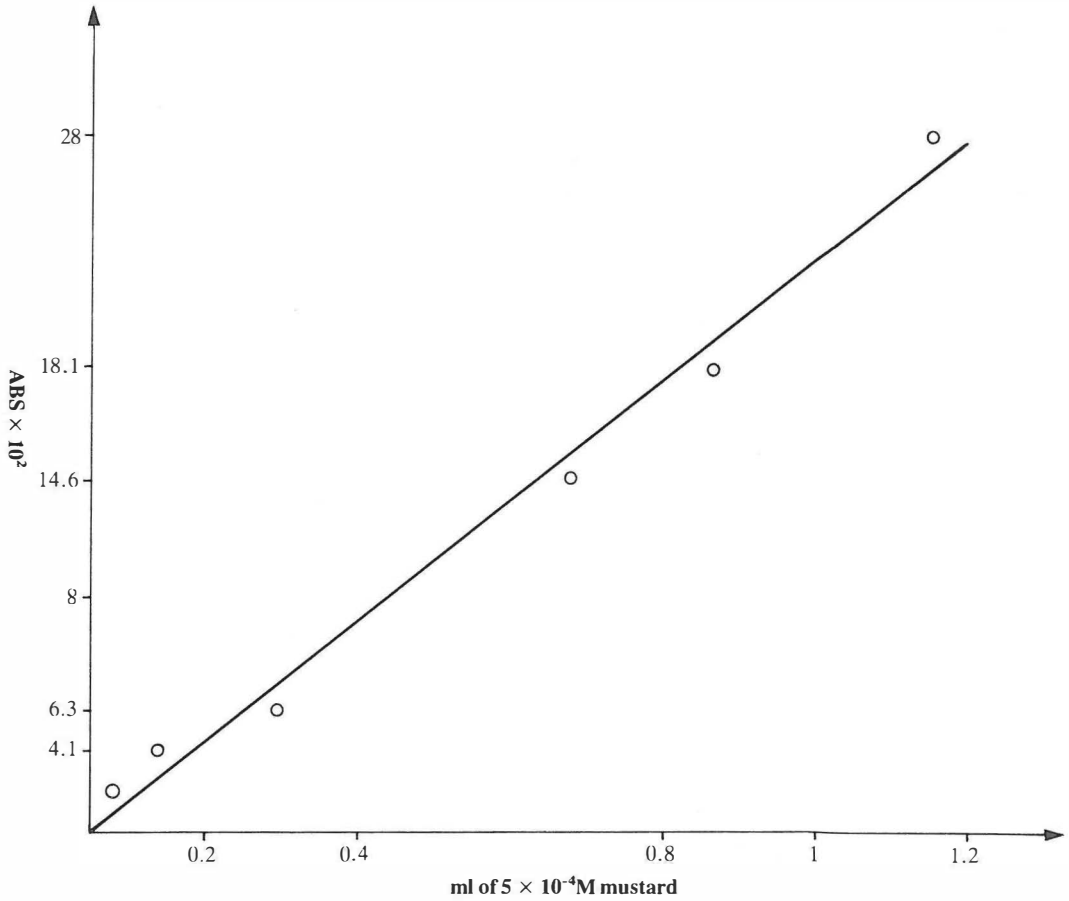


Figure 3. Calibration curve of mustard in urine.

Table II. Variation of absorbance with mustard concentration in urine.

ml. of 5×10^{-4} M of Mustard in Urine	Absorbance
0.1	0.021
0.2	0.041
0.4	0.063
0.8	0.146
1	0.181
1.2	0.277

reagents, however contained no mustard. All solutions were filtered before measurement. The results are summarized in Table II and Figure 3 (the calculation is based on least square method).

B: Gas Chromatographic Method:

Equipment

A Varian chromatograph, Model 2800 equipped with FID detector was used. The injection port, column and detector temperatures were kept at 230°C, 110°C and 220°C respectively. The column was OV-101, 100/120. The carrier gas was nitrogen with flow rate of 20 ml/min. 2-morpholinoethanol was used as internal standard.

Preparation of Calibration Curve of Mustard by GC.

Different volumes (0.2-10 μl) of mustard were injected and area under the curve (A.U.C.) was measured. The result is shown in Table III and Figure 4 (The calculation is based on least square method).

