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Determination of chemical warfare agents, their hydrolysis products and related compounds in soil

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ABSTRACT

A procedure based on sequential hexane and dichloromethane extraction followed by trimethylsilyl derivatization and capillary column gas chromatographic–mass spectrometric (GC–MS) confirmation was developed for the verification of chemical warfare agents, their hydrolysis products and related compounds in soil. The chemical warfare agents sarin, soman and mustard and the simulant triethyl phosphate were added to four different soil types at the 50 and 5 μg/g levels and recovered with efficiencies varying from nearly 100% to about 5%. The recovery efficiencies were in the range 50–90% for most soil types contaminated with soman, mustard and triethyl phosphate. Sarin recovery was generally the lowest (5–30%). Hydrolysis products, due to degradation of the spiked chemical warfare agents during the course of the experiments, were detected and confirmed as trimethylsilyl derivatives. The developed sample handling and analysis procedure was applied to soil samples in support of range clearance operations. The chemical warfare agent tabun and sixteen related components and their hydrolysis products were identified during capillary column GC–MS analysis of soil extracts.

INTRODUCTION

The use of chemical weapons, although prohibited by the 1925 Geneva Protocol, has been reported recently in several armed conflicts, including the Iran–Iraq war [1]. Verification of the use of chemical warfare agents has often been difficult, in part owing to inadequate battlefield sampling and identification procedures. Soil has been used as a sampling medium for the verification of alleged use of chemical warfare agents and was used as a medium in a recent multi-national round robin exercise coordinated by the Finnish Research Project for Chemical Warfare Verification [2].

A number of methods have been reported for the detection of organophosphorus [3–7] and sulfur vesicant [8–11] chemical warfare agents in soil. Sample handling methods, usually based on solvent extraction, vary considerably and remain a problem for the detection of chemical warfare agents and their hydrolysis products in soil. Prior experience, using dichloromethane as an ultrasonic extraction solvent, was successful for the verification of the sulfur vesicant mustard in soil [9–11]. However, the same extraction method resulted in less satisfactory yields for soil contaminated with the organophosphorus chemical warfare agent sarin. Deficiencies in soil sample handling procedures, and a perceived role for these verification procedures in investigations of allegations of the use of chemical warfare agents, suggested the development of new or improved soil sample handling methods. For these reasons a study was initiated with the primary objective being the development of an efficient sample handling procedure that would allow capillary column gas chromatographic–mass spectrometric (GC–MS) confirmation of chemical warfare agents and their hydrolysis products in soil.

Four different soils, ranging from sand to sandy clay loam were spiked at the 50 and 5 μg/g levels with sarin (isopropyl methylphosphonofluoridate), soman (pinacolyl methylphosphonofluoridate), triethyl phosphate and mustard [bis(2-chloroethyl) sulfide], and extracted with a number of candidate

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solvents. Successive extraction of spiked soil with hexane and dichloromethane, followed by trimethylsilyl derivatization of the soil, appeared to be the most promising approach for the concentration of these chemical warfare agents and their hydrolysis products from soil. The developed method was successfully applied to a series of VX-spiked soil samples during a recent multi-national round-robin analytical exercise [2] and was utilized for the verification of chemical warfare agents and their hydrolysis products in soil contaminated with small amounts of tabun during range clearance operations at the Defence Research Establishment Suffield.

EXPERIMENTAL

Standards

Sarin, soman, mustard and triethyl phosphate were provided by the Organic Chemistry Laboratory, Defence Research Establishment Suffield. Distilled-in-glass solvents were purchased from BDH (Edmonton, Canada). The trimethylsilylation agent bis(trimethylsilyl)trifluoroacetamide (BSTFA) (containing 1% trimethylchlorosilane) and silylation-grade pyridine were purchased from Pierce (Rockford, IL, USA).

Soil Samples and Sample Handling

The four Defence Research Establishment Suffield Experimental Proving Grounds soils, used for spiking experiments, were dried for 3 days, sieved (2 mm) and characterized by the Alberta Environmental Centre (Mr. P. Yeung, Vegreville, Canada) using the Canadian Soil Classification standards.

