

# Effect of variations in gas chromatographic conditions on the linear retention indices of selected chemical warfare agents

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## ABSTRACT

Temperature-programmed gas chromatographic retention indices, relative to *n*-alkane and *n*-alkylbis(trifluoromethyl)phosphine sulphide (M-standard) homologous series, were determined for nine chemical warfare agents using SE-54 fused-silica capillary columns. The influence of changes in the chromatographic conditions on the absolute values and on the reproducibility of the indices was evaluated. Nineteen parameters were investigated, with the purpose of discovering those most critical for the retention index monitoring of chemical warfare agents. The parameters most affecting the absolute value of the indices were the carrier gas flow-rate, the temperature programming rate and the properties of the column. Changes in conditions most strongly affected the indices of the low-volatility compounds O-ethyl S-2-(diisopropylamino)ethyl methylphosphonothioate (VX) and dibenz[*b,f*]-1,4-oxazepin (CR). The reproducibility of the indices was good in every case, even when the absolute values of the indices changed. Retention index monitoring is thus a reliable method for preliminary identification of compounds in mixtures, provided that the chromatographic system is regularly tested with a test mixture and corrections to the chromatographic conditions or the retention index library data are made where necessary.

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## INTRODUCTION

A reliable analytical system is needed for the international verification of chemical weapons disarmament. The Finnish Research Project on the Verification of Chemical Disarmament has been developing screening and identification methods for chemical warfare (CW) agents, their precursors and transformation or degradation products for the past 20 years. High-resolution gas chromatography with retention index monitoring (HRGC–RIM) has been selected as a primary screening technique, for several reasons: it offers separation of the sample components, location of suspected agents in a chromatogram, detection of agents containing specific heteroatoms, testing of agent purity, quantification

and an introduction method to mass (MS) and Fourier transform IR (FTIR) spectrometry for ultimate identification. The RIM method is especially valuable when hundreds of samples must be analysed, since then investigation by MS or FTIR is required only for positive samples or samples whose chromatograms are too complex to be resolved with selective detectors. The retention index window depends on the sample background; in most instances  $\pm 2$  index units is sufficient.

Linear retention indices calculated according to Van den Dool and Kratz [1] are preferred to Kováts isothermal retention indices [2] for the preliminary identification of compounds in complex sample mixtures because they allow the indices of highly volatile and less volatile compounds to be determined during a single analytical run.

Although straight-chain alkanes (C-standards) are the most widely used retention index standards, it is always preferable to employ retention index

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standards that are structurally similar to the compounds of interest. Alkanes, moreover, cannot be detected with the selective detectors needed for CW agents and related compounds. For these reasons, several phosphorus-containing standard series were synthesized in the Finnish Project [3–5]. The series we have been using since 1985 is the multi-detector M-series of alkylbis(trifluoromethyl)phosphine sulphides [6], compounds that are sufficiently volatile for GC and detectable both with selective detectors [alkali thermoionization (ATD), electron capture (ECD), flame photometric (FPD) and photoionization (PID) instruments] and with the universal flame ionization detection (FID) [7]. The M-standards have proved useful in trace analysis and when the matrix is complex.

In theory, a retention index system can meet the requirements of a universal data-generating system if the indices are sufficiently reproducible. The Sadtler retention index library for capillary columns is nowadays commercially available [8]. The database, which is based on the C-standards, consists of both isothermal and linear temperature-programmed indices. The retention indices are highly reproducible so long as similar conditions are employed.

As our M-standards are not used elsewhere in the identification of CW agents, we were obliged to create our own database from the retention indices of authentic CW agents and related compounds [3,9,10]. The retention indices were determined by two-channel GC making use of two similar columns or two columns of different polarity. Columns were connected to the same injector and to two similar or two different detectors. Monitored compounds are identified using a computer program, which searches for the retention index pattern, calculates the retention indices of all peaks in the chromatogram and compares these with the indices in the database. RIM provides preliminary identification without the need for authentic compounds and it allows quantitative results from the same run.

Although retention indices have been extensively investigated, as three reviews show [11–13], few studies have dealt with the retention indices of CW agents. Moreover, in these studies, indices were determined using the on-column injection mode and relative to homologous *n*-alkanes [14–18]. An essential question for any interlaboratory screening system is whether reproducible temperature-pro-

grammed retention indices can be achieved under variable chromatographic conditions. Some aspects have been investigated [19–21], but not for CW agents and related compounds.

In this study we investigated those most critical parameters of GC conditions and laboratory set-ups that could be expected to affect the absolute values and reproducibility of linear HRGC retention indices of selected CW agents. The effects of the following factors were evaluated: (1) carrier gas flow-rate; (2) temperature programming rate; (3) starting point of the temperature programme; (4) multi-step temperature programming; (5) injection volume; (6) injection mode; (7) sample solvent; (8) background; (9) column length; (10) inside diameter of the column; (11) film thickness; (12) repeated use of the same column; (13) individual columns; (14) columns from different manufacturers; (15) make of instrument; (16) different operators; (17) different laboratories; (18) one or two retention index standard series in the same run; and (19) reduced number of retention index standards.

The C-series was included in the study along with the M-series not only because it is widely used but also because we wished to know of any important differences in the behaviour of the two sets of indices ( $I_C$  and  $I_M$ ) under identical chromatographic conditions. The experiments in this work show that the two sets of indices can be derived from one another when the same column is used. Further, they demonstrate the essential reproducibility of retention indices and the reliability of the Project's approach to screening for CW agents.

## EXPERIMENTAL

### *Instrumentation and chromatographic conditions*

Our instruments were four Micromat HRGC 412 microcomputer-controlled gas chromatographs (HNU-Nordion) with two-channel integration and printing software, one Carlo Erba Fractovap 2900 capillary column gas chromatograph with HP 3390 A integrator and one Hewlett-Packard 5890 A gas chromatograph with HP 3392 A integrator. The accuracy of the temperature programmes of the chromatographs was checked with a Technoterm type 9500 digital instant-action thermometer.

Several different fused-silica capillary columns were employed: three 15 m × 0.32 mm I.D. cross-

linked SE-54 (5% phenyl methyl polysiloxane with 1% vinyl groups) columns with 0.25- $\mu\text{m}$  film, from Orion Analytica (OA); an SE-54 column, 15 m  $\times$  0.20 mm I.D. with 0.25- $\mu\text{m}$  film, also from Orion Analytica; a DB-5 column, 30 m  $\times$  0.32 mm I.D. with 0.25- $\mu\text{m}$  film, from J & W Scientific (SE-54 type); and a cross-linked SE-54 type column, 25 m  $\times$  0.31 mm I.D. with 0.17- $\mu\text{m}$  film, from Hewlett-Packard (HP).

Standard chromatographic conditions with the 15 m  $\times$  0.32 mm I.D. columns were as follows: injector and detector temperature (FID or ATD) 250°C; carrier gas flow-rate 2 ml/min; splitting ratio, 1:10; septum purge, 10 ml/min; starting point of the temperature programme, 40°C; temperature programming rate, 5 or 10°C/min, and end temperature of the programme, 260, 270 or 300°C, depending on the programming rate.

### Chemicals

The M- and C-series provided the retention index

standard compounds for the investigation. Alkyl-bis(trifluoromethyl)phosphine sulphides,  $(\text{CF}_3)_2\text{P}(\text{S})(\text{CH}_2)_n\text{CH}_3$ ,  $n = 2-19$  (M-standards), were diluted in ethyl acetate and *n*-alkanes (C-standards) in hexane. The M-series is commercially available from HNU-Nordion. The test compounds were isopropyl methylphosphonofluoridate (sarin), 1,2,2-trimethylpropyl methylphosphonofluoridate (soman), ethyl N,N-dimethylphosphoramidocyanidate (tabun), O-ethyl S-2-(diisopropylamino)ethyl methylphosphonothioate (VX), bis(2-chloroethyl) sulphide (mustard), 2-chlorobenzalmalononitrile (CS),  $\alpha$ -chloroacetophenone (CN) and dibenz[*b,f*]-1,4-oxazepin (CR), all obtained from the Research Centre of the Defence Forces of Finland. Not all test compounds were used in all experiments. The stock solutions of test compounds were diluted with ethyl acetate. Fresh solutions were prepared from time to time because of the slowly occurring decomposition (especially of VX). The concentration of each compound was about 50 ng/ $\mu\text{l}$ .

