ELSEVIER

Journal of Chromatography A, 907 (2001) 221–227

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Extraction of thiodiglycol from soil using pressurised liquid extraction

Nicola V. Beck, Wendy A. Carrick*, David B. Cooper, Bob Muir

Chemical and Biological Defence Sector, Defence Evaluation and Research Agency, Porton Down, Salisbury, Wiltshire SP4 0JQ, UK

Received 18 April 2000; received in revised form 12 October 2000; accepted 12 October 2000

Abstract

Thiodiglycol (TDG) is the predominant hydrolysis product of the chemical warfare agent sulfur mustard. The extraction of TDG was investigated using pressurised liquid extraction and the results compared for a variety of different solvents and soils. TDG was analysed underivatised by gas chromatography with flame photometric detection. A mixture of methanol–water (9:1), proved to be the most efficient extracting solvent for TDG at a temperature of 150°C and 10 MPa. © 2001 Published by Elsevier Science B.V.

Keywords: Soil; Pressurised liquid extraction; Extraction methods; Chemical warfare agents; Thiodiglycol

1. Introduction

Sulfur mustard, bis(2-chloroethyl) sulfide was first used as a chemical warfare agent in July 1917. It is a potent vesicant and alkylating agent. When dissolved or dispersed in aqueous media, mustard is very susceptible to hydrolysis. Thiodiglycol (TDG), the predominant hydrolysis product, is more likely to be encountered in the natural environment than mustard; it is also the major precursor for the industrial manufacture of mustard. The Chemical Weapons Convention (CWC) classes TDG as a Schedule 2B chemical, and its use and availability are monitored to prevent use for purposes not allowed under the CWC. Allegations concerning the use of chemical weapons (CWs) have increased over the last 2 decades, particularly during the Iraq–Iran war [1]. CWs were reported to have been used against the Kurdish population in northern Iraq in August 1988 and this was supported by subsequent analysis [2].

The Chemical and Biological Defence (CBD) Sector, Porton Down is a designated laboratory of the Organisation for the Prohibition of Chemical Weapons (OPCW), and as such is required to provide the unequivable detection and identification of CW agents concerned and/or their degradation products [3] from battlefield samples. Samples collected from a battlefield site for analysis, could be retrieved from bomb casings, soil from bomb craters, unexploded weapons or from human casualties [4]. Detection of TDG in the environment, such as soil samples from a battleground, provides strong supporting evidence in cases of alleged use of mustard [4].

CBD Porton Down also supports the Demilitarisation Soil Remediation program.

Traditional solvent extraction procedures for soil

*Corresponding author. Fax: +44-1980-613-822.

E-mail address: wacarrick@dera.gov.uk (W.A. Carrick).

0021-9673/01/$ – see front matter
PII: S0021-9673(00)01037-2

and other solid samples are Soxtec, Soxhlet, ultrasonic bath or manual extraction. Soxtec extraction can take 6 h and Soxhlet extraction up to 18 h, some analyte is frequently lost, large volumes of solvents are required (up to 400 ml) and extraction of analytes from soil is often incomplete [5–7]. Ultrasound has a rapid extraction time (approx. 10 min), but this technique may release additional binding sites from the soil, and extraction of analytes from soil is usually poor [6]. Manual extraction by hand shaking, although more rapid (25 min) than Soxhlet, often produces poor analyte recoveries and is labour intensive [6,7].

Pressurised liquid extraction (PLE) provides fast automatic extraction of solid samples using small solvent volumes (15–60 ml). PLE uses conventional liquid solvents at elevated pressure (10–14 MPa) and temperatures (50–200°C) to extract solid samples quickly and with much less solvent than traditional techniques [8]. With PLE, a solid sample is placed in a stainless steel vessel that is filled with an extraction solvent and heated. The sample is extracted using a static cycle (solvent soak time) of 5–10 min, with the expanding solvent vented to a collection vial. Compressed nitrogen purges the remaining solvent into the same vial. The whole procedure requires <15 min/sample and approximately 15 ml solvent for a 10 g sample. PLE extraction takes advantage of the increases in analyte solubilities that occur at temperatures above the boiling points of solvents. At these elevated temperatures, the kinetic processes for the desorption of analytes from the matrix are accelerated, compared with conditions when the solvents are used at room temperature. Solvent usage is reduced as a result of the higher analyte solubility in the heated solvent [7].