Samples of 1 g of soil were weighed into 16 mm x 125 mm glass culture tubes, spiked with either 50 μl of a 1 mg/ml standard (spike level 50 μg/g) or 50 μl of a 0.1 mg/ml standard (spike level 5 μg/g) containing sarin, soman, triethyl phosphate and mustard in dichloromethane, and allowed to stand for 60 min at 4°C prior to solvent extraction. Spiked soil samples and blanks were extracted with 2 × 2 ml of hexane by ultrasonic vibration (10 min) and then centrifuged (10 min at 700 *g*). The soil was re-extracted using the above procedure with dichloromethane. Both the hexane and dichloromethane extracts were concentrated separately to 1 ml by nitrogen blowdown. Trimethylsilyl (TMS) derivatization was performed following solvent extraction of the soil samples by adding 100 μl of BSTFA, 100 μl of pyridine and 1 ml of dichloromethane to the soil and allowing it to stand at 60°C for 20 min. Derivatized soil samples were centrifuged for 15 min at 700 *g* and the supernatant was removed, concentrated to 300 μl by nitrogen blowdown and analysed within 24 h to minimize degradation. All soil sample extracts were stored in PTFE-lined screw-capped glass vials at 4°C prior to analysis.

Soil samples, taken in support of range clearance operations, were removed from a site suspected to have been contaminated by a leaking container intended for chemical decontamination. An undried portion of the soil sample was extracted with dichloromethane shortly after receipt and tabun was found to be the principal sample component, based on capillary column GC analysis using phosphorus-mode flame photometric detection. The remaining soil was stored at 4°C and subjected to the developed sample handling procedure about 90 days after initial sampling.

Instrumental

Capillary column GC was performed with a Hewlett Packard 5890 gas chromatograph equipped with an on-column injector of our own design [9] and a flame ionization detector. Data were acquired in triplicate at both spiking levels with percentage recoveries being calculated by external standard calibration. A 15 m × 0.32 mm I.D. DB-1701 J&W capillary column (film thickness 0.25 μm) was used for all GC analyses with the following temperature programs: initial temperature 40 or 50°C (held for 2 min), then increased at 10°C/min to 280°C (held for 0–5 min). Capillary column GC–MS analyses were performed with a Model 70/70E double-focusing mass spectrometer (VG Analytical, Wythenshawe, UK) interfaced to a Varian Model 3700 gas chromatograph. Electron impact MS operating conditions were as follows: emission, 0.1 mA; electron energy, 70 eV; source temperature, 200°C; and source pressure, 2×10^{-6} Torr. Full scanning MS data were acquired at a resolution of 1000 (10% valley definition) with an accelerating voltage of 6 kV over a mass range of 500–40 u.

RESULTS AND DISCUSSION

Four soil types, ranging from sand to sandy clay loam, were spiked at the 50 and 5 μg/g levels with sarin, soman, mustard and triethyl phosphate in a effort to develop a sample handling and analysis procedure that would allow verification of chemical warfare agents and their degradation products in soils. The spiking levels selected were well below typical battlefield contamination levels, which have been estimated to be *ca.* 100–1000 μg/g based on a contamination density of 1–10 g/m^2 (soil density *ca.* 1 g/cm^3 and a sampling depth of 1 cm). The 50 μg/g spiked soil samples were considered to be typical of soil contamination levels hours or days after an attack. The 5 μg/g spiked samples were considered reasonable for soil collected days after an attack or for samples that had undergone natural weathering.

A variety of solvents, including, hexane, dichloromethane, chloroform, acetone, methanol, acetonitrile and water, were investigated for the extraction of compounds of chemical defence interest, with the following scheme being selected on the basis of these preliminary investigations. Soil samples, either spiked or unspiked, were extracted sequentially with hexane and dichloromethane. The extracts were then concentrated and analysed by capillary column GC and GC–MS. The most likely degradation products, due to hydrolysis of sarin and soman would be isopropyl methylphosphonic acid and pinacolyl methylphosphonic acid, respectively, and methylphosphonic acid, whereas thiodiglycol would be expected following mustard hydrolysis [12]. These hydrolysis products, with the exception of thiodiglycol, cannot be determined by direct capillary column GC methods. Thiodiglycol may be determined directly at the nanogram level, converted back to mustard [13] or derivatized with pentafluorobenzoyl chloride [14]. The organophosphorus acids, formed after hydrolysis of sarin and soman, may be determined using GC sample introduction after methylation [15] or *tert.*-butyldimethylsilylation [16]. More recently, liquid chromatography–thermospray mass spectrometry has been used to identify organophosphorus acids [17], organophosphorus pesticides [18–20] and VX [21]. Thermospray MS offers the analyst the possibility of direct MS analysis but at the expense of sensitivity. Typical detection limits are two to three orders of magnitude above those routinely obtained by GC–MS analysis of the derivatives. For this reason, TMS derivatization of the remaining soil, following hexane and dichloromethane extraction, was selected as the final step for the concentration of chemical warfare agents and their degradation products.