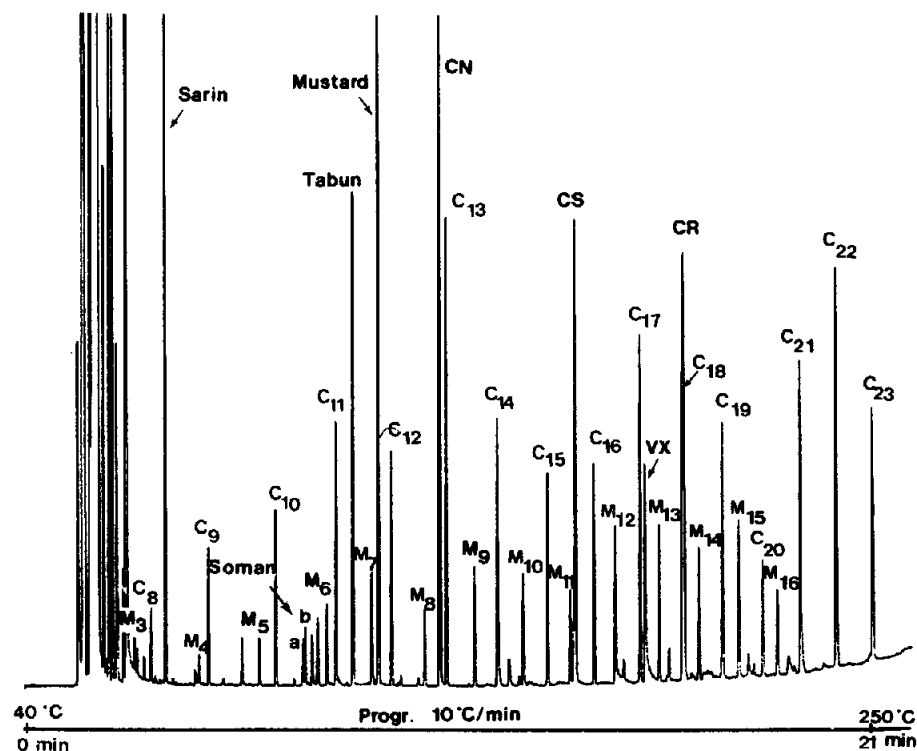


Fig. 1. Typical gas chromatogram of test compounds together with the C- and M-standards recorded under the standard chromatographic conditions: cross-linked fused-silica SE-54, 15 m  $\times$  0.32 mm I.D., 0.25  $\mu\text{m}$ ; FID, attenuation 32; split.

TABLE I  
EFFECT OF CARRIER GAS FLOW-RATE ON RETENTION INDICES

Columns: cross-linked SE-54 (2), 15 m × 0.32 mm I.D., 0.25 μm; cross-linked SE-54 (3), 15 m × 0.32 mm I.D., 0.25 μm. Other conditions as in Table III.

Compound	Parameter	10°C/min									
		0.5 ml/min <sup>a</sup>		1.0 ml/min <sup>a</sup>		1.5 ml/min		2 ml/min		2.5 ml/min	
		<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (3 runs)
Sarin	<i>I</i> <sub>C</sub>	823.5	0.3	823.7	0.1	—	—	—	—	—	—
	<i>I</i> <sub>M</sub>	344.5	1.4	344.0	0.1	326.9	0.1	326.7	0.2	326.2	0.1
Tabun	<i>I</i> <sub>C</sub>	1132.9	0.8	1132.5	0.4	1129.2	0.0	1129.9	0.3	1129.2	0.1
	<i>I</i> <sub>M</sub>	667.9	0.7	666.9	0.4	662.3	0.1	662.0	0.4	660.9	0.1
Mustard	<i>I</i> <sub>C</sub>	1179.0	0.6	1176.2	0.2	1172.9	0.2	1171.8	0.3	1170.6	0.1
	<i>I</i> <sub>M</sub>	716.4	0.6	712.5	0.4	708.4	0.2	706.2	0.3	704.6	0.1
CN	<i>I</i> <sub>C</sub>	1291.4	0.5	1287.4	0.4	1283.6	1.0	1281.4	0.1	1279.8	0.1
	<i>I</i> <sub>M</sub>	834.6	0.9	829.2	0.4	823.7	0.4	821.0	0.0	818.8	0.2
CS	<i>I</i> <sub>C</sub>	1564.2	0.8	1559.6	0.5	1554.4	0.2	1552.2	0.1	1550.7	0.2
	<i>I</i> <sub>M</sub>	1116.6	0.8	1110.6	0.6	1103.8	0.2	1103.2	0.1	1100 <sup>c</sup>	—
VX	<i>I</i> <sub>C</sub>	1716.3	1.0	1712.2	0.6	1705.6	0.4	1703.3	0.4	1700.0 <sup>d</sup>	—
	<i>I</i> <sub>M</sub>	1272.6	1.1	1267.7	0.7	1260.5	0.4	1257.2	0.2	1255.0	1.1
CR	<i>I</i> <sub>C</sub>	1814.8	1.4	1804.8	0.7	1793.2	0.2	1787.6	0.1	1783.7	0.5
	<i>I</i> <sub>M</sub>	1373.9	1.3	1361.0	1.2	1350.0	0.2	1343.5	0.3	1338.7	1.3

<sup>a</sup> Column SE-54 (3).

<sup>b</sup> M<sub>7</sub> and mustard eluted together.

<sup>c</sup> M<sub>11</sub> and CS eluted together.

<sup>d</sup> C<sub>17</sub> and VX eluted together.

### Calculations

The retention data were processed with the Project's LABOS program [22] using on-line or off-line data transfer from the chromatograph to a PDP 11/23+ computer.

Linear retention indices were calculated according to Van den Dool and Kratz's [1] equation:

$$I_{C(\text{or } M)} = 100C_n + 100(C_{n+i} - C_n) \frac{t_{R(x)} - t_{R(n)}}{t_{R(n+i)} - t_{R(n)}}$$

where  $C_n$  and  $C_{n+i}$  are carbon numbers of the C-standards (or carbon numbers of the alkyl chain of M-standards) eluted on either side of the unknown compound,  $t_{R(x)}$  is the retention time of the unknown, and  $t_{R(n)}$  and  $t_{R(n+i)}$  are the retention times of  $C_n$  (or  $M_n$ ) and  $C_{n+i}$  alkanes (or  $M_{n+i}$ ), respectively.

We use linear retention indices because they are easier to calculate than the cubic spline indices [23]. They are also just as reproducible as the cubic spline indices, even though the absolute values may differ slightly.

### RESULTS AND DISCUSSION

#### Carrier gas flow-rate

Although hydrogen is the best carrier gas from a purely chromatographic standpoint, helium was chosen as the carrier gas for safety reasons. Fig. 1 shows a typical gas chromatogram for a test mixture containing the C- and M-standards.

Flow-rates of 0.5, 1.0, 1.5, 2.0 and 2.5 ml/min were studied on 15 m × 0.32 mm I.D. columns; the exact flow-rate was measured with a soap-bubble flow meter at the starting conditions of the temper-

5°C/min					
1.5 ml/min		2 ml/min		2.5 ml/min	
<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (4 runs)
–	–	–	–	820.2	0.1
326.2	0.3	–	–	340.8	1.1
1130.3	0.7	1129.2	0.2	1129.0	0.1
661.2	0.6	658.9	0.3	657.8	0.1
1170.1	0.6	1168.5	0.3	1167.3	0.0
703.1	0.4	700.2	0.2	700.0 <sup>b</sup>	–
1278.8	0.2	1278.0	0.8	1274.5	0.2
816.5	0.6	814.5	0.3	811.5	0.1
1548.3	0.7	1547.7	0.5	1543.9	0.1
1096.0	0.6	1095.7	0.3	1090.3	0.2
1700.0 <sup>d</sup>	–	1699.4	0.4	1695.2	0.3
1251.9	0.1	1250.3	0.1	1246.8	0.2
1777.5	0.4	1774.1	0.1	1767.1	0.1
1330.8	0.4	1328.4	0.1	1319.6	0.1

ature programme. The column inlet pressure was kept constant, so that when the temperature increased in the column oven the viscosity of the carrier gas increased and the carrier gas flow-rate decreased. When the carrier gas flow-rate was increased from 1.5 to 2.5 ml/min and the temperature programming rate was 10°C/min, the retention indices of low-volatility compounds decreased more than those of high-volatility compounds [e.g., CR by about 10 retention index unit (i.u.) and tabun by only 1 i.u.]. The trend was the same when the carrier gas flow-rate was increased from 0.5 to 1.0 ml/min. Although the results of the two experiments cannot properly be combined as the column was different, it is indicative that the  $I_M$  of CR was as much as 35 i.u. smaller at a flow-rate 2.5 ml/min than at 0.5 ml/min. With both programming rates the reproducibility in successive runs was good (S.D. usually 0.1–0.5 i.u.) (Table I).

