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Identification of compounds relevant to the chemical weapons convention using selective gas chromatography detectors, gas chromatography–mass spectrometry and gas chromatography–Fourier transform infrared spectroscopy in an international trial proficiency test

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Abstract

The results of an international trial proficiency test are described. Compounds relevant to the Chemical Weapons Convention were analyzed in rubber, paint and two soil samples by using GC with selective detectors, GC–MS and GC–FTIR. The analytical strategy is presented both for routine analyses and for spectral interpretation of a compound for which no reference spectra are available. Three examples are given of spectral interpretation of alkylphosphonates.

Keywords: Detectors, GC; Trial proficiency test; Gas chromatography–mass spectrometry; Gas chromatography–Fourier transform infrared spectroscopy; Chemical warfare agents

1. Introduction

Chemical weapons were first used on a large scale in World War I. Since then they have been employed several times in conflicts around the world. Most recently, in March 1995, sarin was used in a terrorist attack in Japan.

In January 1993, a comprehensive Chemical Weapons Convention [1] was signed in Paris. For achieving the object and purpose of the Convention, an Organisation for the Prohibition of Chemical Weapons (OPCW) will be established in the Hague to verify the provisions of the Convention. At present, a Provisional Technical Secretariat (PTS) has been established to do the preparatory work required before entry into force of the Convention.

The Convention includes provisions for on-site inspections under which samples may be collected for analysis for the presence or absence of chemicals relevant to the Convention. Samples will be analyzed on-site in order to see whether they will require more detailed analysis in two well-equipped laboratories designated by the OPCW. To be designated by the OPCW, the laboratories must be accredited by an internationally recognized accreditation body, have a

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quality system, and perform successfully in proficiency tests organized by the OPCW.

The first trial proficiency test was preceded by four international inter-laboratory comparison tests (Round-Robins) organized by Finland in 1990–1993 [2–5] and one organized by the PTS in 1994 [6]. In the course of these five tests, recommended operating procedures (ROPs) for preparing and analyzing different sample matrices [7] were developed and tested. The participating laboratories gained experience in analyzing several types of samples by a variety of techniques.

In all analyses performed for the CWC, a laboratory must strive for unambiguous identification of compounds. Unambiguous identification entails confirmation by two different spectrometric techniques. In this context, EI-MS and CI-MS are regarded as separate techniques.

A total of 24 laboratories participated in the first trial proficiency test, which was arranged in January 1995. Laboratories participated in either trial proficiency test mode or in self-assessment mode. In the former mode, in which our laboratory participated, the time available for the test and final reporting was two weeks, starting from the receipt of samples. In the latter mode participants had four weeks for the analysis and two weeks for reporting.

This article describes the analytical work performed by the Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN) during the first trial proficiency test organized by the OPCW.

2. Experimental

2.1. Samples

The samples for the trial proficiency test were prepared by Centre d'Études du Bouchet (CEB; B.P 3, 91710 Vert-le-Petit, France) in cooperation with the PTS. Four unknown sample matrices were spiked: rubber, paint and two soil samples. An identified blank of each material was provided for the participating laboratories.

The paint samples (two parallels) were spiked with 50 μg of sesquimustard [3563-36-8], 200 μg of dibenz[b,f][1,4]oxazepine (CR) [257-07-8] and 200 μg of diesel oil. The rubber sample was spiked with 100 μg of mustard [505-60-2] and 100 μg of cyclohexylmethylethylphosphonate and 2 mg of diesel oil. The first soil sample was spiked with 600 μg each of thiodiglycol [111-48-8], 1,4-dithiane-1-oxide [19087-70-8] and 2-methoxyethylpinacolyl methylphosphonate. The second soil sample was spiked with 400 μg ethylphosphonic acid [6779-09-5] and 750 μg cyclohexylethylphosphonate.

The samples were distributed by CEB on Friday 13 January 1995. The Finnish Institute received the samples on Friday 13 January 1995 and the sample package was opened on Monday 16 January 1995. The analyses were completed by 27 January 1995.

2.2. Sample preparation

The sample preparation methods were based on internationally tested recommended operating procedures [7]. Modifications can be made to the methods as required by the analysis.

Volatile neutral compounds in the samples were screened before sample preparation. This was done by taking a small portion (ca. 0.1 g) of sample and thermally desorbing it with a thermal desorption cold trap system connected to a mass spectrometer.

Both rubber and paint samples were extracted with acetone, then with water. Portions of the acetone extracts were methylated with diazomethane and silylated with BSTFA. The water extracts were evaporated to dryness. The residues were silylated with BSTFA in acetonitrile–dichloromethane.

Four 10-g portions were taken from each of the soil samples. The first portion was extracted with dichloromethane and the second with water. The latter extract was evaporated to dryness, and the residue was dissolved in acidic (HCl) methanol and methylated with diazomethane. The third portion was extracted with water, and the residue after evaporation of water was silylated with BSTFA in acetonitrile–dichloromethane. The fourth portion was extracted with methanol/triethylamine, after which part of the extract was methylated with diazomethane and part was evaporated to dryness. The residue of the evaporation was silylated with BSTFA in acetonitrile–dichloromethane.

Rubber, paint and the first soil portions were extracted with a mixture of acidic (HCl) ethyl acetate and 3,4-mercaptotoluene. The extracts were

tested for lewisites 1 (2-chlorovinylarsine) and 2 (bis(2-chlorovinyl)chloroarsine).

All organic extracts were first analyzed by GC using selective detectors without concentration. Extracts were then concentrated (1:10) and analyzed by GC, GC–MS and GC–FTIR.

