Fresenius J Anal Chem (1995) 352:550–556

Fresenius' Journal of Analytical Chemistry

Identification of some nerve agent homologues and dialkyl methylphosphonates by gas chromatography/Fourier transform infrared spectrometry

Part II: Spectral search with the help of retention indices

Martin T. Söderström, Raimo A. Ketola, Olli Kostiainen

Finnish Institute for Verification at the Chemical Weapons Convention, PO Box-55, FIN-00014 University of Helsinki, Finland

Received: 2 December 1994/Accepted: 16 January 1994

Abstract. GC-FTIR spectra of 55 nerve agent homologues and dialkyl methylphosphonates have been analyzed. Infrared spectral library searches together with retention index searches have been used for identification of nerve agent homologues and dialkyl methylphosphonates.

Introduction

All the 55 nerve agent homologues and dialkyl methylphosphonates analyzed for this study are chemicals covered by the Chemical Weapons Convention (CWC) [1]. The Convention is expected to enter into force in 1995. Several methods for the sample treatment and analysis of chemical warfare agents with different analytical methods have been developed and tested during the four international 'Round-Robin' tests coordinated by Finland [2].

For unambiguous identification of an unknown compound in the 'Round-Robin' tests, identification by two different spectrometric techniques was required together with retention behaviour information. The use of retention indices is the most reliable method for GC identification. The original Kováts' retention indices [3] are determined using a homologue series of *n*-alkanes as reference in an isothermal run. If the boiling points of the compounds in a mixture differ considerably several isothermal runs are needed. Retention indices obtained with a linear temperature programme allow the indices of both more volatile and less volatile compounds to be determined in a single GC run. Shaps et al. [4] used retention indices together with infrared (IR) spectral searches to identify a specific alcohol from a group of compounds with very similar IR spectra. The aim of the current work is to study the use of retention indices in routine GC–FTIR work as additional help and support to the identification of compounds by IR spectra.

The basic structures of the three nerve agent families are shown in Table 1, structures **1**, **2** and **3**. Groups R^1 and R^2 can be saturated, either aliphatic or cyclic, hydrocarbon chains. In treaty-related chemicals the maximum size of group R^1 is restricted to three carbons and group R^2 to ten carbons. Molecules having bigger R^1 or R^2 groups are considered useless as warfare agents. In the VX family (structure **3** in Table 1) the $R^{1'}$ group has the same restrictions as the R^1 group.

Alkylphosphonic acids are the primary hydrolysis products of nerve agents of structure **1** [5]. One way to enable the gas chromatographic analysis of these compounds is to methylate them and thus produce eluable dialkyl alkylphosphonates (structure **4** in Table 1). The groups R^1 and R^2 originate from the alkyl groups of nerve agents or alcohols used in the synthesis. The groups R^2 and $R^{2'}$ can also be the result of the methylation of an acid.

In a previous article [6] the identification of nerve agent homologues and dialkyl methylphosphonates by spectral interpretation was discussed.

Experimental

Infrared spectrometer

This study was performed on a Bio-Rad Tracer gas chromatographic interface system [7, 15]. In this system the compounds eluting from the gas chromatographic column are condensed onto a moving ZnSe slide cooled by liquid nitrogen.

The FTIR spectrometer used was a Bio-Rad FTS-45 equipped with a MCT detector (4000–660 cm^{-1}). During the gas chromatographic run spectra were recorded at 8 cm^{-1} resolution and averaging four scans. Chromatograms produced from the infrared data have one spectrum per second. After the chromatographic run the spectra were rescanned from the ZnSe slide at 4 cm^{-1} resolution and averaging 64 or 256 scans. The data system was a Digilab SPC3200.

Gas chromatograph

The gas chromatograph used was a Hewlett-Packard 5890 Series II gas chromatograph with a split/splitless

Correspondence to: M.T. Söderström

Table 1. Numbering of the analyzed nerve agents and dialkyl methylphosphonate compounds.