#### Temperature programming rate

The Finnish Project uses a temperature programming rate of 10°C/min for rapid screening and characterization of samples and 5°C/min for more detailed studies. We investigated these two rates and, in addition, 2 and 8°C/min, which were used for the Sadtler capillary GC standard retention index library [8].

The retention indices increased with increase in the programming rate. The effect was only a few index units for the highly volatile compounds sarin and tabun. The effects for mustard, CN, CS and VX were all of the same magnitude and slightly greater than the effect for sarin. In contrast, the retention index of CR increased by as much as 40 i.u. when the programming rate was increased from 2 to 10°C/min (Table II). However, the reproducibility was good in every case. The influence of the programming rate on the retention indices was of the same magnitude when a small-bore column was used (Table III).

Golovnya and Uralez [24] found the effect of temperature programming rate (3, 5 and 8°C/min) on *n*-alkyl methyl ketones separated on a packed polar column to be more complicated: whereas the retention indices of low-volatility compounds increased with increase in the temperature programming rate, those of high-volatility compounds decreased.

#### Starting point of the temperature programme

With *n*-alkyl methyl ketones and a polar packed column, Golovnya and Uralez [24] found the values of retention indices to increase when the starting point of the temperature programme was raised from 75 to 100 and 125°C. We studied the effect on retention indices of initial oven temperatures between 30 and 45°C, the range we normally use. Sample components were dissolved in ethyl acetate so that the solvent trapping would work over the whole range. In these experiments mustard sometimes eluted together with M<sub>7</sub>, CS with M<sub>11</sub> and VX with C<sub>17</sub>.

In a first set of measurements carried out at 35, 40 and 45°C, the differences in retention index ( $\Delta I$ ) values due to different initial temperatures were in most instances less than 0.7 i.u. A second set of measurements (Table IV) was carried out with 30 and 40°C as the starting temperatures. The solvent

TABLE II

## EFFECT OF TEMPERATURE PROGRAMMING RATE ON THE REPRODUCIBILITY OF THE RETENTION INDICES

Column: cross-linked SE-54, 25 × 0.32 mm I.D., 0.25 μm. Temperature programmes: I, 40 to 260°C at 10°C/min; II, 40 to 260°C at 8°C/min; III, 40 to 260°C at 5°C/min; IV, 40 to 260°C at 2°C/min. Other conditions as in Table III.

Compound	Parameter	I		II		III		IV		$\Delta I$
		$I$	S.D. (4 runs)	$I$	S.D. (4 runs)	$I$	S.D. (3 runs)	$I$	S.D. (3 runs)	
Sarin	$I_C$	821.5	0.3	822.0	0.2	821.0	0.0	820.8	0.1	0.7
	$I_M$	342.9	0.2	342.7	0.1	341.5	0.0	338.7	0.1	3.1
Tabun	$I_C$	1130.3	0.9	1131.5	0.4	1129.3	0.1	1130.0	0.1	1.3
	$I_M$	664.8	0.8	665.2	0.3	661.8	0.1	658.5	0.1	6.7
Mustard	$I_C$	1176.2	0.7	1175.8	0.3	1171.4	0.1	1167.6	0.0	8.6
	$I_M$	713.2	0.5	711.8	0.1	706.2	0.1	700.0 <sup>a</sup>	–	13.2
CN	$I_C$	1287.2	0.6	1285.7	0.6	1280.5	0.1	1275.0	0.2	12.3
	$I_M$	829.9	0.6	827.8	0.5	820.7	0.1	811.9	0.2	18.0
CS	$I_C$	1559.9	0.6	1558.0	0.5	1551.4	0.1	1544.4	0.3	15.4
	$I_M$	1111.6	0.6	1109.0	0.4	1100.0 <sup>b</sup>	–	1091.1	0.4	20.5
VX	$I_C$	1710.2	0.2	1708.2	0.2	1702.4	0.2	1695.0	0.2	15.2
	$I_M$	1266.7	0.3	1264.1	0.2	1257.0	0.1	1246.7	0.2	19.9
CR	$I_C$	1805.7	0.4	1800.0 <sup>c</sup>	–	1786.8	0.1	1766.5	0.2	39.2
	$I_M$	1364.4	0.4	1358.0	0.2	1342.9	0.1	1319.2	0.2	45.2

<sup>a</sup> M<sub>7</sub> and mustard eluted together.

<sup>b</sup> M<sub>11</sub> and CS eluted together.

<sup>c</sup> C<sub>18</sub> and CR eluted together.

effect more effectively retarded the elution of sarin and soman than the elution of the standards, and the retention indices of sarin and soman were higher at the 30°C starting temperature. VX eluted as a broad peak when the temperature programme was started at 40°C.

#### Multi-step temperature programme

Retention indices in these experiments were calculated using Van den Dool and Kratz's equation, even though the temperature programme was not linear. The effect of different multi-step temperature programmes on the test mixture is shown in Table V. M<sub>3</sub> eluted as a distinct peak after the solvent, and the  $I_M$  of sarin could be calculated reliably when an initial isothermal part was included in the temperature programme or the programming rate was

very slow. The results indicate the usefulness of multi-step temperature programmes in RIM analysis. Peak shapes of low-volatility compounds are improved, allowing more accurate integration, and the analysis time can be shorter. The reproducibility was as good as with a linear temperature programme.

#### Injection volume

Typical injection volumes in our work are 0.5–2 μl and the amounts of the test compounds are 25–50 ng. When the injection volume was increased from 0.5 to 2 μl, the  $\Delta I$  values were less than 1 i.u. An injection volume of 2 μl caused a practical problem, however: M<sub>3</sub> and C<sub>8</sub> were not separated from the solvent peak and the retention indices of sarin could not be determined.

TABLE III

EFFECT OF THE PROGRAMMING RATE ON THE REPRODUCIBILITY OF THE RETENTION INDICES USING COLUMNS OF I.D. 0.32 and 0.20 mm

Instrument: Micromat HRGC 412. Columns: cross-linked SE-54 (2), 15 m × 0.32 mm I.D., 0.25 μm; cross-linked SE-54, 15 m × 0.20 mm I.D., 0.25 μm. Temperature programmes: 40 to 280°C at 10 and 5°C/min. Injector temperature, 250°C; detector temperature, 250°C; Splitting ratio, 1:10; carrier gas, helium at 2 ml/min.

Compound	Parameter	I.D. 0.32 mm					I.D. 0.20 mm				
		10°C/min		5°C/min		$\Delta I$	10°C/min		5°C/min		$\Delta I$
		<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (5 runs)		<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (5 runs)	
Tabun	<i>I<sub>C</sub></i>	1130.0	0.4	1129.2	0.2	0.8	1129.9	0.3	1129.3	0.7	0.5
	<i>I<sub>M</sub></i>	662.3	0.2	658.9	0.3	3.5	663.9	0.3	661.3	0.6	2.6
Mustard	<i>I<sub>C</sub></i>	1173.1	0.9	1168.5	0.3	4.6	1174.8	0.2	1170.4	0.5	4.4
	<i>I<sub>M</sub></i>	707.2	0.1	700.2	0.2	7.1	711.1	0.2	704.6	0.4	6.5
CN	<i>I<sub>C</sub></i>	1283.1	1.5	1277.9	0.8	5.2	1285.8	0.6	1279.5	0.3	6.3
	<i>I<sub>M</sub></i>	822.4	0.2	814.5	0.3	7.9	827.8	0.6	818.7	0.4	9.1
CS	<i>I<sub>C</sub></i>	1553.3	0.5	1547.7	0.5	5.6	1557.9	0.7	1550.2	0.5	7.7
	<i>I<sub>M</sub></i>	1104.5	0.3	1095.7	0.3	8.9	1109.0	0.6	1100.7	0.6	8.4
VX	<i>I<sub>C</sub></i>	1704.9	1.3	1699.4	0.4	5.5	1712.4	0.5	1703.6	0.5	8.9
	<i>I<sub>M</sub></i>	1258.9	0.2	1250.3	0.1	8.7	1264.2	0.3	1255.5	0.6	8.7
CR	<i>I<sub>C</sub></i>	1789.7	0.3	1774.1	0.1	15.7	1803.6	0.7	1783.6	0.4	19.9
	<i>I<sub>M</sub></i>	1346.8	0.2	1328.4	0.1	18.4	1359.2	0.7	1339.1	0.8	20.1

### Injection mode

Retention indices measured with split, splitless and on-column injection modes were compared. We normally use the splitless injection mode for retention index monitoring in trace analysis; split injection is used when the sample is more concentrated. Instruments for these experiments were a Micromat HRGC 412 and Carlo Erba 2900. The Micromat is fully automated, but there is no on-column injector. In the old Carlo Erba 2900, the integrator and temperature programme are started up separately, the splitless time is measured with a stop-watch and the split valve is opened manually. The results obtained with the two instruments are not therefore fully comparable.