2.3. Gas chromatography with selective detectors

The samples were first screened with a two-channel GC (Micromat 412, HNU-Nordion LTD) equipped in one channel with a flame photometric detector (FPD) operating simultaneously in P- and S-modes and in the other with a nitrogen–phosphorus detector (NPD). The GC column (NB-54, 25 m×0.32 mm I.D., 0.25 μm film thickness, manufactured by HNU-Nordion Ltd.) was connected to the detectors via a three-way pressfit connector with capillaries (0.32 mm I.D.) of equal length to achieve a 1:1 split ratio. This arrangement provides a lot of information in a single GC run.

The chromatographic conditions were: injector temperature 250°C, detectors 300°C, carrier gas (He) flow-rate 1.5 ml/min. The GC oven was programmed from 40°C (1 min) to 280°C (10 min) at 10°C/min. Splitless injections of 1–2 μl of sample were used with 0.75 min splitless time.

In addition, the 3,4-dimercaptotoluene derivatives were screened with a flame ionization detector (FID) using the same column and GC conditions as mentioned above. FPD(S) could not be used for these samples because of the sulfur-containing reagent.

2.4. Electron ionization mass spectrometry (EI-MS)

Electron impact mass spectra were generated with a VG AutoSpecQ instrument (EBEQ geometry, VG Analytical; UK). The ion source temperature was set at 200°C, the electron energy at 70 eV. The emission current was 0.24 mA, and the trap current was 200 μA.

The samples were introduced to the mass spectrometer from an HP 5890 Series II gas chromatograph (Hewlett–Packard, USA) equipped with a split/splitless injector and an HP-5 fused-silica capillary column (25 m×0.25 mm I.D., 0.33 μm film thickness). The GC oven was programmed linearly from 40°C (1 min) to 250°C (10 min) at 10°C/min. The injector temperature was 280°C and the temperature of the transfer line between the GC and MS was 260°C. Splitless injections of 1 μl of sample with 0.8 min splitless time were used.

The MS was operated in full scan mode with a mass range from 30 to 500 amu. The scan time was 0.5 s with a delay time of 0.2 s between individual scans. The instrument was tuned to a resolution of 1000. Mass calibration was made with perfluorokerosine (PFK). A recalibration was made after each retuning of the instrument. The performance of the GC–MS system was tested daily using a test solution containing seven test compounds, listed in Table 1, at 1 ng/μl concentration level.

2.5. Thermodesorption electron ionization mass spectrometry (TD-EI-MS), chemical ionization mass spectrometry (CI-MS) and tandem mass spectrometry (CI-MS–MS)

Analysis by TD-EI-MS, CI-MS and CI-MS–MS modes was carried out with a Finnigan MAT triple stage quadrupole (TSQ 45 A) mass spectrometer,

Table 1
The concentration of test compounds for checking the retention indexes and column performance

Compound	FTIR (ng/μl)	EI-MS (ng/μl)	GC (ng/μl)
d_9-Dimethylmethylphosphonate (DMMP)	–	1	2
Trimethylphosphate (TMP)	20	–	–
2,6-Dimethylphenol (DMP)	20	1	–
5-Chloro-methylaniline (CMA)	20	1	20
Tri-*n*-butylphosphonate (TNBP)	20	1	2
Dibenzothiophene (DBT)	20	1	20
Malathion (MAL)	20	1	8
Methyl stearate (MST)	20	1	–

with sample introduction from a Finnigan 9600 gas chromatograph.

A NB-54 fused-silica capillary column (20 m× 0.32 mm I.D., 0.25 μm film thickness) manufactured by HNU-Nordion Ltd. was used for the separation of the analytes. The analytical column was equipped with a retention gap (5 m×0.20 mm I.D.). The GC oven was programmed linearly from 40°C (1 min) to 260°C at 10°C/min, and the injector temperature and the temperature of the transfer line between the GC and MS were 260°C. Splitless injections with 0.8 min splitless time were used. For thermodesorption injections a purge and trap injector manufactured by Chrompack Ltd. was used with the following operating parameters: desorption temperature 150°C for 10 min, cold trap temperature −90°C and injection temperature of cold trap 200°C for 3 min.

The mass spectra were registered with a scan speed of 1.0 s from 45 to 600 amu, from 100 to 600 amu, and from 10 to 300 amu in EI-MS, CI-MS, CI-MS–MS, respectively. Electron energy was 70 eV in EI-MS and 150 eV in CI-MS. The emission current was 0.3 mA in both modes. Source temperature was 120°C in EI-MS and 100°C in CI-MS. Isobutane was used as reactant gas at 0.40 Torr pressure. In CI-MS–MS, argon was used as collision gas at a pressure of 1.0 Torr, and collision energy was −15 eV.

The mass scale was calibrated with perfluorotributylamine (FC43). Each day the performance of the GC–MS instrument was tested with our standard test solution, shown in Table 1, at 1 ng/μl concentration level.

2.6. Fourier transform infrared spectrometry (FTIR)

The GC–FTIR analyses were performed on a Bio-Rad FTS-45 spectrometer connected to a Tracer interface unit. The temperature of the transfer line and the deposition tip was 250°C.

The spectra recorded with this system are condensed phase spectra. The detector was of MCT (mercury–cadmium–telluride) type with a spectral range of 4000–660 cm^{-1}. During the chromatographic run the spectra were recorded using 4 scans and a resolution of 8 cm^{-1}. After the run all relevant peaks were rescanned using 64 scans and a resolution of 4 cm^{-1}.

An HP 5980 series II gas chromatograph equipped with split/splitless injector provided the sample introduction. Splitless injection (1–1.5 μl) was used with 0.8 min splitless time. Separation was made as in the other techniques, with a SE-54 capillary column (20 m×0.32 mm I.D., film thickness 0.25 μm). The carrier gas was helium with an approximate flow of 1.6 ml/min. The GC oven was programmed from 40°C (2 min) to 250°C (10 min) at a rate of 10°C/min.