PLE has been successfully used for the extraction of chlorinated pesticides and hydrocarbon contaminants from contaminated soils and organic contaminants from toxic waste [5,9,10]. PLE gave comparable performance or better compared with Soxhlet and Soxtec with considerable savings in extraction time, 10 min compared to 6 h for Soxtec, or 18 h for Soxhlet [5]. PLE has also proved suitable for the extraction of polychlorinated biphenyls (PCBs), mustard, sarin, organophosphorus pesticides and herbicides [6,7,11,12].

2. Experimental

2.1. Materials

All solvents used (Distol quality) were obtained from Fisher Scientific (Loughborough, UK). TDG (>99%) was purchased from Fluka (Gillingham, UK).

Porton soil (which is classified as a silty clay loam) was collected and prepared at CBD Porton Down. The sandy loam and peaty loam soils were purchased from Levington Agriculture Ltd. (Ipswich, UK). Table 1 displays the soil data. At this point all the soils are free of TDG.

2.2. Soil spiking

Samples (20 g) of Porton soil, peat soil and sand soil were spiked with TDG (10.0 μg/ml) in methanol (20 ml), left open overnight for the solvent to evaporate and stored in a sealed glass jar for specified periods. Five replicate soil samples of each type were spiked.

2.3. Instrument specifications

2.3.1. Soil extraction

The soil extractions were carried out using a Dionex ASE™ Model 200 accelerated solvent extractor with 33 ml stainless steel extraction cells.

2.3.2. Analysis

The gas chromatography (GC) system used was a HP 5890 (Hewlett–Packard) with a flame photometric detector (FPD). The capillary column used was a DB WAX of 15 m×0.53 mm I.D. with 1.0 μm film thickness. The carrier gas was helium (99.996%) and the make-up gas was nitrogen

Table 1
Soil data

Soil type	Composition (%, w/w)			pH	Organic matter (%, w/w)
	Clay	Silt	Sand		
Porton	30.0	53.0	17.0	7.9	5.6
Peat	28.5	50.8	20.7	7.8	24.7
Sand	9.0	28.0	63.0	5.7	1.6

(99.998%). A split–splitless injector was used in the splitless mode and maintained at 230°C. A 1.0-μl volume of sample was injected. The flow was maintained at a constant pressure of 24.82 kPa. Purge gas flow through the injector was 98.7 ml/min, purge time was 1.00 min, and carrier gas flow-rate was 11.1 ml/min. The column temperature programme was as follows: initial temperature 90°C (held for 1.00 min), increased at 30°C/min to 240°C and held at the final temperature for 2.00 min. The detector temperature was 250°C.

2.4. Calibration standards

TDG in methanol was used for the calibration standards at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 and 10.0 μg/ml.

2.5. Method development for TDG extraction from soil by PLE

An extraction method for TDG from Porton soil was developed on the PLE system by varying the parameters for extraction temperature and hold time, static extraction time, and solvent flush volume [8].

A range of solvents, from polar to less polar was investigated to find the optimum solvent for TDG extraction. The solvents investigated (v/v) were: water, methanol–water in proportions 3:1, and 9:1, methanol, ethyl acetate, acetonitrile and acetone–dichloromethane (1:1).

The extraction temperature was investigated at temperatures 150 and 180°C with methanol–water (9:1).

The extraction of TDG was compared for three soil types, Porton soil, a peaty loam and a sandy loam. TDG recovery was investigated for these three soil types at time periods of, 1 day, 7 days and 28 days, using methanol–water (9:1), in order to compare TDG recoveries from different soil types over time. Five replicate soil samples of each type were spiked and extracted.