R^2	Sarin family 1		Tabun family 2		VX family 3						Dialkyl methylphosphonates 4					
	R			R^1	R^1						R^1					
						methyl			ethyl				methyl			
		$R^{1'}$	methyl	ethyl	methyl						R^2	methyl	ethyl	propyl	i-propyl	
methyl		**1a**	**1n**	**2a**		**3a**	**3b**	**3c**		**3f**	**3i**		**4a**			
ethyl		**1b**	**1o**	**2b**					**3d**	**3g**	**3j**		**4b**	**4f**		
propyl		**1c**	**1p**	**2c**									**4c**		**4g**	
i-propyl		**1d**	**1q**	**2d**					**3e**	**3h**	**3k**		**4d**			**4h**
butyl		**1e**	**1r**	**2e**												
i-butyl		**1f**	**1s**	**2f**												
s-butyl		**1g**	**1t**	**2g**												
pentyl		**1h**	**1u**	**2h**												
hexyl		**1i**	**1v**	**2i**												
pinacolyl		**1j**	**1w**										**4e**			
cyclohexyl		**1k**	**1x**													
heptyl		**1l**	**1y**	**2j**												
2-methylcyclohexyl		**1m**														

injector. Splitless injection was used with a splitless time of 0.80 min. The column was a SE-54 fused silica capillary column (25 m, 0.32 mm i.d., 0.25 μm film thickness) from HNU-Nordion, Ltd, Finland.

The column temperature was programmed from 40 °C (2 min) to 250 °C (10 min) at rate of 10 °C/min. The temperature of the transfer line between the gas chromatograph and the spectrometer was kept at 250 °C.

Helium (99.9995%) was used as carrier gas with a flow rate of 1.6 ml/min measured at 40 °C. The carrier gas was dried using a OM-1 tube from Supelco, Inc., USA A typical injection volume was 1 μl and the concentrations of the test compounds were between 100 and 300 ng/μl.

Retention indices

A mixture known as the M-series (available from HNU-Nordion, Ltd, Finland) was used as an internal index reference standard. The mixture consists of alkylbis(trifluoromethyl)phosphite sulphides, $(CF_3)_2P(S)R$, where R is an *n*-alkyl group having 3, 4, 6, 8, ... or 20 carbons [8]. This mixture is being used in our laboratory instead of the more commonly used *n*-alkane series (C-series). M-series can be used in preliminary screening of the samples using selective detectors (e.g. NPD, ECD, FPD, PID) because it contains heteroatoms (S, P, F).

Retention indices (RI) were calculated using the linear polygon method [9]:

$$RI = 100i\frac{X - M_n}{M_{n+i} - M_n} + 100n$$

where X is the retention time of the unknown compound, M_n and M_{n+i} are the retention times of the M-series compounds with indices n and n + i. The usefulness of the retention indices is increased considerably if computer programs are used for the calculation and searching of the retention indices. During the creation of the RI library with GC–FID the Sunicom 3.0 chromatographic data handling programme (Sunicom Oy, Finland) was used for the calculation of the retention indices. In the GC–FTIR a programme written with the GRAMS/386 (Galactic Industries Corporation, USA) macro programming language was used for calculation and searching.

Results and discussion

Creation of a spectral library

A spectral library containing 214 chemical warfare agents and related compounds was created. The library contains 55 compounds of the structures **1**, **2**, **3** or **4**. The maximum absorbances of the spectra accepted to the library were between 0.30 and 1.20 absorbance units. All spectra were examined and the run was repeated if any abnormalities were observed in the spectra. The baseline of each spectrum was manually corrected.

Creation of retention index library

A retention index library containing 145 chemical warfare agents or related compounds [2] has been established in our laboratory. Retention indices have been measured with FID against both M-series and C-series using two different column types (SE-54 and OV-1701; 25 m, i.d. 0.32 mm, 0.25 μm film thickness). The index value added to the library is the mean of three measurements. A typical standard error for a retention index value was between 0.1 and 0.3 index units. Index values depend on several GC parameters [10]. Therefore GC conditions should be kept as constant as possible while performing retention index monitoring.

The correlation between retention indices measured using the M-series and the C-series is good. When all library values for SE-54 column within M4–M20 and C8–C24 (100 compounds) are analyzed using linear regression, the r^2 value is 0.99991 and the standard deviation of the error between measured and calculated value is 3.5 index units. The C-series indices (RI_C) can be calculated from the M-series indices (RI_M) using the following equation

$$RI_C = 0.9666 \cdot RI_M + 487.6$$

This result is consistent with those obtained by Kokko [10] using 7 compounds with columns from three different manufacturers and also with our previous results [11]. Values for RI_C were estimated using RI_M values 300, 1000 and 2000 with the four equations. Differences between the highest and lowest estimation were 2.1, 2.0 and 2.4, respectively.