Library searches were made by using the square derivative search algorithm [8]. All spectra were first searched without masking of any spectral regions. If there were considerable interferences in the spectrum the search was repeated with the vibrations due to ice (3900–3100 and 2000–1600 cm^{-1}) and carbon dioxide (2450–2250 cm^{-1}) masked. Ice was formed from water molecules in the surrounding atmosphere condensing on the deposition surface, and carbon dioxide was due to variations in the purge air.

2.7. Retention index monitoring (RIM)

The M-series [9] (commercially available from HNU-Nordion Ltd.) was used as a retention index standard. The identification was performed with the aid of temperature-programmed retention indexes [10] with the M-series as standards and an index window of ten index units. External RIM was chosen for identification. In this method the retention times of the standards are measured in a separate calibration run. Retention index standards were run at least after every ten runs and the nearest run was used for the calculation of indexes of the sample. The chromatographic data were processed on a PC with a SuniCom workstation program (SuniCom Oy, Finland) containing a program for automated RIM and a retention index library of 130 treaty-related compounds.

2.8. Quality control

A solvent blank run was used to monitor any possible contamination arising from outside the sample itself, e.g. from the syringe or the memory effect of the equipment. The pure solvent used in the sample preparation was run as blank at the beginning of each sample series and whenever there was any suspicion of contamination.

In GC with selective detectors a sensitivity test solution was used to check the sensitivity of the detectors. This test solution was run after the installation of detectors or columns, and with each new set of samples at a minimum after every 20th sample.

Column performance and retention index stability were tested with a test mixture of seven compounds (Table 1), and on the basis of the test a retention index window was selected for the sample analysis. The test mixture was prepared in ethyl acetate.

The test mixture was run before the analysis of samples and between sample analyses, with the test mixture included at least after each 20th sample. Retention indexes for test compounds were calculated by the external standard method.

When the peak shape of d_9-dimethylmethylphosphonate or trimethylphosphate was poor, three meters were cut from the front end of the analytical column and three meters of the same type of column were connected to the rear end of the column using a press-fit connector.

2.9. Synthesis of reference compounds

Cyclohexylmethylethylphosphonate was synthesized from cyclohexylethylphosphonofluoridate after this was hydrolyzed in 0.17 *M* NaOH. The resulting acid was evaporated to dryness, dissolved in acidic methanol and methylated with diazomethane.

2-Methoxyethylpinacolylmethylphosphonate was synthesized from methylphosphonic dichloride. Methylphosphonic dichloride and 2-methoxyethanol were mixed in equivalent amounts in $CDCl_3$–pyridine at 60°C. After this, an equivalent amount of pinacolyl alcohol was added to the mixture.

Methyltrimethylsilylethylphosphonate was synthesized from methylethylphosphonofluoridate, after this was hydrolyzed in 0.1 *M* NaOH. The resulting acid was evaporated to dryness, dichloromethane–acetonitrile was added and the solution was silylated with BSTFA.

3. Results and discussion

3.1. Retention indexes

Many studies have been made during the last ten years on the application of retention indexes in the identification of organic compounds. The retention indexes of some CW agents with respect to *n*-alkanes (C-series) have been reported [11–14]. Unfortunately the *n*-alkanes are not detectable with selective detectors and other series have had to be developed. Selective detectors play an important part in the verification of treaty-related compounds, because the compounds are rich in heteroatoms. Thus, one of the most attractive series is the M-series of alkylbis(trifluoromethyl)phosphine sulfides, whose heteroatoms (S, P, and F) make them detectable with selective detectors.

The reproducibility of retention indexes makes it possible to create a useful retention index library on a computer. The reliability and simplicity of index monitoring is increased significantly by using a computer program that searches for the retention index pattern, calculates the retention indexes for all peaks in the chromatogram and then compares the indexes with the library data.

In addition to the screening of compounds of interest according to their retention indexes, retention indexes can be used to locate the correct peak in other analytical techniques.

3.2. Identification strategy

Our identification strategy was to make good use of all techniques available in a complementary way. Spectral and chromatographic data were obtained with as many techniques as possible to produce sufficient information for reliable structure elucidation. Where no reference spectrum was available a reference compound was synthesized.

Before sample preparation, all the samples were screened for volatile compounds by EI-MS using thermodesorption as sample introduction method. After sample preparation the samples were screened by GC with selective detectors. Also nuclear magnetic resonance (NMR) was used in the analysis of the samples, but these results will be published in a separate article [15]. The screening gave retention indexes for compounds containing phosphorus, sulfur or nitrogen. After this the samples were analyzed by EI-MS and FTIR and then by CI-MS or CI-MS–MS depending on the concentration of the analytes.

An unambiguous identification requires at least two different spectrometric methods. The methods available to us were EI-MS, CI-MS (or CI-MS–MS),

FTIR and NMR. Any combination of two of these methods giving consistent results is regarded as unambiguous. In making a spectral interpretation, however, as much data as possible should be available before synthesis of the reference chemicals. The fuller the compound information, the easier and more reliable will be the interpretation. Fig. 1 shows the steps in the interpretation of spectra of an unknown compound.

Retention indexes play an important role in all of the methods based on gas chromatographic sample introduction. The retention index data obtained from the GC screening are passed on to all other instruments together with the sample. In this way it is ensured that all techniques focus on the same peaks even in complex samples.

3.3. Analytical results

In this article we discuss only those instrumental techniques for which a gas chromatograph provided sample introduction. Results of the analyses are summarized in Table 2.