Soil Spiking Experiments

Table I lists the recoveries of sarin, soman, mustard and triethyl phosphate from the four different soils. The principal hydrolysis products of sarin, soman and mustard were screened for as their TMS derivatives along with the spiked agents in all the TMS extracts. An estimate of hydrolysis product content was made by comparing the detector response of these products to that of the corresponding chemical warfare agent. Trace amounts of chemical warfare agents or hydrolysis products indicate confirmed mass spectrometric detection, but at levels below 1% of the initial agent spike level.

Sarin was generally difficult to extract efficiently from all the soil types. The recovery was usually highest in the dichloromethane extract, with total recoveries of 30–40% for the recovery of sarin from the loamy sand and sandy loam. The TMS derivative of isopropyl methylphosphonic acid was only detected in the loamy sand and sandy loam TMS extracts after spiking at the 50 μg/g level.

Soman was recovered fairly efficiently from all four soil types, with total recoveries typically in the 30–90% range. Most of the soman was found in the dichloromethane extracts of the soils. Soman and the TMS derivative of pinacolyl methylphosphonic acid were generally detected in the TMS extract, with the greatest recoveries being associated with the higher spike levels.

The soman simulant triethyl phosphate was recovered during the dichloromethane and TMS steps with good efficiency (50–90%) at both the 5 and 50 μg/g level in all soil types except for the sand. This soil appears to be a difficult medium as the recovery of all the spiked agents was generally lower than that obtained for the other three soil types. Hydrolysis of triethyl phosphate was considered unlikely under these experimental conditions and possible hydrolysis products were not screened for.

Mustard recovery was good with total recoveries in the 50–90% range for all soil types except the sandy clay loam. Mustard was generally recovered

TABLE I

EXTRACTION DATA FOR SARIN, SOMAN, TRIETHYL PHOSPHATE AND MUSTARD ADDED TO SOILS AT 50 AND 5 μg/g

Compound	Soil	Level (μg/g)	Recovery after each step (%)[a]			Total recovery (%)
			Hexane	CH_2Cl_2	TMS	
Sarin	Sand	50	ND	5±1	ND (1[b])	5
		5	ND	ND	ND	0
	Loamy sand	50	6.7±0.2	32.7±0.7	ND (trace[b])	39
		5	2.0±0.7	23±7	ND	25
	Sandy loam	50	1±0.3	31±5	ND (trace[b])	32
		5	ND	30±4	ND	30
	Sandy clay loam	50	ND	6.7±0.2	ND	7
		5	ND	Trace	ND	Trace
Soman	Sand	50	1.0±0.3	24±3	12.5±0.5 (8±3[b])	37
		5	ND	17±1	2±2	19
	Loamy sand	50	23.1±0.8	50.7±0.4	12.5±0.5 (10±2[b])	86
		5	3±1	58±5	Trace	61
	Sandy loam	50	4±1	56±8	8±2 (trace[b])	68
		5	Trace	67±7	Trace	67
	Sandy clay loam	50	ND	30±1	3.2±0.6	33
		5	ND	22±5	7.6±0.4	30
Triethyl phosphate	Sand	50	ND	4±1	32±6	36
		5	ND	ND	25±13	25
	Loamy sand	50	1.4±0.2	30±1	32±4	63
		5	ND	27±9	30±3	57
	Sandy loam	50	ND	25±6	40±10	65
		5	ND	28±5	51±7	79
	Sandy clay loam	50	ND	6±1	56±4	62
		5	ND	5.6±0.3	80±7	86
Mustard	Sand	50	39±6	5±1	ND (10.6±0.1[b])	44
		5	44±5	3.5±0.2	ND (17±5[b])	48
	Loamy sand	50	80±3	4.7±0.9	ND (5±2[b])	85
		5	75±4	5.1±0.5	ND	80
	Sandy loam	50	50±5	7±2	ND (3.1±0.6[b])	57
		5	74±3	5±1	ND	79
	Sandy clay loam	50	10.9±0.6	6.0±0.7	ND (7±2[b])	17
		5	14±2	ND	ND (27±10[b])	14

[a] Mean ± S.D. ($n=3$); ND = not detected; trace = <1%

[b] Detected as TMS derivative of hydrolysis product(s) (not included in total recovery).

in the hexane extract and the presence of the mustard hydrolysis product thiodiglycol was confirmed in most of the TMS extracts. Thiodiglycol recovery, although not included in the total mustard recovery estimates, was fairly high for the two soil types (sand and sandy clay loam) for which the mustard recovery was lower.