Chromatography of homologues

Two different methods were used for creating chromatograms from the infrared data: the Gram-Schmidt reconstruction method and the functional group chromatogram method. We found that for nerve agents and dialkyl methylphosphonates the functional group chromatograms measured from regions around the absorption bands of P=O and P–O bonds (1300–950 cm^{-1} was used in this study) produce the best sensitivity: about three times better signal-to-noise ratio than in Gram-Schmidt chromatogram. For compounds of the tabun family the region around the C≡N bond absorption (2250–2150 cm^{-1}) is also useful in higher concentrations.

The resolution of the Tracer chromatographic interface is slightly lower than that of a high resolution gas chromatograph (HRGC) [12] and the resolution degradation is

$$R_d = \frac{R_{CHROM}}{R_{TRACER}} = 1.8$$

where R_{CHROM} is the resolution of a HRGC and R_{TRACER} is the resolution of the Tracer.

Homologues of the same family having straight carbon chains can easily be identified by retention indices. The retention index increases by about 100 index units when the group R^2 is increased by one CH_2 unit. The relationship between retention index and the length of the R^2 group is quite linear as can be seen in Fig. 1. In the sarin family the change of R^1 from methyl to ethyl will increase the retention index by 100 index units. For the linear chain homologues the retention indices of the long chain (six or more carbons) compounds can be predicted quite accurately from the RI of the short chain (from three to five or six carbons) compounds as can be seen in Table 2. The measured and the predicted retention index values differ at maximum only by 3.9 index units. In the case of branched chain esters the estimation of retention indices is more difficult.

Verweij et al. reported [13] that compounds belonging to the sarin family elute as two peaks using nonchiral

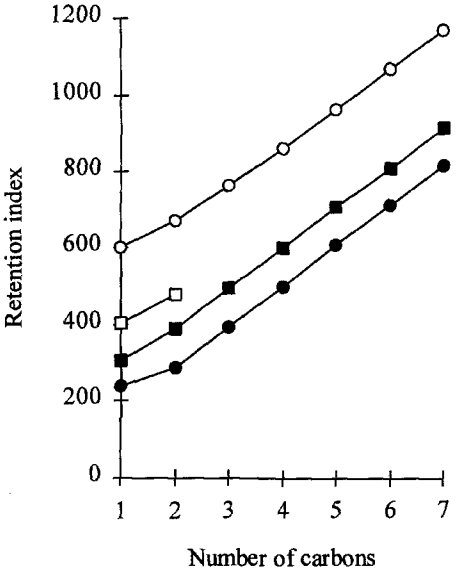

Fig. 1. Linearity of retention indices of different homologue series with a straight R^2 chain: alkyl methylphosphonofluoridates (**1**, R^1 = methyl, ●), alkyl ethylphosphonofluoridates (**1**, R^1 = ethyl, ■), dimethylamino alkyl phosphoramidocyanidates (**2**, ○), and alkyl methyl methylphosphonates (**4**, □)

Table 2. Prediction of retention indices of long-chain homologues. The predictions are based on retention indices of other homologues with at least three carbons

Homologue series	R^2 = hexyl (n = 6)		R^2 = heptyl (n = 7)		Equation[a]
	Predicted	Measured	Predicted	Measured	
1 R^1 = Me	708.4	709.5	813.8	812.8	105.49 $n \pm$ 75.41
1 R^1 = Et	806.1	805.0	910.0	910.8	103.88 $n \pm$ 182.86
2 R^1 = Me	1063.0	1062.9	1164.1	1165.4	101.12 n + 456.28

[a] Equation for prediction of retention indices of long-chain compounds (correlation > 0.999 for n = 3 – 7)

columns, if the group R^1 is methyl and the carbon next to oxygen in the group R^2 is chiral. These compounds are diastereomers because there are two chiral atoms: phosphorus and carbon. In the current study three of these compounds were analyzed: **1g** (retention index difference between diastereomers: 2.2), **1j** (4.9), and **1m** (2.0). It was found that also in the tabun family the separation of diastereomers can be observed: compound **2g** with a separation of 3.4 index units. Verweij et al. stated that the separation is completely lost when the P–F group is substituted with a P–OCH_3 group. In the current study it was noted that at least compound **4e**, which contains both P–O–pinacolyl and P–O–methyl, gives two peaks with a separation of 7.8 index units recorded on a SE-54 type column. In compounds where group R^1 is ethyl, separation of the diastereomers **1t** and **1w** was not observed with the nonchiral column.