All nine compounds spiked to the samples, and three non-spiked degradation products (2, 10 and 11), were found. Thiodiglycol (2), a degradation product of mustard produced by hydrolysis of the C–Cl bond, was found in the rubber sample. Methylethylphosphonate (11), also found in the rubber

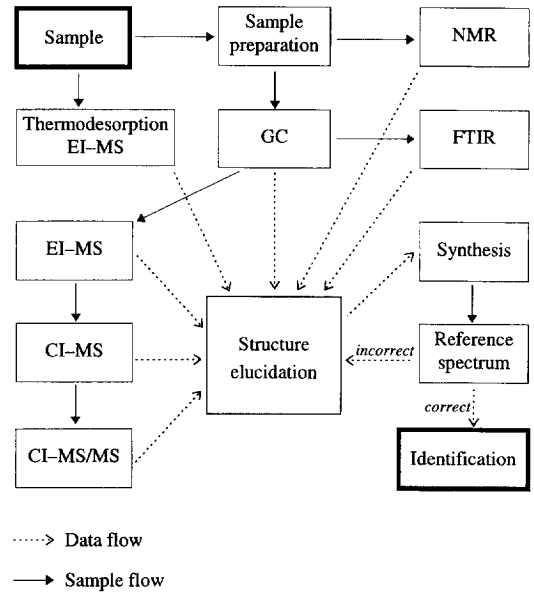


Fig. 1. Flow chart of the interpretation process for an unknown compound.

Table 2
Summary of identification results

Sample	Compound[a]	Spiked chemical	Identification	
			Techniques	Type
Rubber	1. Mustard	Yes	MS(EI, CI), IR[b]	Search
	2. Thiodiglycol (S)	No	MS(EI, CI), IR	Search
	3. Cyclohexyl methyl ethylphosphonate	Yes	MS(EI, CI), IR	Interpretation
	11. Methyl ethylphosphonate (S)	No	MS(EI, CI-MS–MS), IR	Interpretation
Paint	4. Sesquimustard	Yes	MS(EI, CI), IR[b]	Search
	5. CR	Yes	MS(EI, CI), IR[b]	Search
Soil 1	2. Thiodiglycol	Yes	MS(EI), IR	Search
	Thiodiglycol (S)	Yes	MS(EI, CI), IR	Search
	6. 2-Methoxyethyl pinacolyl methylphosphonate[c]	Yes	MS(EI, CI), IR	Interpretation
	7. 1,4-Dithiane-1-oxide	Yes	MS(EI, CI), IR	Search
	10. Pinacolyl methylphosphonate (M)[c]	No	MS(EI, CI-MS–MS)	Search
Soil 2	8. Ethylphosphonic acid (M)	Yes	MS(EI, CI), IR	Search
	Ethylphosphonic acid (S)	Yes	MS(EI, CI), IR	Search
	9. Cyclohexyl ethylphosphonate (M)	Yes	MS(EI, CI), IR	Intepretation
	Cyclohexyl ethylphosphonate (S)	Yes	MS(EI, CI)	Interpretation

[a] (S) analyzed as TMS derivative, (M) analyzed as methylated.
[b] Compound also identified using thermodesorption-EI-MS.
[c] Diastereomeric compounds: letters A and B are later used to identify the different diastereomers.

sample, is a degradation product of cyclohexylmethylethylphosphonate produced when the ester bond to cyclohexyl breaks. Pinacolylmethylphosphonate (10), found in the first soil sample, is formed in a similar way, through the break-up of the ester bond to 2-methoxyethyl.

Three compounds were identified in thermodesorption-EI-MS: mustard in the rubber sample, and sesquimustard and CR in the paint sample.

Except thiodiglycol in the first soil sample in this test all identified compounds were seen with selective GC detectors. Thiodiglycol was not seen without derivatization with BSTFA because it is a highly adsorptive compound and difficult to detect in low concentrations if the column is not in excellent condition. The relative response of thiodiglycol is lower in FPD than in EI-MS. It must always be remembered that there are some compounds relevant to chemical warfare agents that are not visible to selective detectors, e.g. benzilic acid, phosgene and a number of compounds containing arsenic. These compounds can be screened using EI-MS in selective ion monitoring (SIM) mode.

Compounds with a reference spectrum in a library can be identified routinely. Seven of the identified compounds (1, 2, 4, 5, 7, 8, and 10) were analyzed routinely through spectral searches or comparison with hard copy spectra. In EI-MS, four of the compounds (1, 2, 4, and 5) were identified by spectral search made by computer, and the rest through reference to hard copy spectra recorded from authentic samples at the Institute. In FTIR, five compounds (1, 2, 4, 5, and 8) were identified by spectral search. 1,4-Dithiane-1-oxide (7) was identified by comparison with a published reference spectrum [6]. All routine identification data are summarized in Table 3.

Interpretation of spectra was required for four of the compounds (3, 6, 9 and 11), and three reference chemicals (3, 6, and 11) had to be synthesized. (After methylation compound 9 was identical with compound 3.) The compounds to be synthesized were chosen on the basis of the interpretation of the spectral data. The synthesis products were investigated by EI-MS and FTIR, and silylated methylethylphosphonate (11) also by CI-MS–MS. The spectra were added to spectral libraries, and the sample spectra were once again searched in the libraries. Table 4 summarizes the results of these searches.