2.6. Preparation of PLE soil extracts for analysis

A 5-ml volume of the total extracts (~45 ml) was removed and filtered using a 5-ml Plastipak disposable syringe, with Whatman PTFE or nylon syringe filters (25 mm, 0.2 μm pore size).

The 1-day aged soil extracts were analysed without further concentration, as the extracts were likely to contain high amounts of recovered TDG. Extracts from 7-day and 28-day aged soil samples were subjected to concentration under nitrogen, as they were likely to contain much lower amounts of recovered TDG.

2.7. TDG extraction from soil by hand shaking

Manual extraction of TDG from soil (Porton, peat and sand) by hand shaking was carried out for comparison with PLE. Although it is widely known that hand shaking is not a very effective method, this investigation aimed to show exactly how much more efficient PLE was when compared to hand shaking.

Soils (20 g) were extracted with methanol–water (9:1) (30 ml), shaken for 1 min by hand and left to stand for 5 min. The extracts were filtered and analysed in exactly the same way as the PLE extracts. Five replicate soil samples of each type were spiked, extracted and analysed.

2.8. Statistical methods

TDG concentration data were found to be log-normally distributed and hence this transformation was applied prior to statistical manipulation. Each set of concentration data was examined for outliers by the Dixon's Q-test. This resulted in two observations being excluded from statistical analysis. Normality of the log transformed concentration data was then confirmed using the Anderson–Darling normality test at the 95% confidence level. Normalised data had a $P>0.05$ confirming normal distribution.

Analysis of variance (ANOVA) was performed to establish which experimental factors influenced TDG recovery by fitting a general linear model to a two-way ANOVA which also included a second-order interaction term. ANOVA was performed at the 95% confidence level. Bonferroni simultaneous confidence intervals were also generated as part of the ANOVA to allow comparison of sample means.

3. Results and discussion

3.1. Analysis

3.1.1. Analysis of TDG calibration standards

The observed detector response was found to be quadratic with the *F*-statistic for ANOVA being <0.0001 and the *P* value for the intercept term being <0.05. The precision of the method was evaluated using four independently-prepared replicates of TDG at concentrations ranging between 0.5 and 10.0 μg/ml. The relative standard deviation at 0.5 μg/ml was 16%, and all the other tested values ranged between 6 and 11%.

3.1.2. Analysis of TDG extracts

Traditionally, TDG is analysed as its trimethylsilyl (TMS) or its bis(*tert*.-butyldimethylsilyl) (TBDMS) derivative. However, this method is time consuming [6] and gave poor precision with soil residues, probably due to extraneous materials interfering with the derivatisation. An alternative method was developed which allowed analysis of intact TDG. Analysis of the filtered PLE and hand extracts was performed underivatised by GC–FPD. Five replicate soil extracts were analysed.

Analysis of the TDG extracts using GC–FPD without derivatisation proved to be a rapid, robust and moderately sensitive method. When the analysis of the TDG extracts analysed underivatised by GC–FPD was compared with the analysis of derivatised extracts by GC–mass spectrometry (MS) (selected ion mode), the GC–MS method did provide lower detection limits. However, the sensitivity of the GC–FPD method was sufficient for this investigation, with a detection limit of 1.13 μg/g soil (S/N~10).

3.1.3. Method development for TDG extraction from soil by PLE

Preliminary work with TDG recovery from soil by PLE produced an optimised method regarding hold time, static extraction time and solvent flush volume. Two operating temperatures were investigated with the above parameters, 150°C and 200°C. At 200°C, TDG was found to degrade and resulted in lower recoveries at 200°C compared to 150°C.

A range of solvents, from polar to less polar, was investigated in order to determine the optimum solvent for TDG extraction. The presence of water in the extracting solvent did not pose a problem. Mixtures of aqueous and organic solvents can assist in the extraction of wet samples [8], although in this case, all soil samples were dry and free flowing.