Spectral searching

The best library search method for the identification of different homologues was found to be the derivative least squares method [14] with a modified algorithm:

$$(\mathrm{HQI})^2 = \sum_{\mathrm{W}} \left(\frac{\mathrm{dU_W}}{|\mathrm{U}|} - \frac{\mathrm{dL_W}}{|\mathrm{L}|} \right)^2$$

where data points (W) of derivative spectra of unknown (dU_W) and library (dL_W) compound are divided by the maximum values (|U| and |L|) of the respective spectra. This method minimizes the effects of a sloping baseline and broad non-specific spectral features. In the derivative least squares method the hit quality indices (HQI) are higher than in other methods tested, but the difference between the right compound and the next hit is larger than in other methods tested. In this method the HQI of 0.0 is the perfect match and 1.0 is already quite poor match.

In general using this search method the limit for acceptable HQI when the background of the spectrum is good was found to be 0.60. It was noticed that the search result must always be inspected visually before acceptance.

The amount of the sample has a direct effect on the HQI. The spectra of tabun (**2b**) measured "on-the-fly" from 100 ng, 10 ng and 500 pg with HQIs of 0.53, 0.60 and 1.26, respectively, are shown in Fig. 2. The "on-the-fly" spectrum of 500 pg of tabun was considered too noisy for a reliable identification. Therefore the spectrum was rescanned using 400 scans. The HQI for the rescanned spectrum was 1.08. The region for frozen water (3700–3100 cm^{-1}) was left out from the search of the 10 ng and 500 pg spectra.

Retention index searching

The indices for the analyzed compounds [2] are listed in Table 3. A retention index window of $\pm$ 10 index units has been found suitable for the search. Because chemical warfare agents are rich in heteroatoms including P, N, S, Cl, F and O among others, the use of selective GC detectors have an important role in verification analysis. When samples are first analyzed with GC, the compounds of interest can easily be selected from the GC–FTIR chromatogram using retention indices as a reference.

The retention index standards can be used both as an internal or external reference for the calculation of indices. If the reference compounds are run in a different run than the sample the GC conditions must be similar. The solvent used for the index standard mixture must be the same as (or similar to) the solvent in the sample to minimize the different solvent effects. When retention index monitoring is used only as a screening method with a large RI window ($\pm$ 10) small variations between consecutive runs do not have any practical effect.