Figs. 1 and 2 illustrate typical capillary column gas chromatograms obtained for the 50 μg/g spike of the sandy clay loam and the loamy sand respectively. These chromatograms clearly illustrate the presence of the spiked agents and their hydrolysis products in each of the three sample extracts. All the spiked agents and the mustard hydrolysis product thiodiglycol were detected in the sandy clay loam extracts. These agents and the hydrolysis product related to soman, pinacolyl methylphosphonic acid, were observed in the loamy sand ex-

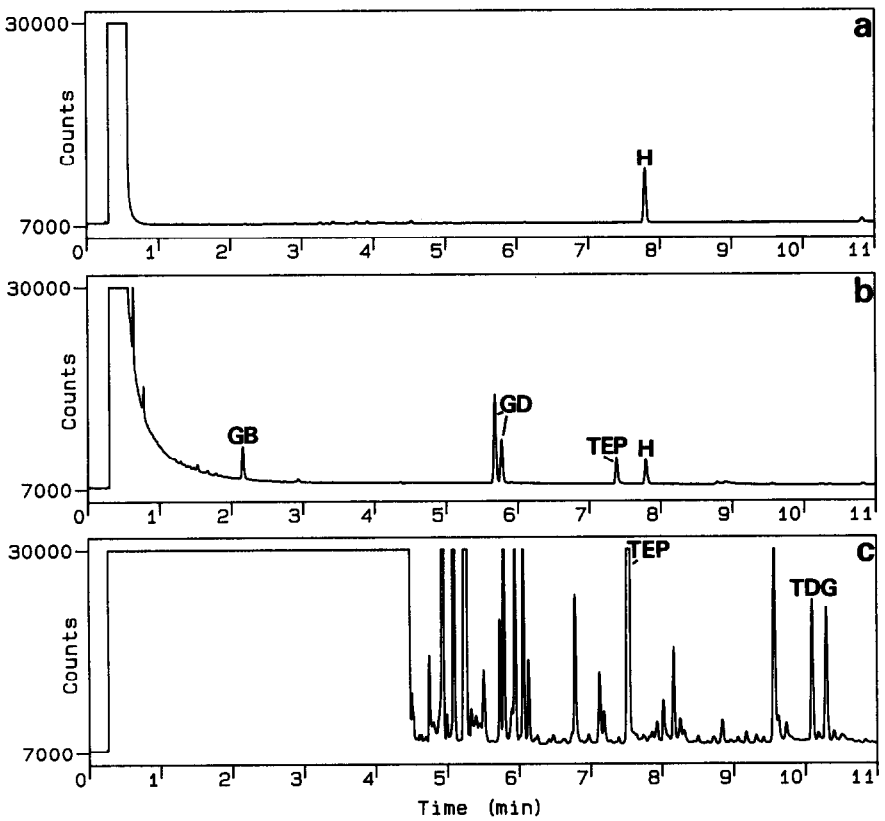

Fig. 1. Capillary column gas chromatograms of successive extraction of 50 μg/g of sarin (GB), soman (GD), triethyl phosphate (TEP) and mustard (H) added to sandy clay loam with (a) hexane, (b) dichloromethane and (c) TMS derivatizing agent. TDG = di-TMS derivatative of thiodiglycol. For conditions, see text.

tracts. Compounds were confirmed by capillary column GC–MS analysis.

Application

The developed procedure has been used for the detection of chemical warfare agents, their degradation products and related compounds in soil during range clearance operations. This sample contained tabun, a chemical warfare agent not used for method development, and sixteen compounds related to tabun. Most of the compounds, identified in the dichloromethane and TMS extracts (Fig. 3) and listed in Table II, have been previously characterized during analysis of munitions grade tabun [22]. Evidence of tabun hydrolysis was evident during GC–MS analysis of the TMS extract. Tabun, present at a trace level (Fig. 4a), and the TMS derivatives of three hydrolysis products, ethyl hydrogendimethylphosphoramidate (Fig. 4b), diethyl hydrogenphosphate (Fig. 4c) and ethyl dihydrogenphosphate (Fig. 4d) were identified on the basis of the acquired electron impact mass spectra (Table III). The first and third hydrolysis products were due to hydrolysis of the cyano and both the cyano and amine functional groups, respectively, of tabun, whereas the second product was probably due to hydrolysis of the amine function group of the principal impurity of munitions grade tabun, diethyl dimethylphosphoramidate [22].