More solvent goes into the column in the splitless than in the split injection mode and the phase ratio of the column is temporarily changed. Whether or not the solvent has a similar effect on the sample

compounds and the retention index standards depends on the solvent and the structure of the compounds. According to Grob [25], the longer the time needed to evaporate the solvent, the greater are the retention times of compounds. Measured on the Micromat, retention indices of the test compounds were slightly greater (about 0.1–2.0 i.u.) in the splitless than in the split injection mode.

The flow-rate was slightly lower in measurements done on the Carlo Erba 2900 instrument and the indices were therefore higher than those measured on the Micromat. The retention indices were highest when measured on the Carlo Erba 2900 with the split injection mode and the 10°C/min temperature programme. With the 5°C/min temperature programme the retention indices were nearly the same with the split and splitless injection modes. At both programming rates the retention indices were smallest with on-column injection.

TABLE IV

EFFECT OF THE INITIAL TEMPERATURE (30 AND 40°C) OF THE TEMPERATURE PROGRAMME ON THE RETENTION INDICES USING DIFFERENT PROGRAMMING RATES

Column: cross-linked SE-54 (1), 15 m × 0.32 mm I.D., 0.25 μm. Temperature programmes: 30 to 300°C at 5 and 10°C/min; 40 to 300°C at 5 and 10°C/min. Other conditions as in Table III.

Compound	Parameter	5°C/min					10°C/min				
		30°C		40°C		$\Delta I$	30°C		40°C		$\Delta I$
		$I$	S.D. (5 runs)	$I$	S.D. (5 runs)		$I$	S.D. (5 runs)	$I$	S.D. (5 runs)	
Sarin	$I_C$	823.9	0.3	821.4	0.1	2.5	823.1	0.1	821.6	0.3	1.5
	$I_M$	351.0	0.3	341.9	0.1	9.1	345.1	0.1	—	—	—
Soman a	$I_C$	1042.8	0.6	1040.5	0.2	2.0	1041.6	0.1	1040.7	0.1	0.9
	$I_M$	568.0	0.6	566.2	0.2	1.8	568.9	0.1	568.2	0.1	0.7
Soman b	$I_C$	1047.3	0.6	1045.0	0.2	2.3	1046.0	0.1	1045.1	0.1	0.9
	$I_M$	572.8	0.6	571.1	0.2	1.7	573.7	0.1	573.0	0.1	0.7
Tabun	$I_C$	1130.5	0.5	1133.3	0.2	-2.7	1130.3	0.1	1132.6	0.4	-2.3
	$I_M$	660.1	0.4	662.7	0.2	-2.6	662.4	0.1	664.6	0.3	-2.2
Mustard	$I_C$	1168.0	0.1	1170.5	0.1	-1.5	1171.9	0.1	1173.7	0.3	-1.8
	$I_M$	700 <sup>a</sup>	—	702.7	0.1	—	706.4	0.1	707.9	0.2	-1.6
CN	$I_C$	1276.7	0.4	1279.4	0.1	-2.7	1281.6	0.2	1283.8	0.3	-2.1
	$I_M$	813.9	0.3	816.7	0.1	-2.8	821.4	0.1	823.7	0.3	-2.3
CS	$I_C$	1546.8	0.4	1548.8	0.2	-2.0	1553.1	0.2	1554.8	0.5	-1.7
	$I_M$	1093.9	0.5	1095.7	0.1	-1.8	1100 <sup>c</sup>	—	1103.6	0.5	—
VX	$I_C$	1700 <sup>b</sup>	—	1708.6 <sup>d</sup>	2.2	—	1704.0	0.8	1722.7 <sup>d</sup>	3.6	—
	$I_M$	1250.6	0.9	1260.2 <sup>d</sup>	2.2	—	1258.0	0.8	1276.4 <sup>d</sup>	3.7	—
CR	$I_C$	1773.2	0.4	1774.4	0.1	-1.2	1788.4	0.2	1789.8	0.2	-1.4
	$I_M$	1325.9	0.3	1327.1	0.2	-1.3	1344.3	0.2	1345.5	0.2	-1.2

<sup>a</sup> M<sub>7</sub> and mustard eluted together.

<sup>b</sup> C<sub>17</sub> and VX eluted together.

<sup>c</sup> M<sub>11</sub> and CS eluted together.

<sup>d</sup> Broad peak.

#### Sample solvent

Solvents ethyl acetate, diethyl ether and acetone were chosen for the investigation, bearing in mind the preferred solvents for sample preparation procedures in GC analysis. Measurements with ethyl acetate and diethyl ether were done using a 35 or 40°C initial oven temperature and 10 and 5°C/min temperature programmes (Table VI), and measurements with diethyl ether and acetone using a 30°C initial temperature and 10°C/min temperature programme. The reproducibility was better for the 10°C/min temperature programme: the  $\Delta I$  values

for different solvents were within the standard deviation. With the 5°C/min temperature programme the  $\Delta I$  values were occasionally over 3 i.u. CR was most affected.

#### Background

In complex matrices, the amount of some component may be so great that it behaves like a solvent plug and temporarily alters the stationary phase. In this way it could have a reverse or normal solvent effect, respectively, on compounds that elute just before or after it [26]. Peak shifting between two



TABLE V

## EFFECT OF THE MULTI-STEP TEMPERATURE PROGRAMME ON THE RETENTION INDICES

Columns: cross-linked SE-54 (1), 15 m × 0.32 mm I.D., 0.25 μm; cross-linked DB-5, 30 m × 0.3 mm I.D., 0.25 μm (DB-5 is a bonded phase from J & W Scientific corresponding to SE-54).

Temperature programmes:

I = 30°C  $\xrightarrow{20^\circ\text{C}/\text{min}}$  150°C  $\xrightarrow{10^\circ\text{C}/\text{min}}$  300°C;

II = 30°C (2 min)  $\xrightarrow{10^\circ\text{C}/\text{min}}$  150°C  $\xrightarrow{5^\circ\text{C}/\text{min}}$  250°C (5 min);

III = 30°C  $\xrightarrow{1^\circ\text{C}/\text{min}}$  32°C  $\xrightarrow{2^\circ\text{C}/\text{min}}$  35°C  $\xrightarrow{5^\circ\text{C}/\text{min}}$  40°C  $\xrightarrow{10^\circ\text{C}/\text{min}}$  100°C  $\xrightarrow{15^\circ\text{C}/\text{min}}$  200°C  $\xrightarrow{20^\circ\text{C}/\text{min}}$  250°C (10 min);

IV = 30°C  $\xrightarrow{1^\circ\text{C}/\text{min}}$  32°C  $\xrightarrow{2^\circ\text{C}/\text{min}}$  35°C  $\xrightarrow{5^\circ\text{C}/\text{min}}$  40°C  $\xrightarrow{8^\circ\text{C}/\text{min}}$  60°C  $\xrightarrow{10^\circ\text{C}/\text{min}}$  100°C  $\xrightarrow{15^\circ\text{C}/\text{min}}$  200°C  $\xrightarrow{20^\circ\text{C}/\text{min}}$  280°C (5 min).

Other conditions as in Table III.