Table 3
Summary of compounds identified on the basis of retention indexes and reference spectra

Sample	Compound[a]	RI Search (RI/ΔRI)		EI Search Purity[b,c]	CI M_r	IR Search HQI/Mask[c,d]
		GC	FTIR			
Rubber	1. Mustard	715.9/+0.4	711.9/−3.6	993	158	0.41/Yes
	2. Thiodiglycol (S)	973.0/−1.6	973.5/−1.1	HC	266	0.50/Yes
Paint	4. Sesquimustard	1262.3/+4.7	1253.4/−4.2	993	218	0.87/Yes
	5. CR	1367.3/−2.2	1369.9/+0.4	997	195	0.45/No
Soil 1	2. Thiodiglycol	–	741.9/+18.5	918	–	0.72/Yes
	Thiodiglycol (S)	973.7/−0.9	976.1/+1.5	HC	266	0.17/No
	7. 1,4-Dithiane-1-oxide	934.8/–	951.8/–	HC	136	HC
Soil 2	8. Ethylphosphonic acid (M)	498.0/–	504.2/–	HC	138	0.72/Yes
	Ethylphosphonic acid (S)	763.1/–	–	–	–	–
	10. Pinacolyl methylphosphonate-A (M)	745.0/+4.1	–	HC	MS–MS	–
	Pinacolyl methylphosphonate-B (M)	751.8/+3.1	–	HC	MS–MS	–

[a] (S) analyzed as TMS derivative, (M) analyzed as methylated.
[b] Purity 0–1000, 1000 perfect match.
[c] HC=Compared to hard copy spectrum.
[d] Hit quality index, 0.00 perfect match, 0.01–0.60 good match, >1.00 very poor match. Mask: No: no masking used, Yes: 3900–3100, 2450–2250, 2000–1600 cm^{-1} masked.

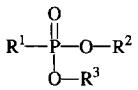
Table 4
Summary of compounds identified on the basis spectral identification

Sample	Compound[a]	RI value		EI Search (purity[b])	CI (M_r)	IR Search (HQI/Mask[c])
		GC	FTIR			
Rubber	3. Cyclohexyl methyl ethylphosphonate	1010.6	1012.8	984	206	0.50/Yes
	11. Methyl ethylphosphonate (S)	645.8	634.1	913	MS–MS	0.08/No
Soil 1	6. 2-Methoxyethyl pinacolyl methylphosphonate-A	998.5	1000.7	960	238	0.60/No
	2-Methoxyethyl pinacolyl methylphosphonate-B	1013.5	1014.7	993	238	0.47/No
Soil 2	9. Cyclohexyl ethylphosphonate (M)	1011.7	1013.3	994	206	0.21/No
	Cyclohexyl ethylphosphonate (S)	1111.6	–	–	–	–

The spectral searches were made after the spectra of the authentic compounds had been added to spectral libraries.
[a] (S) analyzed as TMS derivative, (M) analyzed as methylated.
[b] Purity 0–1000, 1000 perfect match.
[c] Hit quality index, 0.00 perfect match, 0.01–0.60 good match. >1.00 very poor match. Mask: No masking used, Yes: 3900–3100, 2450–2250, 2000–1600 cm^{-1} masked.

3.4. Interpretation of the structure of dialkyl alkylphosphonates by IR and MS

IR spectrometry is extremely well suited for the interpretation of the structure of dialkyl alkylphosphonates. There are three alkyl groups (R^1, R^2, and R^3) to be identified in these compounds:



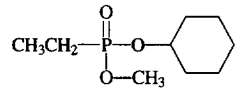
If R^1 is methyl or ethyl there is a single or double peak near the P=O absorption at 1320–1280 cm^{-1}. The ester groups R^2 and R^3 can be identified in the region 1250–1050 cm^{-1} between the strong P=O and P–O–C absorption peaks [16].

In both EI-MS and CI-MS, lower molecular mass (M_r<200) dialkylphosphonate compounds produce the common ion $R^1P(OH)_3^+$, m/z 82+R (m/z 97 for methylphosphonates and m/z 111 for ethylphosphonates) [17–19]. In some of the compounds the ion $R^1P(OR^2)(OH)_2^+$ is found as well [18]. Alkyl methyl alkylphosphonates where R^1 is methyl or ethyl produce in EI-MS at least the ions $R^1P(OCH_3)(OH)_2^+$ (m/z 111 or 125), $CH_3P(O)OCH_3^+$ (m/z 93 or 107) and $CH_3P(O)OH^+$ (m/z 79) [20].

3.5. Identification of cyclohexyl methyl ethylphosphonate (3)

An intense peak was found by GC–FPD(P) and GC–NPD in the neutral extract of the rubber sample and the methylated water extract of the second soil sample, but not in the neutral extract of the second soil. This suggested that the peak was produced by a methyl ester of a phosphorus-containing acid, and confirmation was provided by the identical MS–EI and FTIR spectra recorded for the neutral extract of the rubber sample and the methylated extract of the soil sample.

The infrared spectral search gave cyclohexyl methyl methylphosphonate as the first hit, but the compound was clearly not the methylphosphonate. Examination of the 1250–1050 cm^{-1} region of the FTIR spectrum (Fig. 3a) showed it to be an alkylphosphonic acid ester with both methoxy and cyclohexoxy groups at the phosphorus [16], and as the peak structure in the region 1300–1220 cm^{-1} was similar to that of dimethyl ethylphosphonate, it must be an ethylphosphonate:



From this, the compound in the rubber sample was concluded to be cyclohexylmethylethylphosphonate and the compound in the second soil sample to be cyclohexylethylphosphonate.

For cyclohexylmethylethylphosphonate, a reference EI-MS spectrum was found in the literature [20] with a good visual match with the obtained spectrum (Fig. 3b). The CI-MS spectrum (Fig. 3c) confirmed the molecular mass deduced to be 206 (MH^+ m/z 207).

The compound was synthesized and EI-MS and

FTIR spectra were recorded. Both techniques showed the proposed structure to be the correct one.

3.6. Identification of 2-methoxyethylpinacolyl-methylphosphonate (6)

Both GC–FPD(P) (Fig. 2b) and GC–NPD showed a strong peak pair in the neutral extract of the first soil sample with peak height ratio of 1:2 and a retention index difference of 15 index units. No signal was observed on the sulfur channel of the FPD (Fig. 2a).