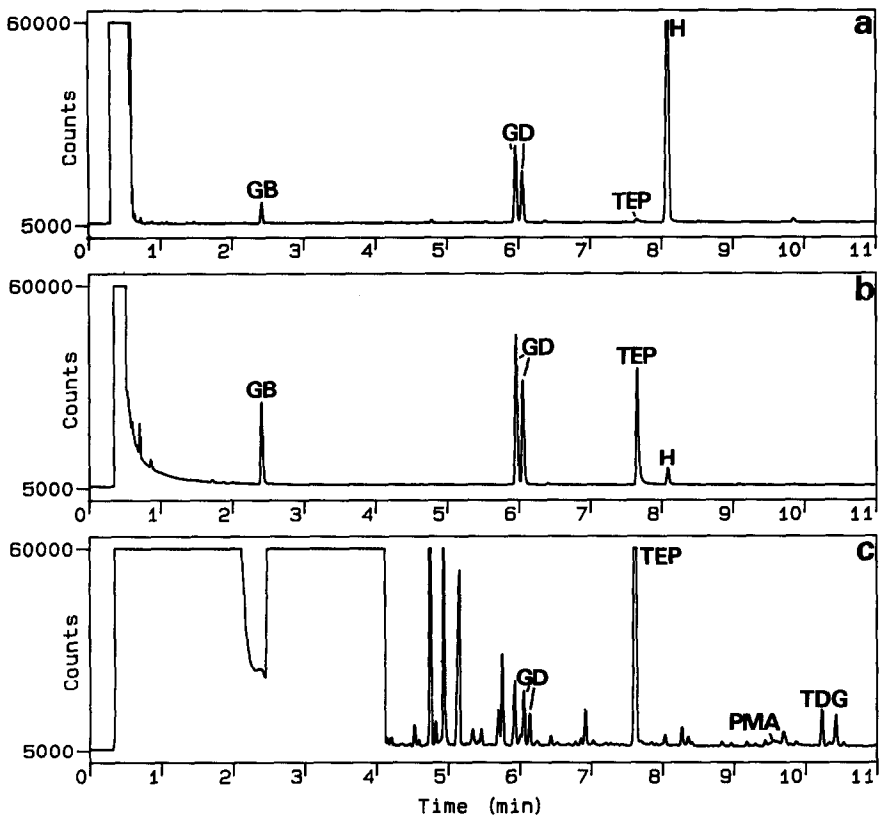

Fig. 2. Capillary column gas chromatograms of successive extraction of 50 μg/g of sarin (GB), soman (GD), triethyl phosphate (TEP) and mustard (H) added to loamy sand with (a) hexane, (b) dichloromethane and (c) TMS derivatizing agent. TDG = di-TMS derivative of thiodiglycol; PMA = TMS derivative of pinacolyl methylphosphonic acid. For conditions, see text.

TABLE II

COMPOUNDS IDENTIFIED IN RANGE CLEARANCE SOIL SAMPLES

Peak No.[a]	Mol. Wt.	Compound
1	181	Diethyl dimethylphosphoramidate
2	182	Triethyl phosphate
3	180	Ethyl tetramethylphosphorodiamidate
4	161	Tetramethylphosphorodiamidic cyanide
5	288	Bis(ethyl dimethylphosphoramidic) anhydride
6	289	Dimethyl phosphoric ethyl dimethylphosphoramidic anhydride
7	287	Ethyl dimethylphosphoramidic tetramethylphosphorodiamidic anhydride
8	219[b]	Unknown
9	395	Bis(ethyl dimethylamidophosphonyl) dimethylamidophosphonate
10	360[b]	Unknown
11	225[c]	TMS derivative of ethyl hydrogendimethylphosphoramidate
12	162	Ethyl dimethylphosphoroamidocyanidate (tabun)
13	226[c]	TMS derivative of diethyl hydrogenphosphate
14	270[c]	Di-TMS derivative of ethyl dihydrogenphosphate
15	—	Phthalate
16	386[b]	TMS derivative
17	330[b]	TMS derivative

[a] See the chromatograms in Fig. 3.
[b] Based on electron impact MS data.
[c] Identification based on interpretation of acquired MS data (Table III).