Compound	Parameter	I <sup>a</sup>		II		III		IV	
		<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (5 runs)
Sarin	<i>I<sub>C</sub></i>	823.4	0.2	823.2	0.1	824.8	0.1	824.2	0.1
	<i>I<sub>M</sub></i>	346.6	0.0	359.9	0.1	361.5	0.2	359.9	0.2
Soman a	<i>I<sub>C</sub></i>	1042.3	0.1	1041.9	0.1	1044.1	0.2	1044.1	0.1
	<i>I<sub>M</sub></i>	571.7	0.2	570.7	0.1	571.7	0.1	571.3	0.2
Soman b	<i>I<sub>C</sub></i>	1046.8	0.1	1046.4	0.1	1048.7	0.1	1048.7	0.1
	<i>I<sub>M</sub></i>	576.4	0.2	575.4	0.2	576.3	0.1	575.9	0.2
Tabun	<i>I<sub>C</sub></i>	1132.5	0.6	1131.9	0.1	1133.3	0.3	1133.6	0.2
	<i>I<sub>M</sub></i>	666.7	0.5	666.5	0.1	667.8	0.2	667.8	0.2
Mustard	<i>I<sub>C</sub></i>	1177.4	0.4	1179.2	0.1	1180.1	0.2	1179.9	0.2
	<i>I<sub>M</sub></i>	713.9	0.3	716.4	0.2	717.1	0.3	716.5	0.3
CN	<i>I<sub>C</sub></i>	1288.7	0.4	1291.3	0.1	1292.9	0.2	1292.6	0.2
	<i>I<sub>M</sub></i>	831.3	0.3	833.9	0.1	837.2	0.2	836.8	0.2
CS	<i>I<sub>C</sub></i>	1558.5	0.3	1561.4	0.2	1569.7	0.3	1569.9	0.2
	<i>I<sub>M</sub></i>	1110.4	0.2	1114.3	0.3	1123.5	0.3	1123.6	0.2
VX	<i>I<sub>C</sub></i>	1711.7	0.4	1708.6	0.3	1718.3	0.1	1718.2	0.1
	<i>I<sub>M</sub></i>	1266.3	0.3	1264.2	0.3	1276.3	0.2	1276.2	0.2
CR	<i>I<sub>C</sub></i>	1796.8	0.5	1803.6	0.5	1827.3	0.5	1828.7	0.3
	<i>I<sub>M</sub></i>	1353.9	0.4	1361.1	0.4	1388.2	0.4	1388.8	0.4

<sup>a</sup> SE-54 column.

components not fully separated from each other might also occur. The retention behaviour of sarin, soman, tabun, CS and CR was studied in an air background.

An air sample extract was concentrated so that 1 μl corresponded to 0.8 m<sup>3</sup> of air, and then diluted so that the air background was present in a ratio of 1:2:4 in three different samples. Each sample was

spiked with 500 ng each of sarin and tabun, 1.5 ng of soman and 2.5 ng of CS. The retention indices of sarin and soman were affected more ( $\Delta I_M = 5.2$  and 0.9 i.u.) than those of tabun and CS (Fig. 2). Soman eluted partly together with a compound in the background. As expected, the reproducibility in successive runs was better when the amount of the air sample extract was low.

TABLE VI

## EFFECT OF SOLVENT ON THE RETENTION INDICES WITH ETHYL ACETATE AND DIETHYL ETHER AS SOLVENTS

Column: cross-linked SE-54 (2), 15 m × 0.32 I.D., 0.25 μm. Temperature programmes: 35 to 280°C at 10 and 5°C/min; 40 to 280°C at 10 and 5°C/min. Other conditions as in Table III.

Compound	Parameter	35 to 280°C at 10°C/min					40 to 280°C at 10°C/min				
		Ethyl acetate		Diethyl ether		$\Delta I$	Ethyl acetate		Diethyl ether		$\Delta I$
		<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (5 runs)		<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (5 runs)	
Sarin	<i>I<sub>C</sub></i>	824.6	0.1	824.3	0.1	0.2	823.1	0.3	822.9	0.3	0.2
	<i>I<sub>M</sub></i>	—	—	—	—	—	—	—	—	—	—
Soman a	<i>I<sub>C</sub></i>	1043.3	0.1	1043.1	0.0	0.2	1042.7	0.1	1042.5	0.1	0.2
	<i>I<sub>M</sub></i>	570.3	0.1	570.1	0.1	0.3	570.1	0.1	570.0	0.1	0.2
Soman b	<i>I<sub>C</sub></i>	1047.6	0.1	1047.5	0.1	0.1	1047.1	0.1	1046.9	0.1	0.2
	<i>I<sub>M</sub></i>	575.0	0.1	574.8	0.1	0.3	574.9	0.1	574.7	0.1	0.2
Tabun	<i>I<sub>C</sub></i>	1130.4	0.2	1130.5	0.1	0.1	1130.5	0.3	1130.2	0.3	0.3
	<i>I<sub>M</sub></i>	662.5	0.2	662.6	0.1	0.1	662.8	0.3	662.4	0.3	0.4
Mustard	<i>I<sub>C</sub></i>	1171.7	0.2	1171.9	0.1	0.1	1172.0	0.2	1171.9	0.1	0.1
	<i>I<sub>M</sub></i>	706.1	0.1	706.2	0.1	0.1	706.5	0.2	706.5	0.1	0.0
CN	<i>I<sub>C</sub></i>	1281.3	0.1	1281.4	0.1	0.2	1281.5	0.2	1281.4	0.2	0.1
	<i>I<sub>M</sub></i>	821.0	0.2	821.1	0.2	0.1	821.2	0.3	1281.4	0.2	0.0
CS	<i>I<sub>C</sub></i>	1552.6	0.3	1552.8	0.2	0.2	1552.9	0.3	1552.6	0.2	0.3
	<i>I<sub>M</sub></i>	1100 <sup>b</sup>	—	1100 <sup>b</sup>	—	—	1100 <sup>b</sup>	—	1100 <sup>b</sup>	—	—
VX	<i>I<sub>C</sub></i>	1704.2	0.0	1703.9	0.3	0.2	1703.9	0.2	1700 <sup>c</sup>	—	—
	<i>I<sub>M</sub></i>	1258.2	0.0	1258.2	0.3	0.02	1258.0	0.2	1258.0	0.2	0.0
CR	<i>I<sub>C</sub></i>	1788.1	0.0	1788.5	0.4	0.4	1788.1	0.2	1788.2	0.3	0.1
	<i>I<sub>M</sub></i>	1343.8	0.1	1344.4	0.3	0.5	1344.0	0.2	1344.2	0.4	0.2

<sup>a</sup> M<sub>7</sub> and mustard eluted together.

<sup>b</sup> M<sub>11</sub> and CS eluted together.

<sup>c</sup> C<sub>17</sub> and VX eluted together.

### Column length

The experiments were done using a 25-m column, which was shortened by 5 m after each series of measurements, the column lengths thus studied being 25, 20 and 15 m. The absolute values of the retention indices decreased as the columns become shorter (Table VII). The decrease was small for sarin and tabun and greater for compounds with long retention times. Table VII shows that when the compound elutes near the retention index standard (VX and C<sub>17</sub> at a temperature programming rate of 5°C/min), the retention index does not change with column length. The retention index standard always interfered with the elution of a CW agent eluting

nearby, even when the concentration of the compounds was low.

### Inside diameter of the column

In general, as the diameter of the column decreases the resolution increases and columns can be shorter. The capacity of the column also decreases, however. Two columns differing only in diameter (0.32 and 0.20 mm) were investigated. The average linear velocity (in cm/s) of the carrier gas was adjusted so as to be the same in the two columns. This meant that the flow-rate at the outlet was only 0.72 ml/min for the 0.20 mm I.D. column. Because such a small value is difficult to measure, we used the

35 to 280°C at 5°C/min					40 to 280°C at 5°C/min				
Ethyl acetate		Diethyl ether		$\Delta I$	Ethyl acetate		Diethyl ether		$\Delta I$
<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (5 runs)		<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (5 runs)	
—	—	823.6	0.4	—	821.2	0.1	822.2	0.4	1.0
—	—	—	—	—	—	—	—	—	—
—	—	1042.7	0.5	—	1042.8	0.1	1042.1	0.2	0.7
—	—	567.8	0.4	—	566.9	0.1	567.8	0.2	0.9
—	—	1047.2	0.5	—	1045.1	0.1	1046.5	0.2	1.4
—	—	572.7	0.4	—	571.6	0.1	572.7	0.2	1.0
1129.6	0.5	1130.1	0.2	0.6	1130.0	0.1	1129.8	0.2	0.2
659.5	0.5	659.0	0.2	0.5	660.0	0.1	658.7	0.2	1.3
1168.3	0.3	1167.7	0.1	0.6	1168.8	0.1	1167.7	0.1	1.1
700 <sup>a</sup>	—	700 <sup>a</sup>	—	—	700 <sup>a</sup>	—	700 <sup>a</sup>	—	—
1277.3	0.7	1275.6	0.2	1.7	1276.7	0.2	1275.8	0.2	0.8
814.3	0.3	812.4	0.2	1.8	814.3	0.2	812.9	0.2	1.3
1547.0	0.6	1545.3	0.6	1.7	1546.3	0.0	1545.5	0.2	0.8
1095.7	0.3	1092.0	0.7	3.7	1093.5	0.1	1092.3	0.2	1.2
1700 <sup>c</sup>	—	1700 <sup>c</sup>	—	—	1702.8	0.1	1700 <sup>c</sup>	—	—
1250.7	0.4	1249.3	0.6	1.4	1252.4	0.1	1249.2	0.4	3.3
1774.3	0.3	1772.3	0.6	1.9	1773.2	0.1	1771.7	0.2	1.5
1328.6	0.2	1324.6	0.6	4.0	1326.0	0.2	1324.3	0.4	1.7

retention time of propane to ensure that the velocity through the columns was the same.