Table 2 shows the amount of TDG recovered in μg/g from Porton soil at 1 day aged using a range of solvents, after spiking with 10 μg TDG/g ($n=5$). For 100% methanol $n=4$, as one sample suffered from leakage whilst in the PLE, hence was not included in the analysis. The most efficient solvent for TDG extraction was methanol–water (9:1). Two-factor ANOVA performed on solvent composition and TDG recovery shows that the mean recovery of TDG using methanol–water (9:1) differs significantly from all other solvent systems ($P=0.05$) and gives the greatest TDG recovery. A plot of recovery vs. replicate number (Fig. 1) clearly shows that the optimum solvent for extraction of TDG from Porton soil is methanol–water (9:1). Error bars indicate standard deviation of extracted concentration. Error bars for acetonitrile are omitted for clarity.

Bonferroni simultaneous comparison of means reveals that extraction efficiency decreases in the order: methanol–water (9:1)>acetone–dichloromethane (1:1)>acetonitrile >ethyl acetate>methanol (100%)>methanol–water (3:1)>water (100%).

In order to optimise the oven temperature of the PLE, TDG extraction from Porton soil at 150°C and 180°C was performed. TDG was extracted from soil spiked with 10 μg TDG/g at 1 day aged, using methanol–water (9:1).

At 180°C TDG recoveries were 6.82 μg/g (0.62) and at 150°C 8.89 μg/g (0.29); mean values fol-

Table 2
Mean and SD of TDG recovered in μg/g of Porton soil, at 1 day, using a range of solvents, after spiking with 10 μg TDG/g soil[a]

Solvent	Recovery (μg/g)
Methanol–water (9:1)	8.89 (0.29)
Acetone–dichloromethane (1:1)	5.45 (0.90)
Acetonitrile	2.81 (1.15)
Ethyl acetate	2.95 (0.58)
Methanol (100%)	2.16 (0.51)[b]
Methanol–water (3:1)	4.97 (1.65)
Water (100%)	1.10 (0.17)

[a] Extraction temperature was 150°C. Mean values with SD in parentheses, $n=5$ except where indicated.
[b] $n=4$.

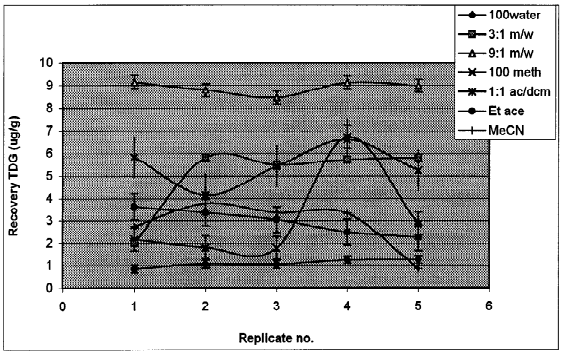

Fig. 1. Recovery of TDG from spiked Porton soil using various solvent systems by PLE. m/w=Methanol–water; meth=methanol; ac/dcm=acetone–dichloromethane; Et ace=ethyl acetate; MeCN=acetonitrile.

lowed by standard deviation (SD) in parentheses. ANOVA was performed using temperature and solvent composition as variables. A second-order term (temperature×solvent composition) was also included. The resulting ANOVA indicates that both temperature and solvent composition influence the recovery of TDG. Interestingly however, there is no interaction between these terms. ANOVA also indicates a significant difference in mean TDG recovery at both temperatures and solvent compositions.

Mean TDG recovery was higher at the lower temperature (150°C). This perhaps suggests thermal adsorption or decomposition of TDG at elevated temperatures. From these results and the preliminary work, the optimum extraction temperature was taken as 150°C. In summary, optimum PLE parameters for TDG extraction from soil were found to be: (a) heat sample from room temperature to 150°C and hold for 7 min; (b) static extraction for 15 min at 150°C and 10 MPa; (c) solvent flush of 40% volume with nitrogen purge for 60 s; (d) two cycles.