EI-MS and FTIR spectra of the two peaks were the same, suggesting that the compound was a diastereomeric alkylphosphonic acid ester. A com-

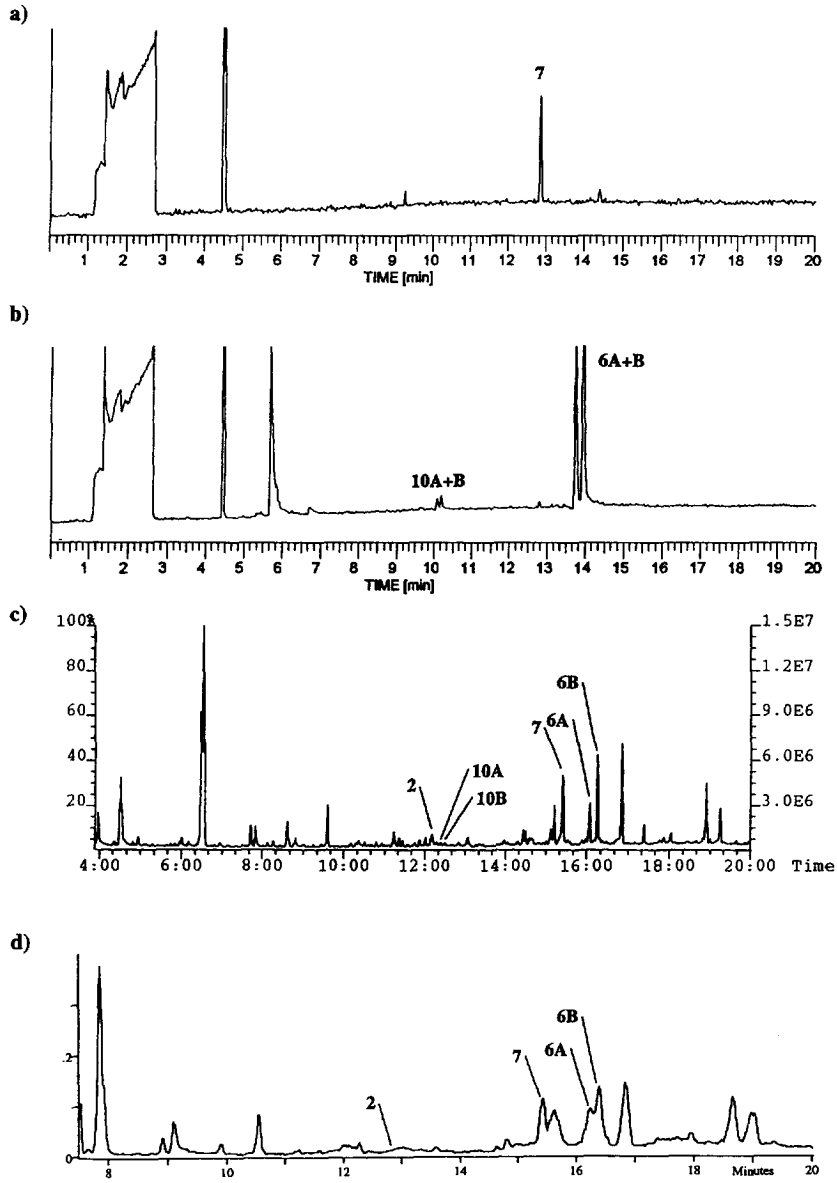


Fig. 2. Chromatograms of methylated and concentrated water extract of the first soil sample: (a) FPD S-channel, (b) FPD P-channel, (c) EI-MS total ion chromatogram, and (d) FTIR Gram–Schmidt chromatogram.

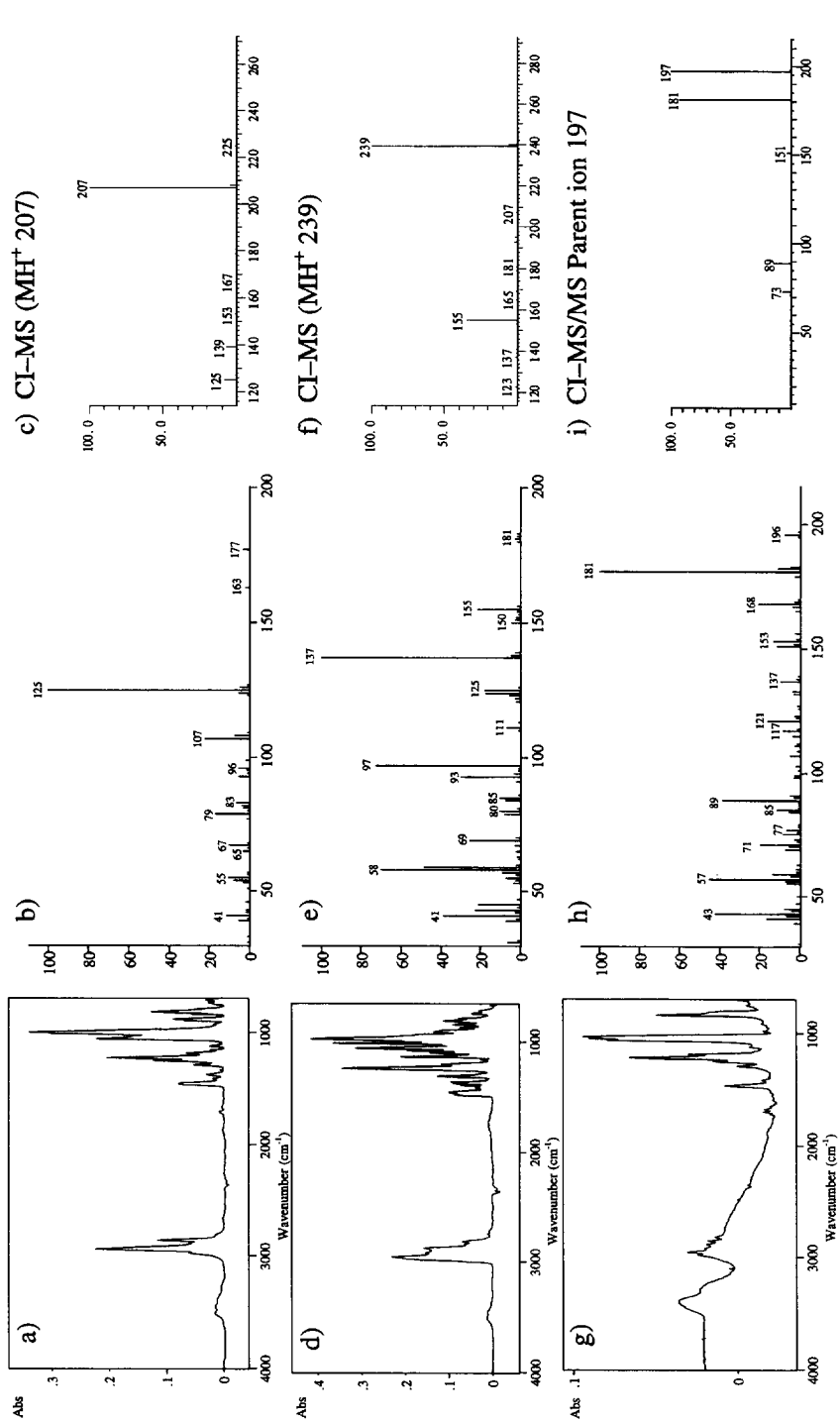
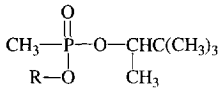