The peak shapes of test compounds were slightly better with the 0.20 mm I.D. column. The reproducibility of successive runs was not as good for the small-bore column as for the 0.32 mm I.D. column. The retention indices of the test compounds were larger in the column with smaller inside diameter (see Table III).

#### Film thickness

Film thickness affects the column capacity. With a thinner film the indices are more susceptible to the

amount of solute. The column capacity is lower for compounds with high retention indices than for compounds with low retention indices [27,28].

On the SE-52 column, the reproducibility of retention indices for non-phosphorus CW agents is better with a thicker film. The retention indices are lower with the thinner film [29]. In our experiment, the columns of different film thickness were of the same type but from different manufacturers. The retention indices of all compounds except sarin and tabun were lower on the thinner film (0.17  $\mu\text{m}$ ) HP column than on the OA SE-54 (0.25  $\mu\text{m}$ ) column (see Table VIII).

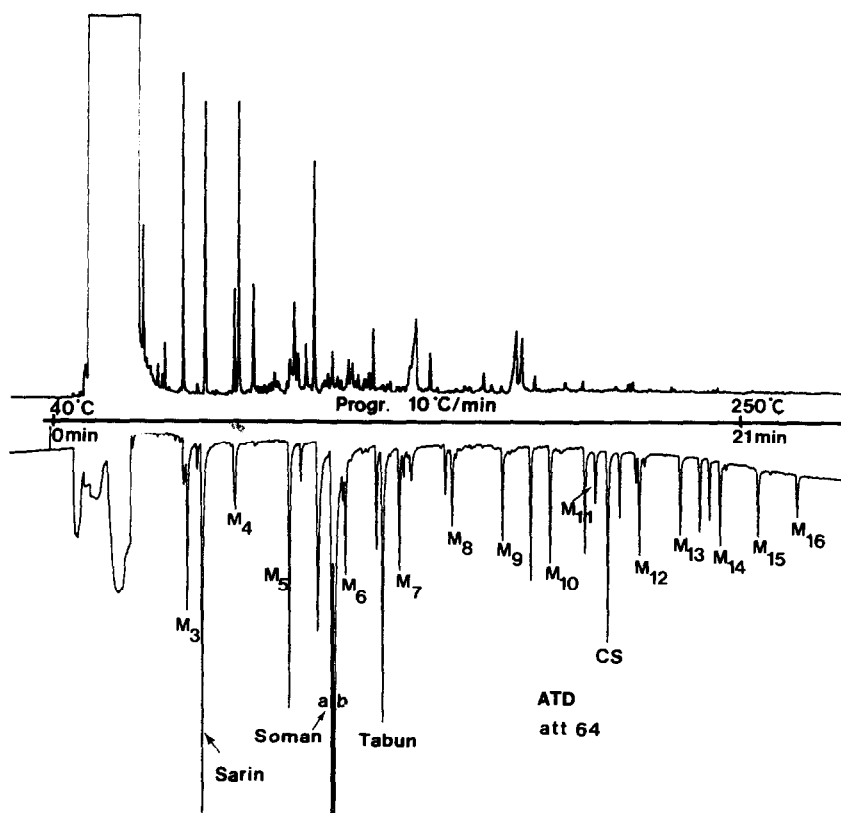


Fig. 2. Chromatogram of test mixture and M-standards with air background. Cross-linked fused-silica SE-54, 30 m  $\times$  0.32 mm I.D., 1.0  $\mu$ m; effluent splitter; FID, attenuation 128.

#### Repeated use of the same column

Measurements were done over 5 months using the same individual SE-54 column and the same gas chromatograph. The column was in use most of the time for other experiments and from time to time was taken for reproducibility tests.

The properties of the column did not change much with time. However, the peak shape of VX, which is most sensitive to changes in the stationary phase, was poor in the third series of experiments. The  $\Delta I$  values for different experiment series were less than 1.8 i.u., except for VX and CR, where the  $\Delta I$  value was sometimes about 4 i.u. The stability of the cross-linked SE-54 column was better than that of the SE-52 and OV-1 columns used earlier. On these columns, retention indices decreased during long-term use as the film became thinner [30].

#### Individual columns

Our standard method for RIM analysis requires two similar or different columns attached in parallel to a common injector and two different or similar detectors. Even though two columns are attached to the same injector, the carrier gas flow-rate may be slightly different. Also, even though columns should be similar, the actual lengths, phase thicknesses or inside diameters may deviate slightly, resulting in non-identical flow.

The first experiments were made with one column installed into different detectors in different runs. In other experiments two similar individual columns were used in parallel in the same run. Retention indices for the same individual column when it was used alone and together with another similar column were within 0.8 i.u., except for tabun, where the difference was within 1.2 i.u.

TABLE VII  
EFFECT OF COLUMN LENGTH ON RETENTION INDICES

Column: cross-linked SE-54, 25, 20 and 15 m  $\times$  0.32 mm I.D., 0.25  $\mu$ m. Other conditions as in Table III.

Compound	Parameter	25 m			20 m			15 m					
		10°C/min			5°C/min			10°C/min			5°C/min		
		I	S.D.	I	S.D.	I	S.D.	I	S.D.	I	S.D.	I	S.D.
Sarin	$I_c$	821.5	0.3	821.0	0.0	821.4	0.1	—	—	822.0	0.2	821.2	0.1
	$I_M$	342.9	0.2	341.5	0.0	342.4	0.2	—	—	342.1	0.2	340.4	0.1
Soman a	$I_c$	1042.4	—	—	—	1042.1	0.1	1041.4	0.1	1041.6	0.1	1040.8	0.1
	$I_M$	572.5	—	—	—	570.8	0.1	567.9	0.1	569.1	0.1	566.9	0.1
Soman b	$I_c$	1046.8	—	—	—	1046.4	0.1	1046.5	0.1	1045.0	0.1	1045.1	0.1
	$I_M$	575.9	—	—	—	575.4	0.1	572.7	0.1	573.8	0.1	571.6	0.1
Tabun	$I_c$	1130.3	0.9	1129.3	0.1	1130.1	0.2	1129.5	0.3	1130.8	0.5	1130.0	0.1
	$I_M$	664.8	0.8	661.8	0.1	663.8	0.2	661.2	0.2	663.2	0.5	660.0	0.1
Mustard	$I_c$	1176.2	0.7	1171.4	0.1	1174.7	0.2	1170.6	0.2	1173.0	0.4	1168.8	0.1
	$I_M$	713.2	0.5	706.2	0.1	710.8	0.2	704.7	0.2	707.6	0.3	700.0 <sup>a</sup>	—
CN	$I_c$	1287.2	0.6	1280.5	0.1	1285.0	0.1	1279.3	0.1	1282.2	0.3	1276.7	0.2
	$I_M$	829.9	0.6	820.7	0.1	826.7	0.2	819.8	0.2	822.3	0.3	814.3	0.2
CS	$I_c$	1559.9	0.6	1551.4	0.1	1556.9	0.1	1549.9	0.2	1553.1	0.4	1546.3	0.0
	$I_M$	1111.6	0.6	1100.0 <sup>b</sup>	—	1107.7	0.2	1100.0 <sup>b</sup>	—	1100.0 <sup>b</sup>	—	1093.5	0.1
VX	$I_c$	1710.2	0.2	1702.4	0.2	1708.2	0.3	1702.7	0.1	1702.6	0.2	1702.8	0.1
	$I_M$	1266.7	0.3	1256.9	0.1	1263.6	0.3	1254.1	0.2	1258.2	0.4	1252.4	0.1
CR	$I_c$	1805.7	0.4	1786.8	0.1	1800.0 <sup>c</sup>	—	1782.6	0.3	1788.9	0.5	1773.2	0.1
	$I_M$	1364.4	0.4	1342.9	0.1	1357.2	0.2	1337.4	0.3	1345.1	0.5	1326.0	0.2

<sup>a</sup> M<sub>7</sub> and mustard eluted together.

<sup>b</sup> M<sub>11</sub> and CS eluted together.

<sup>c</sup> C<sub>18</sub> and CR eluted together.

TABLE VIII  
EFFECT ON RETENTION INDICES OF COLUMNS FROM DIFFERENT MANUFACTURERS

Column: cross-linked SE-54, 25 m × 0.32 mm I.D., 0.25 μm (Orion Analytica; OA); cross-linked 5% phenyl-methyl silicone, 25 m × 0.31 mm I.D., 0.17 μm (Hewlett-Packard; HP); DB-5, 30 m × 0.33 mm I.D., 0.25 μm (J & W Scientific; J & W). Other conditions as in Table III.