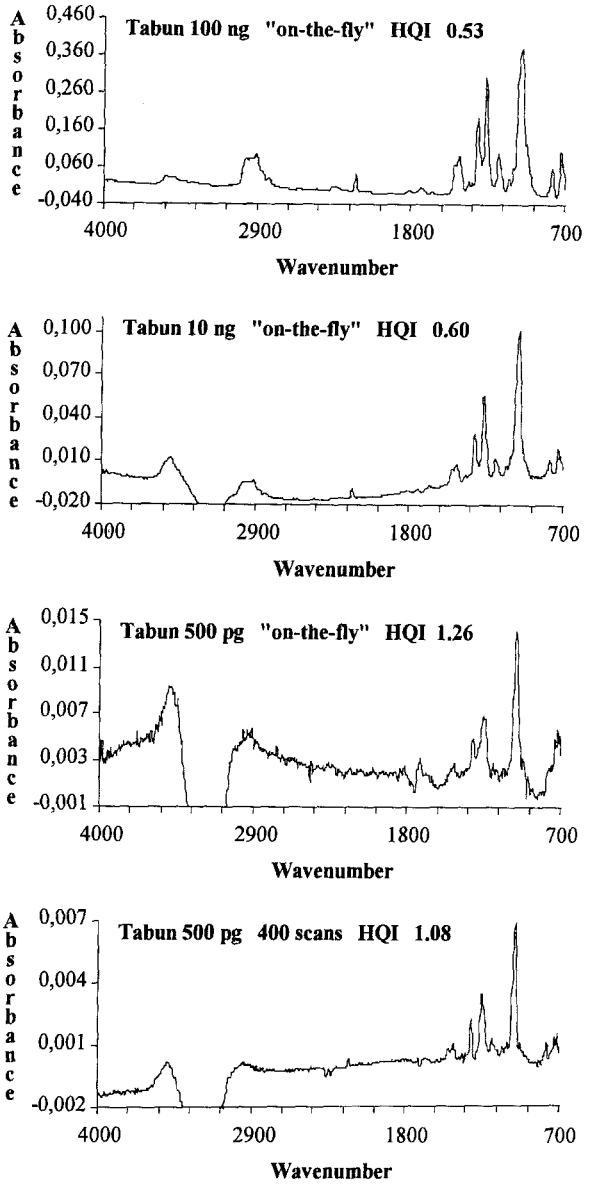

Fig. 2. Spectra of tabun (**2b**) measured with three different quantities. The negative peak between 3600–3200 cm^{-1} is from frozen water in the background spectrum and was omitted from the infrared search of the 10 ng and 500 pg spectra

Table 3. Retention indices of some nerve agents and dialkyl methylphosphonates. Retention indices have been measured against both M- and C-series. Compounds are listed in ascending retention index order. Typical standard error in retention index values is 0.1–0.3 index units

Compound	Retention index		Compound	Retention index		Compound	Retention index	
	M-series	C-series		M-series	C-series		M-series	C-series
1a	238.8	586.6	**1j**[a]	576.8	1043.7	**2f**	812.2	1272.9
1b	285.6	754.9	**1r**	597.8	1067.2	**1l**	812.8	1272.4
1n	306.3	802.7	**2a**	600.0	1070.2	**1x**	849.9	1307.1
1d	329.3	820.2	**1h**	604.4	1073.4	**2e**	859.7	1317.0
1o	388.9	865.7	**4h**	607.4	1072.7	**1y**	910.8	1364.2
1c	392.6	868.9	**2b**	668.1	1133.4	**2h**	960.0	1411.8
4a	403.9	880.9	**1w**	677.4	1141.4	**3a**	989.6	1442.5
1q	432.2	906.9	**2d**	697.3	1161.0	**2i**	1062.9	1512.9
1g[a]	438.2	915.4	**1u**	702.7	1165.2	**3d**	1072.1	1521.7
1g[a]	440.4	917.9	**1i**	709.5	1171.5	**3e**	1083.6	1532.4
4b	474.9	951.5	**4e**[a]	740.9	1200.0	**3b**	1148.9	1594.5
1p	491.3	967.1	**4e**[a]	748.7	1208.3	**2j**	1165.4	1610.9
1e	495.0	971.2	**2c**	761.4	1222.1	**3f**	1175.5	1621.7
4d	512.1	988.6	**2g**[a]	794.9	1256.1	**3g**	1224.7	1670.9
1t	540.5	1015.0	**1m**[a]	798.0	1260.2	**3h**	1234.0	1680.0
4f	544.5	1015.1	**2g**[a]	798.3	1259.3	**3c**	1271.1	1713.0
1s	554.3	1027.7	**1m**[a]	800.0	1261.7	**3i**	1298.0	1741.0
1j[a]	571.9	1048.1	**1v**	805.0	1265.3	**3k**	1348.6	1789.3