Fig. 3. FTIR, EI-MS, CI-MS, and CI-MS–MS spectra of interpreted compounds: (a–c) cyclohexylmethylethylphosphonate (3); (d–f) 2-methoxyethylpinacolylmethylphosphonate (6); (g–i) methyltrimethylsilylethylphosphonate (11).

mon group producing this type of strong GC separation in chemical weapons is the pinacolyl group [$-CH(CH_3)C(CH_3)_3$].

The FTIR spectrum (Fig. 3d) showed peaks typical of the pinacolyl group in region 1300–1220 cm^{-1} [16]. From the spectrum it was clear that the molecule also contained a second ester group not previously observed (i.e. not alkyl group C_1–C_4, straight chain alkyl group C_5–C_{10}, pinacolyl, or cyclohexyl) [16]. The peak at 1311 cm^{-1} indicated a methyl group directly connected to the phosphorus. Thus the structure of the compound was deduced to be of the following type:



A CI-MS spectrum (Fig. 3f) gave the molecular mass 238 (MH^+ m/z 239). Subtraction the known part of the molecule [$CH_3P(O)OCH(CH_3)C(CH_3)_3$] from this indicated a mass of 59 for the unknown ester group, for which the simplest formula is C_3H_7O.

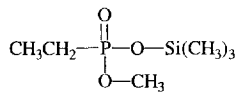
An MS–EI spectrum (Fig. 3e) showed, among others, ions m/z 155 and m/z 137. The ion m/z 155 is in agreement with a cleavage of the pinacolyl group producing ion $CH_3P(OH)_2(OR)^+$, which also was clearly present in the CI-MS spectrum. The ion m/z 137 could have been $CH_3P(O)(OR)^+$, formed from ion m/z 155 via cleavage of a water molecule, while ion m/z 181 could have been $CH_3P(OH)_2(OCH(CH_3)C(CH_3)_3)^+$, formed by loss of the unknown ester group from molecular the ion. The ion for the pinacolyl fragment m/z 84 also was present in the spectrum, but the ion of the unknown ester group, m/z 58, was of stronger intensity.

If the nerve agent soman (pinacolylmethylphosphonofluoridate) is decontaminated with DS2 decontamination solution, the fluorine atom is replaced by a 2-methoxyethoxy group [21,22]. This primary decomposition product (2-methoxyethylpinacolylmethylphosphonate) was synthesized, and both EI-MS and FTIR confirmed it to be the unknown compound in the first soil sample. The synthesized compound contained the diastereomers in equal amounts.

3.7. Identification of methylethylphosphonate (11)

Dimethylethylphosphonate was seen in both EI-MS and FTIR spectra of the methylated water extract of the rubber sample, and was confirmed in library searches. The compound was not seen in the neutral extract, however. In the silylated water extract an additional phosphorus peak was found by both GC–FPD(P) and GC–NPD. This suggested the possibility of methylethylphosphonate because the compound was not ethylphosphonic acid for which a reference spectrum was available.

The FTIR spectrum (Fig. 3g) of the silylated compound contained a peak at 1186 cm^{-1} corresponding to methoxy group connected to phosphorus. The peak was of lower intensity than that produced by dimethylethylphosphonate, however, indicating that only one methoxy group was present in the molecule. The region 1300–1220 cm^{-1} was the same as in cyclohexylmethylethylphosphonate and dimethylethylphosphonate, which suggested that the compound was an ethylphosphonate. There was also a peak at 851 cm^{-1}, which can be seen also in other trimethylsilylated alkylphosphonic acids. Based on the FTIR data, the structure of the compound was thus



The EI-MS spectrum (Fig. 3h) of the silylated compound included a possible molecular ion, m/z 196, with m/z 181 as the base peak. Also present was ion m/z 73, typical for silylated compounds. A CI-MS–MS spectrum (Fig. 3i) was measured from the suspected MH^+ ion m/z 197. Methyltrimethylsilylethylphosphonate was synthesized, and EI-MS, CI-MS–MS, and FTIR spectra confirmed the compound to be the correct one. The conclusion was that the original compound in the sample was methylethylphosphonate.

3.8. Detection limits

The detection limit of the total analysis depends on the particular combination of spectrometric tech-

niques employed. The detection limit is lower when routine identifications are performed with just EI-MS and CI-MS than when structure elucidation requires the use of FTIR spectra in addition.