Table III. Variation of A.U.C. with mustard concentration

ml of Mustard	A.U.C.
0.2	216
0.3	292
0.4	432
0.5	537
0.6	672
0.7	796
0.8	918
0.9	1020
1	1143

Sample Preparation for Direct Mustard Extraction

20ml of urine was placed into a 50ml flask. After addition of crystalline sodium chloride until saturation 20ml of chloroform was added and stirred for 40 minutes. The mixture was centrifuged for 3 minutes at 2000 rpm. For clean up, to the organic layer 200mg

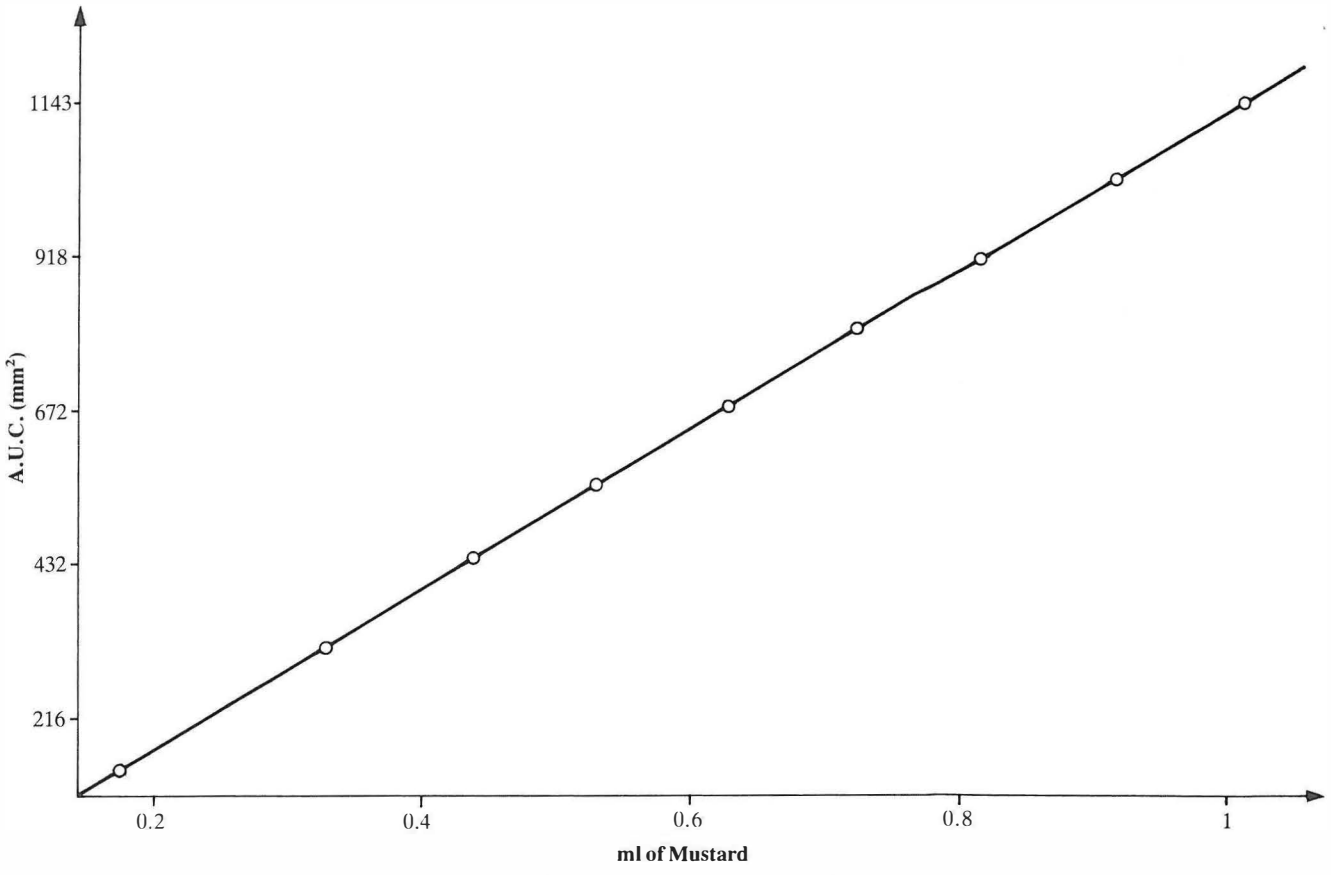


Figure 4. Calibration curve of mustard by GC.

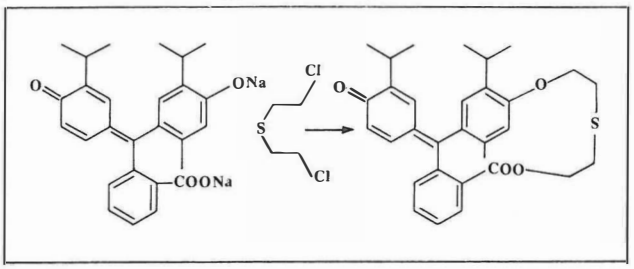
silica gel was added, stirred for 5 minutes and filtered. The solvent was evaporated under the reduced pressure and the residue was taken up in 20 to 100ml of dichloromethane containing internal standard. One to 2ml of this solution was injected to gas chromatograph.

Conversion of Thiodiglycol to Mustard in Urine.