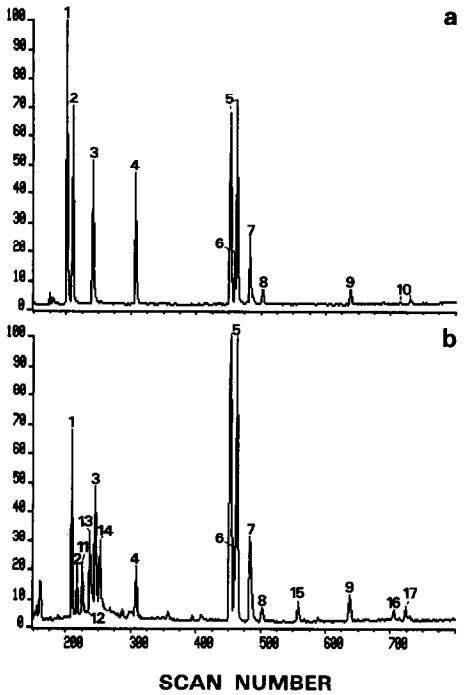

Fig. 3. Capillary column GC–total ion current (500–40 u) MS of range clearance soil sample after successive extraction with (a) dichloromethane and (b) TMS derivatizing agent. Tabun and sixteen related compounds were identified (see Table II). For conditions, see text; one MS scan = 1.8 s.

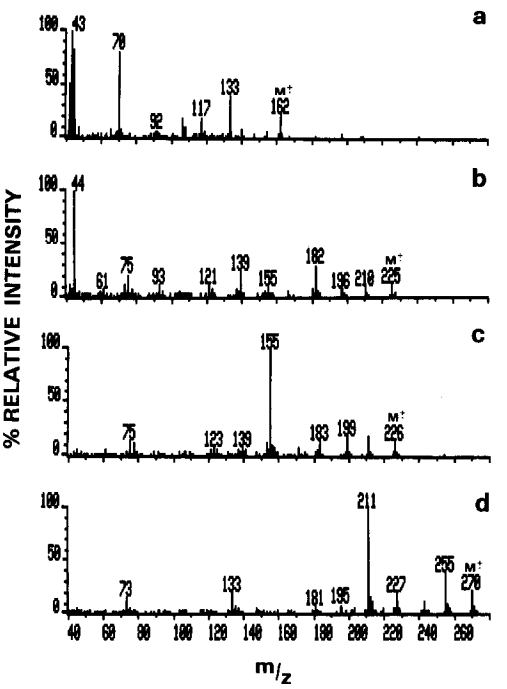

Fig. 4. Electron impact mass spectra of (a) tabun, (b) TMS derivative of ethyl hydrogendimethylphosphoramidate, (c) TMS derivative of diethyl hydrogenphosphate and (d) di-TMS derivative of ethyl dihydrogenphosphate acquired during analysis of range clearance soil sample (Fig. 3).

TABLE III

PRINCIPAL ELECTRON IMPACT IONS FOR HYDROLYSIS PRODUCTS OF TABUN (PEAK NUMBERS 11, 13 AND 14)

Peak 11 = TMS derivative of ethyl hydrogendimethylphosphoramidate [$(C_2H_5O)(O)P(OTMS)(N(CH_3)_2)$]; 13 = TMS derivative of diethyl hydrogenphosphate [$(C_2H_5O)_2(O)P(OTMS)$]; 14 = di-TMS derivative of ethyl dihydrogenphosphate [$(C_2H_5O)(O)P(OTMS)_2$].

Possible ion structure	*m/z* (relative intensity, %)		
	Peak 11	Peak 13	Peak 14
M^+	225 (11)	226 (12)	270 (21)
$[M-CH_3]^+$	210 (8)	211 (17)	255 (38)
$[M-C_2H_3]^+$	198 (2)	199 (17)	243 (10)
$[M-OC_2H_3]^+$	182 (30)	183 (12)	227 (18)
$[N(CH_3)_2]^+$	44 (100)		
$[(OH)_2P(OC_2H_5)_2]^+$		155 (100)	
$(M-CH_3-OC_2H_4]^+$			211 (100)

CONCLUSIONS

A procedure based on sequential hexane and dichloromethane extraction followed by trimethylsilyl derivatization and capillary column GC–MS confirmation has been developed for the verification of chemical warfare agents, their hydrolysis products and related compounds in soil. The developed procedure was applied to soil samples in support of range clearance operations. Trace amounts of tabun and sixteen tabun related components and their hydrolysis products were identified in the range clearance samples. The ability of the developed procedure to identify the chemical warfare agent tabun, not evaluated during spiking, and its degradation products and related compounds in uncharacterized soil clearly illustrates its potential for the verification of chemical warfare agents, their

hydrolysis products and related compounds at trace levels in soil.

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