The resulting extract (~45 ml per sample) was collected in 60 ml vials.

The nominal sample-to-sample processing time was 50 min.

The ability of methanol–water (9:1) to extract TDG was compared on three different soil types: Porton, peat and sand. These 10 μg TDG/g spiked soils were extracted with methanol–water (9:1), after 1 day, 7 days and 28 days, as aged samples are more akin to real life situations [2,4]. Table 3 displays

Table 3
Mean and SD of TDG recovered in μg/g of Porton, peat and sand soils at 1 day, 7 days and 28 days using methanol–water (9:1), after spiking with 10 μg TDG/g soil[a]

Soil type	Recovery (μg/g)		
	1 Day	7 Days	28 Days
Porton	8.89 (0.29)	4.84 (1.02)	1.16 (0.24)[b]
Peat	5.68 (1.14)	6.75 (1.31)	2.23 (0.08)
Sand	7.19 (0.64)	5.18 (0.65)	5.56 (0.16)

[a] Extraction temperature was 150°C. Mean values with SD in parentheses, $n=5$ except where indicated.
[b] $n=3$.

TDG recoveries in μg/g from Porton, peat and sand soils at 1 day, 7 days and 28 days using methanol–water (9:1), after spiking at 10 μg TDG/g soil. $n=3$ for 28-day Porton as two samples suffered from leakage whilst in the PLE, hence were not included in the analysis. ANOVA indicates that soil type, number of days after spiking and the second order term (soil type×number of days after spiking) all have a significant effect on average TDG recovery. Porton and peat soils give statistically similar mean TDG recoveries over 28 days but sand behaves differently from these soils exhibiting a higher overall recovery. All soils show a decrease in mean TDG recovery between 1 and 7 days after spiking. This is also noted between 7 and 28 days indicating a gradual decrease in TDG recovery on aged soil. TDG reduced recoveries could be attributed to the formation of TDG sulfoxide in the soil matrix, which was not investigated in this application. The higher TDG recovery on sand could be due to sand containing less active sites than other soil types. A chromatogram of 28-day aged TDG spiked sandy loam soil extracted with methanol–water (9:1) is displayed in Fig. 2.

Performance of the optimised PLE extraction system for all three soil types was then compared to manual extraction (hand shaking) from 1 to 28 days after spiking. Table 4 shows comparisons of TDG recoveries on the three different soil types by PLE and manual extraction using methanol–water (9:1). ANOVA indicated soil type, extraction method and the second-order term again influence the recovery of TDG from spiked soils. Recovery of TDG from Porton soil is less than for peat and sand while the latter soils exhibit similar mean recoveries. PLE and

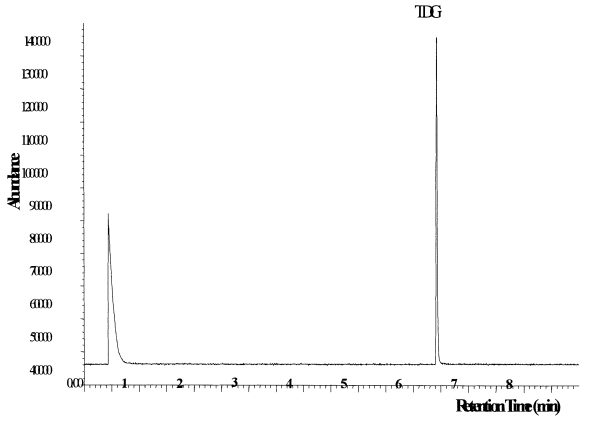

Fig. 2. Chromatogram of 28-day aged TDG spiked sandy soil extracted using methanol–water (9:1) by PLE.

manual extraction give significantly different recoveries from all soil types with PLE giving the highest mean recoveries for each soil type. For the majority of samples, PLE does provide improved TDG recoveries from Porton, peat and sand type soils when compared to manual extraction by hand shaking.