Compound	Parameter	J & W			OA			HP					
		10°C/min	5°C/min	S.D.	10°C/min	5°C/min	S.D.	10°C/min	5°C/min	S.D.			
Sarin	$I_c$	819.5	819.5	0.8	821.5	821.0	0.3	829.0	821.0	0.0	829.0	829.0	3.3
	$I_M$	—	—	—	342.9	341.5	0.2	—	—	—	—	—	—
Tabun	$I_c$	1131.0	1130.2	0.2	1130.3	1129.3	0.9	1132.4	1129.3	0.1	1132.4	1132.1	0.1
	$I_M$	666.4	663.2	0.5	664.8	661.8	0.8	666.1	661.8	0.1	666.1	663.6	0.2
Mustard	$I_c$	1178.7	1174.0	0.1	1176.2	1171.4	0.7	1174.7	1171.4	0.1	1174.7	1170.5	0.2
	$I_M$	716.7	709.4	0.3	713.2	706.2	0.5	710.8	706.2	0.1	710.8	704.4	0.2
CN	$I_c$	1290.6	1284.0	0.2	1287.2	1280.5	0.6	1285.5	1280.5	0.1	1285.5	1279.8	0.4
	$I_M$	834.2	824.8	0.5	829.9	820.7	0.6	827.1	820.7	0.1	827.1	818.8	0.3
CS	$I_c$	1563.5	1555.5	0.2	1559.9	1551.4	0.6	1557.2	1551.4	0.1	1557.2	1550.2	0.3
	$I_M$	1116.3	1105.7	0.5	1111.6	1100 <sup>a</sup>	0.6	1108.1	1100 <sup>a</sup>	—	1108.1	1100 <sup>a</sup>	—
VX	$I_c$	1712.6	1704.4	0.1	1710.2	1700 <sup>b</sup>	0.2	1708.2	1700 <sup>b</sup>	—	1708.2	1700 <sup>b</sup>	—
	$I_M$	1269.7	1259.6	0.2	1266.7	1256.9	0.3	1263.7	1256.9	0.1	1263.7	1254.7	0.7
CR	$I_c$	1814.3	1794.6	0.4	1805.7	1786.8	0.4	1800 <sup>c</sup>	1786.8	0.1	1800 <sup>c</sup>	1782.2	0.2
	$I_M$	1373.6	1351.6	0.3	1364.4	1342.9	0.4	1357.1	1342.9	0.1	1357.1	1337.2	0.7

<sup>a</sup> M<sub>11</sub> and CS eluted together.

<sup>b</sup> C<sub>17</sub> and VX eluted together.

<sup>c</sup> C<sub>18</sub> and CR eluted together.

### Make of column

Stationary phases and columns marketed under the same product name may have different properties. Columns of the SE-54 type with bonded phenyl methyl polysiloxane phases, purchased from J & W, HP and OA (see Experimental), were compared.

All test compounds were separated with good resolution from retention index standards at a 10 °C/min programming rate on the J & W (30 m) and OA (25 m) columns and at 5 °C/min on the J & W column. On the HP (25 m) column the peak of sarin was badly tailed and the peaks of tabun and VX were slightly tailed at both rates. Perhaps the thinner film causes wall effects to appear. The peak of VX was slightly tailed on the OA column at a programming rate of 5 °C/min.

There were no great differences in the reproducibility of successive runs on the different columns, even on the column from HP which had a thinner film (Table VIII). The absolute values of the retention indices were lower when measured on the HP column, except for sarin and tabun, which eluted slightly slower from the HP than from the OA column. This effect may be due to adsorption or different polarity of the columns. Values of retention indices obtained for the J & W column were higher than values obtained for the HP and OA columns, except for the retention indices of sarin and tabun. In part this may be because the J & W column was longer than the others.

The two sets of retention indices,  $I_M$  and  $I_C$ , showed a linear dependence on different columns. The correlation coefficients were high in each instance, however, and when  $I_M$  is known,  $I_C$  can be calculated, and *vice versa*. The linear regression equations were calculated from the results in Table VIII for the 10 °C/min temperature programme, as follows:

$$\text{OA column (25 m):} \\ I_C = 0.9656I_M + 487.2 \quad r = 0.99999$$

$$\text{J & W column (30 m):} \\ I_C = 0.9663I_M + 485.9 \quad r = 0.99999$$

$$\text{HP column (25 m):} \\ I_C = 0.9660I_M + 487.8 \quad r = 0.99999$$

Retention indices ( $I_C$ ) calculated with these equations differed by 0.2-1.3 i.u. from the measured values, except for sarin, with a difference of 3.2 i.u.

### Make of instrument

This study was done using two Micromat HRGC 412 instruments, a Hewlett-Packard (HP) 5890 A and a Carlo Erba Fractovap 2900. Peak shapes were good in the chromatograms obtained with all the instruments.

As all screening for CW agents involves temperature programming, it was also of interest to know exactly how the oven of the gas chromatograph used follows the temperature programme. The oven temperature of the Micromat 412 rose more slowly and that of the Carlo Erba 2900 faster than the programmed rate. The oven temperature of the HP 5890 A followed the programmed rate (10 °C/min) within  $\pm 1$  °C, measured at the centre of the column coil. When the actual programming rate was calculated at a set value of 10 °C/min it was 9.25 °C/min for the Micromat HRGC 412 and 11 °C/min for the Carlo Erba 2900; at a set value of 5 °C/min the actual rate was 4.65 °C/min for the Micromat and 5.33 °C/min for the Carlo Erba instrument. As noted above, the retention indices of the test compounds increased when the rate of the temperature programme increased, and this may be one reason why the retention indices determined on the Hewlett-Packard and Carlo Erba instruments were slightly higher than those determined on the Micromat HRGC 412. The reproducibility, however, was good on all instruments.

### Different operators

The retention indices measured by three operators on one instrument and on one individual column in most instances differed by less than 1 i.u. The slightly larger variations in the indices for CS and VX were due to the peaks not being fully separated from those of retention index standards in all runs.

### Different laboratories

The reproducibility of the retention indices of the test compounds was good in three different laboratories using the same individual column (Table IX). The reproducibility was slightly better with the 10 °C/min temperature programme.  $\Delta I$  values were usually less than 1 i.u. With the 5 °C/min temperature programme the  $\Delta I$  value for soman was nearly 3 i.u.

TABLE IX

## DIFFERENCES IN RETENTION INDICES RECORDED IN DIFFERENT LABORATORIES

Column: cross-linked SE-54 (2), 15 × 0.32 mm I.D., 0.25 μm. Other conditions as in Table III.

Compound	Parameter	10°C/min							
		Laboratory 1		Laboratory 2		Laboratory 3		Different laboratories	
		<i>I</i>	S.D. (6 runs)	<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (6 runs)	$\Delta I$	$\Delta I$
Sarin	<i>I<sub>C</sub></i>	822.4	0.2	823.10	0.3	—	—	—	1.4
	<i>I<sub>M</sub></i>	—	—	—	—	—	—	—	—
Soman a	<i>I<sub>C</sub></i>	1042.0	0.2	1042.7	0.1	1042.4	0.3	0.7	0.7
	<i>I<sub>M</sub></i>	569.7	0.2	570.1	0.1	570.6	0.2	0.7	0.9
Soman b	<i>I<sub>C</sub></i>	1046.3	0.2	1047.1	0.1	1047.2	0.3	0.8	0.9
	<i>I<sub>M</sub></i>	574.4	0.2	574.9	0.1	575.3	0.3	0.7	0.9
Tabun	<i>I<sub>C</sub></i>	1129.6	0.1	1130.5	0.3	1130.8	0.5	1.3	1.2
	<i>I<sub>M</sub></i>	662.1	0.1	662.8	0.3	663.1	0.4	1.2	1.0
Mustard	<i>I<sub>C</sub></i>	1171.6	0.1	1172.0	0.2	1172.2	0.4	0.9	0.6
	<i>I<sub>M</sub></i>	706.5	0.1	706.5	0.2	706.8	0.3	0.7	0.3
CN	<i>I<sub>C</sub></i>	1281.0	0.1	1281.5	0.2	1281.5	0.3	0.7	0.5
	<i>I<sub>M</sub></i>	821.0	0.1	821.2	0.3	821.4	0.3	0.6	—
CS	<i>I<sub>C</sub></i>	1551.3	0.6	1552.9	0.3	1551.0	—	—	—
	<i>I<sub>M</sub></i>	1102.6	0.2	1104.0	0.4	1100 <sup>b</sup>	—	—	—
VX	<i>I<sub>C</sub></i>	1702.8	0.4	1703.9	0.2	1707.0	0.4	1.1	4.2
	<i>I<sub>M</sub></i>	1258.1	0.6	1258.0	0.2	1258.8	0.4	1.0	0.8
CR	<i>I<sub>C</sub></i>	1787.3	0.2	1788.1	0.2	1788.9	0.4	1.2	1.6
	<i>I<sub>M</sub></i>	1343.8	0.7	1344.0	0.2	1344.5	0.4	1.2	0.7

<sup>a</sup> M<sub>7</sub> and mustard eluted together.<sup>b</sup> M<sub>11</sub> and CS eluted together.*One or two retention index standard series in the same run*

The C- and M-series behaved very similarly and they did not seem to interfere with each other, with the consequence that the reproducibility of retention indices was the same whether one or both series were present in the run. The absolute values of the retention indices were nearly the same in the two instances.