[a] Diastereomers give two peaks

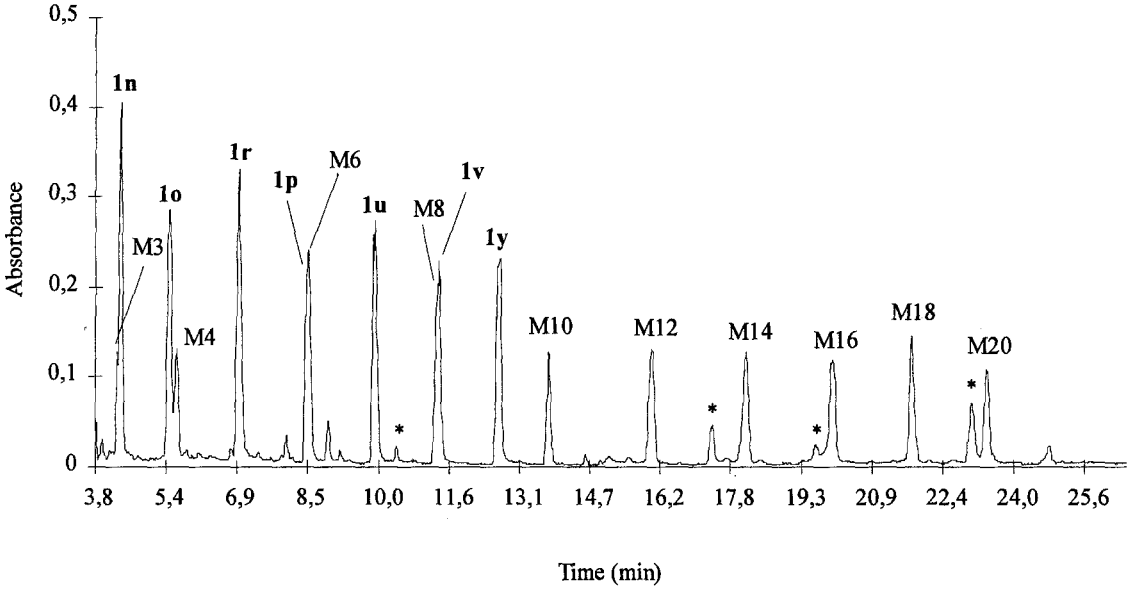

Fig. 3. Gram-Schmidt chromatogram of a sample containing M-series and alkyl ethylphosphonofluoridates. Peaks marked with an asterisk are impurities in M-series standard

Some environmental sample materials such as soil may slowly destroy the column material and the retention index value may change. Especially dialkyl methyl phosphonates have adsorptive properties at low concentration levels. Thus testing of column activity is one of the most critical aspects in guaranteeing the correctness of the analytical results. To improve the elution of polar compounds silylation can be used instead of methylation. Silylated methyl phosphonates have excellent chromatographic properties, and therefore the adsorption on the column material is no longer so critical.

If the retention index standard solution is injected together with the sample, some peaks may overlap as in Fig. 3 where three of the seven fluoridate peaks overlap completely (**1n**, **1p** and **1v**) and one partially (**1o**) with the RI standard compound. Therefore it is difficult to obtain pure spectra for compounds **1n**, **1p** and **1v**. In addition to M-series standard compounds the index standard mixture contains some impurities from the synthesis of the M-series that may also interfere with the analysis. Thus it is advisable to use a retention index standard in separate GC runs when analyzing complex samples such as environmental samples.

The result of a retention index search for the mixture of homologues in Fig. 3 is presented together with spectral search results in Table 4. In this example all test

Table 4. Results of an infrared library search and a retention index search on a sample containing *n*-alkyl methylphosphonofluoridates using the M-series as the internal index reference. The searches were made separately and the results were combined manually. Compounds actually in the sample are marked with asterisk. Compounds for which an HQI value is reported were found only in the retention index search. Retention indices in parentheses were not reported in the retention index search but have been calculated manually from the library values