To 20ml of urine sample 10ml of concentrated hydrochloric acid was added and refluxed for minutes. After cooling it was extracted with chloroform and worked up as explained above.

RESULTS AND DISCUSSION

Sulfur mustard reacts with thymolphthalein in alkaline media producing a yellow colour after acidification. The following equation was suggested for the reaction of reagent with mustard:



Beer's law is obeyed for 80 ng/ml to 16ng/ml (Figures 1-3). The detection limit of mustard in the urine by this method was 80 ng/ml.

In Figure 5 the retention time of mustard and internal standard by gas chromatography is shown.

17 urine samples obtained from Iranian victims all males were analyzed for mustard by gas chromatogra-

Table IV. Mustard concentration (ng/ml) Found in Urine Samples.

No.	Mustard concentration	Mustard concentration after conversion of Thiodiglycol
1	22	
2	124	
3	60	
4	33	
5	56	
6	37	
7	-	193
8	-	80
9	-	57
10	-	82
11	-	135
12	-	22
13	-	62
14	-	63
15	-	-
16	-	-
17	-	-

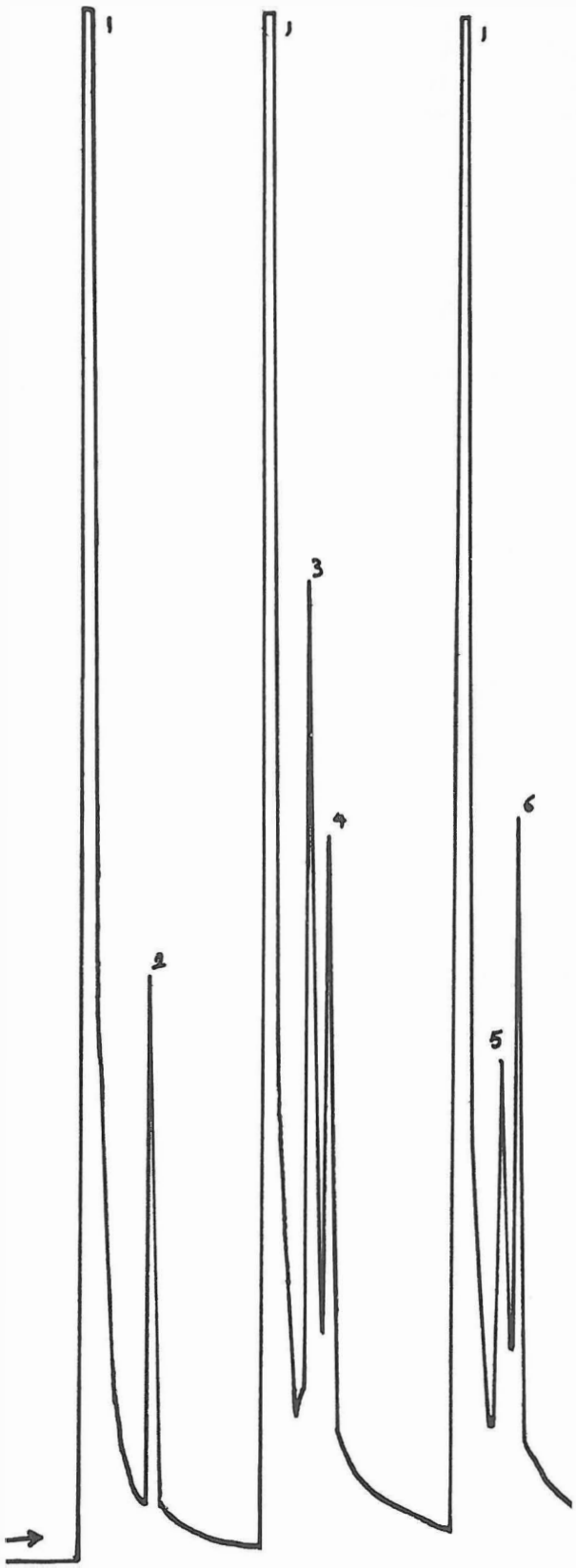


Figure 5. FID detection of mustard and Internal standard. Peaks: 1 = solvent (CH_2Cl_2); 2,4 and 6= mustard (1μl of 0.3%); 3 = Internal standard (1μl of 0.7%); 5 = Internal standard (1μl of 0.4%); relative retention time of mustard to Internal standard = 1.54.

phy. From 17 samples we could detect mustard in six of them. The result is shown in Table IV. The thiodiglycol was converted to mustard in the 11 urine samples of those patients which were negative for mustard (see experimental), and we detected mustard in eight of them (Table IV).

CONCLUSION

Although the colorimetric method is a simple and fast method for detection and assay of mustard gas, we have shown however that gas chromatography is a sensitive method of choice for detection and assay of mustard and its metabolite in the urine. The detection of mustard or thiodiglycol in a patient's urine is indicative of mustard gas poisoning.

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