*Reduced number of retention index standards*

The Project usually employs the internal standard method for RIM, where the retention index standards and sample compounds are present in the same mixture. In screening for a certain compound,

known to elute partially or totally overlapped with a retention index standard, it may be useful to omit this standard away. In the extreme case, the relative retention times of other compounds can be determined relative to only a single retention index standard [31].

One method for determining the relative retention times or retention indices of early eluting compounds is the calibration detector method [10,32]. A single column with effluent splitter and FID/ATD or FID/ECD detection may be used. FID is employed for detection of C-standards and ATD or ECD for detection of compounds of analytical interest. The ATD instrument is also used as a selective calibration detector for detecting M-standards,



5°C/min							
Laboratory 1		Laboratory 2		Laboratory 3		Different laboratories	
<i>I</i>	S.D. (5 runs)	<i>I</i>	S.D. (3 runs)	<i>I</i>	S.D. (5 runs)	$\Delta I$	$\Delta I$
821.0	0.6	821.2	0.1	—	—	—	0.2
—	—	—	—	—	—	—	—
1040.9	0.3	1042.8	0.1	1042.8	0.4	1.0	1.9
567.2	0.1	566.9	0.1	569.6	0.4	0.5	2.7
1045.2	0.3	1045.1	0.1	1047.1	0.4	1.0	2.0
572.0	0.1	571.6	0.1	574.4	0.4	1.0	2.8
1129.0	0.3	1130.0	0.1	1130.4	0.1	0.2	0.4
658.4	0.3	660.0	0.1	660.0	0.0	0.4	1.6
1167.0	0.2	1168.8	0.1	1167.8	0.4	0.8	1.0
698.4	0.1	700 <sup>a</sup>	—	700 <sup>a</sup>	—	—	—
1275.6	0.2	1276.7	0.2	1276.3	0.1	0.3	0.4
813.4	0.2	814.3	0.2	813.8	0.1	0.3	0.5
1545.0	0.3	1546.3	0.0	1545.4	0.1	0.4	1.3
1092.6	0.2	1093.5	0.1	1092.5	0.2	0.5	1.0
1703.8	0.5	1702.8	0.1	1701.8	0.7	0.3	2.0
1253.2	0.5	1252.4	0.1	1251.6	0.7	1.1	1.6
1771.9	0.3	1773.2	0.1	1773.1	0.2	0.3	1.3
1324.6	0.2	1326.0	0.2	1325.6	0.4	0.4	1.3

and FID or ECD provides analytical detection.

The absolute value of the retention index of a compound differed according to the number of standards used, but the reproducibility of retention indices was equally good whether every, every second, every third or every fourth member of the standard series was used. If the retention index library for RIM is created using every member of the standard series, wider index windows will be necessary for RIM with every second or third member of the series.

Table X presents a summary of the mean values of the retention indices of CW agents as these are affected by factors 1–3, 5, 7, 9, 10, 12, 13 and 16–18 (see Introduction). Only values recorded for the

flow-rate of 2 ml/min and programming rates of 10 and 5°C/min are included in the calculation. As can be seen, except for sarin the  $\Delta I$  values were consistently less than  $\pm 2$  i.u.. Standards M<sub>3</sub> and C<sub>8</sub>, which elute near sarin, also elute very near to the solvent peak.

#### CONCLUSIONS

The results of this work indicate that, in retention index monitoring (RIM) of the selected CW agents, the determination of retention indices is only slightly affected by the operator, different instruments from the same manufacturer with the same column in one or several laboratories, individual columns

TABLE X

THE MEAN VALUES OF RETENTION INDICES MEASURED UNDER SELECTED GAS CHROMATOGRAPHIC CONDITIONS

Instrument: Micromat HRGC 412. Column: cross-linked SE-54, 15 m × 0.32 mm I.D., 0.25 μm. For other conditions, see text.

Compound	Parameter	10°C/min		5°C/min	
		<i>I</i>	S.D.	<i>I</i>	S.D.
Sarin	<i>I<sub>C</sub></i>	823.5	3.9	821.6	1.1
	<i>I<sub>M</sub></i>	337.1	6.8	342.5	4.2
Soman a	<i>I<sub>C</sub></i>	1043.3	2.1	1042.2	1.0
	<i>I<sub>M</sub></i>	569.8	0.8	567.6	1.0
Soman b	<i>I<sub>C</sub></i>	1046.7	0.8	1046.1	1.1
	<i>I<sub>M</sub></i>	574.5	0.8	572.4	1.0
Tabun	<i>I<sub>C</sub></i>	1130.5	0.7	1130.0	0.9
	<i>I<sub>M</sub></i>	662.9	1.0	659.8	1.2
Mustard	<i>I<sub>C</sub></i>	1172.6	0.8	1168.7	1.0
	<i>I<sub>M</sub></i>	707.2	1.1	701.2	1.9
CN	<i>I<sub>C</sub></i>	1282.3	1.1	1277.4	1.2
	<i>I<sub>M</sub></i>	822.4	1.7	815.0	1.8
CS	<i>I<sub>C</sub></i>	1553.5	1.4	1547.4	1.4
	<i>I<sub>M</sub></i>	1104.2	1.8	1095.4	2.0
VX	<i>I<sub>C</sub></i>	1704.5	1.3	1701.9	1.5
	<i>I<sub>M</sub></i>	1258.7	1.0	1251.1	1.3
CR	<i>I<sub>C</sub></i>	1789.3	1.4	1773.7	1.1
	<i>I<sub>M</sub></i>	1345.4	1.9	1327.2	1.8

from the same manufacturer, different solvents (diethyl ether, acetone and ethyl acetate), use of M- and C-standards alone or together, use of a multi-step temperature programme and use of a Micromat 412 HRGC or HP 5890 A instrument. The same column could be used for 5 months without any serious deterioration in performance, except for sarin, which elutes at the beginning of the chromatogram near the solvent peak. Changes in the retention properties of the column may be due to deposition of non-volatile impurities at the column inlet. There was no significant difference in the reproducibility of one- and two-channel RIM.

The most critical parameters affecting the retention indices were the carrier gas flow-rate, the temperature programming rate and the properties of the column. The absolute values of the retention indices changed in response to these parameters anywhere from a few index units to 20–40 i.u., but

the reproducibility remained good. CR and VX were most affected in the case of nearly every parameter. Both are low-volatility compounds; CR contains aromatic rings, which means high sensitivity to temperature changes, and VX is a very large molecule. Where a compound eluted together with or very near a retention index standard, peak shifting tended to occur and the exact retention index was difficult to determine without omitting this standard.

The standard column used in this study was so short that the injection volume and the starting point of the temperature programme were important for the separation of M<sub>3</sub> and C<sub>8</sub> from the solvent peak and hence for the determination of the retention index of sarin. The background in the air sample extract strongly affected the detection of sarin and soman and longer columns are recommended for the screening of highly volatile agents.

There was no significant difference in the reproducibility of retention indices determined with the two index standard series. M-standards are relatively non-polar and on non-polar SE-54 phase behaved little differently from C-standards. Particularly the higher homologues of the M-standards behaved similarly to the C-standards. The most important property of the M-standards is that they are detectable by selective detectors at trace levels.

Overall we have found that RIM is a reliable and rapid method for the preliminary identification of CW agents, not requiring the use of authentic reference compounds provided that the chromatographic method is regularly tested with a test mixture and the chromatographic conditions or the retention index library are corrected if the deviations are too large. The retention index window  $\pm 2$  i.u. is suitable for all test compounds except VX and CR, for which a larger window of  $\pm 5$  i.u. may be necessary. The ultimate identification must, of course, be confirmed by another independent technique such as MS or FTIR.

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