The detection limits of the selective GC detectors are a limiting factor. Since any compound present in the sample below these limits will not be seen care must always be taken to search for them in the raw MS data. The approximate detection limits of the GC screening are 10 pg (NPD) and 50 pg (FPD) for phosphorus compounds, 500 pg (FPD) for sulfur compounds, and 300 pg (NPD) for nitrogen compounds.

The detection limit in full scan EI-MS is approximately 300 pg and the detection limits in CI-MS and CI-MS–MS methods are about 1 ng/μl and 0.2 ng/μl, respectively. The detection limit in FTIR depends on the structure of the compound. The approximate detection limit during the test was 0.5–1 ng for phosphonate compounds and 3–5 ng for mustard.

4. Conclusions

The time frame of two weeks for the identification of compounds by several different spectrometric techniques, the spectral interpretation of unknowns, and the synthesis of reference compounds requires a high level of instrumentation, skilled and experienced personnel, and a well-planned analytical strategy.

In routine analysis of chemical weapons related compounds, detection and identification of relevant chemicals are normally based on a comparison of the experimental data with data recorded in the database of the instrument. Unambiguous identification requires that results be obtained by at least two spectrometric techniques (EI-MS, CI-MS, FTIR or NMR).

If reference spectra are not available, the data obtained from the different techniques must be interpreted. In this case, use of the same column and retention indexes with all instruments will ensure that the data obtained from different instruments refer to the same peak. The compound deduced to be present must then be synthesized to allow that the corresponding reference spectra to be measured. For quick synthesis of reference compounds, it is advisable to have some basic precursors available in the laboratory.

In the present test, once all possible chromatographic and spectroscopic information was available, the structure elucidation proved to be fairly straightforward. It is worth emphasizing that GC–FTIR spectrometry alone provided nearly complete structures of the three interpreted phosphonate compounds. It was only when the ester chain of the phosphonate was not present in a library compound (e.g. 2-methoxyethoxy) that IR spectrometry could not provide the full structure.

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References

[1] Report of the Ad Hoc Committee on Chemical Weapons, CD/1170, 26 August 1992.
[2] M. Rautio (Editor), Inter-laboratory Comparison (Round-Robin) Test for the Verification of Chemical Disarmament, F.1. Testing of Existing Procedures, The Ministry for Foreign Affairs of Finland, Helsinki, 1990.
[3] M. Rautio (Editor), Interlaboratory Comparison (Round-Robin) Test for the Verification of Chemical Disarmament, F.4. Validating of Procedures for Water and Soil Samples, The Ministry for Foreign Affairs of Finland, Helsinki, 1993.
[4] M. Rautio (Editor), Interlaboratory Comparison (Round-Robin) Test for the verification of Chemical Disarmament, F.3 Testing of Procedures on Simulated Military Facility Samples, The Ministry for Foreign Affairs of Finland,Helsinki, 1992.
[5] M. Rautio (Editor), Interlaboratory Comparison (Round-Robin) Test for the Verification of Chemical Disarmament, F.4. Validating of Procedures for Water and Soil Samples, The Ministry for Foreign Affairs of Finland, Helsinki, 1993.
[6] M. Rautio (Editor), Interlaboratory Comparison Test Coordinated by the Provisional Technical Secretariat for the Preparatory Commission for the Organization for the Prohibition of Chemical Weapons, H.1 First Interlaboratory Comparison Test, The Ministry for Foreign Affairs of Finland, Helsinki, 1994.

[7] M. Rautio (Editor), Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament, 1994 Edition, The Ministry for Foreign Affairs of Finland, Helsinki, 1994.
[8] S.R. Lowry and D.A. Huppler, Anal. Chem., 53 (1981) 889–893.
[9] A. Manninen, M-L. Kuitunen and L. Julin, J. Chromatogr., 394 (1987) 465–471.
[10] H. van den Dool and P.D. Kratz, J. Chromatogr., 11 (1963) 463–471.
[11] P.A. D'Agostino and L.R. Provost, J. Chromatogr., 31 (1985) 47.
[12] P.A. D'Agostino, L.R. Provost and J. Visentini, J. Chromatogr., 402 (1987) 221.
[13] P.A. D'Agostino, L.R. Provost and J. Visentini, J. Chromatogr., 436 (1987) 399.
[14] M.T. Söderström, R.A. Ketola and O. Kostiainen, Fresenius J. Anal. Chem., 352 (1995) 550.
[15] M. Mesilaakso and E.-L. Tolppa, in preparation.
[16] M.T. Söderström and R.A. Ketola, Fresenius J. Anal. Chem., 350 (1994) 162–167.
[17] J.L. Occolowitz and G.L. White, Anal. Chem., 35 (1963) 1179.
[18] J.L. Occolowitz and J.M. Swan, Aust. J. Chem., 19 (1966) 1187–1195.
[19] S. Sass and T.L. Fisher, Org. Mass Spectr., 14 (1979) 257–264.
[20] Identification of Degradation Products of Potential Organophosphorus Warfare Agents, An Approach for the Standardization of Techniques and Reference Data, The Ministry for Foreign Affairs of Finland, Helsinki, 1980.
[21] W.T. Beaudry, L.L. Szafraniec, D.K. Rohrbaugh and D.R. Leslie, in P. Reeves (Editor), Proceedings of the 1990 CRDEC Scientific Conference on Chemical Defense Research, CRDEC, Aberdeen Proving Ground, MD, 1990, pp. 815–821.
[22] W.T. Beaudry, L.L. Szafraniec and D.R. Leslie, Reactions of Chemical Warfare Agents with DS2: Product Identification by NMR, I. Organophosphorus Compounds, Report (1992), CRDEC-TR-364; Order No. AD-A254284, 49 pp. Avail: NTIS, CRDEC, Aberdeen Proving Ground, MD, 1992.