Peak	Retention index	Compound name	Infrared library search HQI[a]	Retention index search ΔRI[b]
1	305.9	* Methyl ethylphosphonofluoridate (**1n**)	0.42	− 0.4
		Pentyl ethylphosphonofluoridate (**1u**)	0.93	(396.8)
		Propyl ethylphosphonofluoridate (**1p**)	0.95	(185.4)
2	387.8	* Ethyl ethylphosphonofluoridate (**1o**)	0.56	− 1.1
		Isobutyl ethylphosphonofluoridate (**1s**)	0.95	(166.5)
		Heptyl ethylphosphonofluoridate (**1y**)	1.06	(523.0)
3	492.8	* Propyl ethylphosphonofluoridate (**1p**)	0.46	1.5
		Methyl ethylphosphonofluoridate (**1n**)	0.93	(− 186.5)
		Butyl ethylphosphonofluoridate (**1r**)	0.94	(105.0)
		Butyl methylphosphonofluoridate (**1e**)	—	2.2
4	597.4	* Butyl ethylphosphonofluoridate (**1r**)	0.42	− 0.4
		Hexyl ethylphosphonofluoridate (**1v**)	0.91	(205.1)
		Propyl ethylphosphonofluoridate (**1p**)	0.96	(− 108.6)
		1,4-Dithiacyclohexane	—	0.6
		M6	—	− 2.6
		Methyl N,N-dimethylphosphoramidocyanidate (**2a**)	—	− 2.6
5	700.9	* Pentyl ethylphosphonofluoridate (**1u**)	0.54	− 1.8
		Hexyl ethylphosphonofluoridate (**1v**)	0.85	(104.1)
		Heptyl ethylphosphonofluoridate (**1y**)	0.91	(209.9)
		Isopropyl N,N-dimethylphosphoramidocyanidate (**2d**)	—	3.6
6	802.8	* Hexyl ethylphosphonofluoridate (**1v**)	0.38	− 2.2
		Heptyl ethylphosphonofluoridate (**1y**)	0.87	(108.0)
		Pentyl ethylphosphonofluoridate (**1u**)	0.92	(− 100.1)
		M8	—	2.8
		2-Methylcyclohexyl methylphosphonofluoridate (**1m**)	—	2.8
7	911.2	* Heptyl ethylphosphonofluoridate (**1y**)	0.46	0.4
		Hexyl ethylphosphonofluoridate (**1v**)	0.81	(− 106.2)
		Pentyl ethylphosphonofluoridate (**1u**)	0.92	(− 208.5)

[a] Hit Quality Index obtained from derivative least squares search (three best hits). The smaller the HQI the better the hit
[b] Retention index difference between library values and measured values (search window ± 4 index units)

compounds were found as first hits in both searches. Maximum difference in the measured retention index value compared to the library value was 2.2 index units although the library values were recorded with a different instrument and column.

Conclusions

A combination of retention index searches and infrared spectrum searches provides a reliable identification method for compounds included in both libraries. Rough identification limits were found for both searches. The hit quality index must be less than 0.60 and the retention index must be within a window of ± 10 index units before the compound can be identified unambigously. Visual inspection of the spectral search results is necessary.

The retention index standard can also be used as an external standard in a separate run from the actual sample without considerably losing retention index accuracy. In complex samples this is even recommendable.

The retention indices are used in spectral interpretation for locating the chromatographic peaks of interest for various spectrometric techniques. For the interpretation it is important to be sure that all data are recorded from the same peak.

Acknowledgement. The NC Laboratory of Defense Technology and Procurement Agency in Spiez, Switzerland, is gratefully acknowledged for synthesizing some of the compounds discussed in this article.

References

1. Report of the Ad Hoc Committee on Chemical Weapons CD/1170 26 August 1992
2. Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament (1994) Ministry for Foreign Affairs of Finland, Helsinki
3. Kováts E (1958) Helv Chim Acta VII: 1915–1932
4. Shaps RH, Boruta M, Varano A (1988) Mikrochim Acta I: 109–112

5. Franke S (1977) Lehrbuch der Militärchemie, Bd 1. Militärverlag, Berlin
6. Söderström MT, Ketola RA (1994) Fresenius J Anal Chem 350: 162–167
7. Haefner AM, Norton KL, Griffiths PR, Bourne S, Curbelo R (1988) Anal Chem 60: 2441–2444
8. Manninen A, Kuitunen M-L, Julin L (1987) J Chromatogr 394: 465–471
9. van den Dool H, Kratz PD (1963) J Chromatogr 11: 463–471
10. Kokko MJ (1993) J Chromatogr 640: 231–249
11. Air monitoring as a Means for Verification of Chemical Disarmament (1985) Ministry for Foreign Affairs of Finland, Helsinki
12. White R (1990) Chromatography/Fourier transform infrared spectroscopy and its applications. Dekker, New York
13. Verweij A, Burghardt E, Koonings AW (1971) J Chromatogr 54: 151–156
14. Lowry SR, Huppler DA (1981) Anal Chem 53: 889–893
15. Griffiths PR, Pentoney SL, Pariente GL, Norton KL (1987) Mikrochim Acta III: 47–62