4. Conclusion

The most efficient solvent for TDG extraction from soil was found to be methanol–water (9:1). Optimum PLE parameters employing this solvent were: (a) heat sample from room temperature to 150°C and hold for 7 min; (b) static extraction for 15 min at 150°C and 10 MPa; (c) solvent flush of 40% volume with nitrogen purge for 60 s and (d) two cycles.

The resulting extract (~45 ml per sample) was collected in 60 ml vials.

The nominal sample-to-sample processing time was 50 min.

TDG could still be recovered from Porton, peat and sand type soils, 28 days after they were spiked, by PLE and to a lesser extent manual extraction. TDG was most easily recovered from sand, probably due to less active sites in sand compared to Porton and peat soils. PLE provided improved TDG recoveries from Porton, peat and sand, when compared to manual extraction by hand shaking, from 1-day to 28-days aged.

Analysis of the TDG extracts, underivatised by GC–FPD provided a rapid and sensitive method with a detection limit of 1.13 μg/g soil. PLE enables automated extraction of soils, with typical sample turnaround time approximately 1 h including analysis.

Table 4
Mean and SD of TDG recovered in μg/g of Porton, peat and sand soils, at 1 day, 7 days and 28 days by PLE and manual extraction, after spiking with 10 μg TDG/g soil[a]

Soil	Period (days)	TDG recovery (μg/g)	
		PLE	Manual Extraction
Porton	1	8.89 (0.29)	3.11 (0.08)
	7	4.84 (1.02)	2.84 (0.28)
	28	1.16 (0.24)	1.21 (0.05)
Peat	1	5.68 (1.15)	2.57 (0.35)
	7	6.75 (1.31)	2.86 (0.21)
	28	2.23 (0.08)	0.80 (0.15)
Sand	1	7.19 (0.64)	4.20 (0.30)
	7	5.18 (0.65)	5.82 (0.46)
	28	5.56 (0.16)	2.06 (0.08)

[a] Extraction temperature was 150°C and solvent used was methanol–water (9:1). Mean values with SD in parentheses, $n=5$.

Acknowledgements

The authors would like to thank the following members of staff at CBD Porton Down: Mrs. Moira Burton for her preliminary results of TDG extraction from Porton soil using PLE, Mr. Bob Gwyther for his statistical consultation and Dr. Robin Black for his advice and support in producing this manuscript.

References

[1] S.J. Lundin, in: SIPRI Yearbook (1989). World Armaments and Disarmament, Oxford University Press, New York, 1989, Chapter 4.
[2] R.M. Black, R.J. Clarke, D.B. Cooper, R.W. Read, D. Utley, J. Chromatogr. A 637 (1993) 71.
[3] R.M. Black, G.S. Pearson, Chem. Br. 29 (1993) 584.
[4] R.M. Black, R.J. Clarke, R.W. Read, M.T.J. Reid, J. Chromatogr. A 662 (1994) 301.

[5] P. Popp, P. Keil, M. Moder, A. Paschke, U. Thuss, J. Chromatogr. A 774 (1997) 203.
[6] B.A. Tomkins, G.A. Sega, S.J. Macnaughton, Anal. Lett. 31 (1998) 1603.
[7] J. Ezzell, B. Richer, W. Felix, S. Black, J. Meikle, LC–GC 13 (1995) 390.
[8] Application Note 208, Methods Optimization in ASE, Dionex, Sunnyvale, CA, 1997.
[9] Application Note 324, ASE of Hydrocarbon Contaminants (BTEX, Diesel and TPH) in Soils, Dionex, Sunnyvale, CA, 1996.
[10] S. Pyle, A. Marcus, J. Mass Spectrom. 32 (1997) 897.
[11] Application Note 319, Extraction of Organophosphorus Pesticides Using ASE, Dionex, Sunnyvale, CA, 1996.
[12] Application Note 316, Extraction of PCBs from Environmental Samples Using ASE, Dionex, Sunnyvale, CA, 1996.