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GAS CHROMATOGRAPHIC RETENTION INDICES OF CHEMICAL WARFARE AGENTS AND SIMULANTS

P. A. D'AGOSTINO* and L. R. PROVOST

Defence Research Establishment Suffield, Ralston, Alberta T0J 2N0 (Canada)

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SUMMARY

Temperature-programmed retention indices, relative to a homologous *n*-alkane series, were determined for twenty-two chemical warfare agents and simulants using fused-silica capillary columns coated with DB-1, DB-5, DB-1701 and DBWAX films. Reproducibility of retention indices, based on Van den Dool's equation, was excellent over the course of the study. The applicability of this method for compound verification was demonstrated by the analysis of a soil sample containing chemical warfare agents. In addition, an on-column injector of our own design was evaluated for use with wide bore fused-silica capillary columns.

INTRODUCTION

The alleged use of chemical warfare agents in recent conflicts highlights the need for detection and identification procedures¹. Fused-silica capillary column gas chromatography (GC) is an excellent method for this application since it is capable of separating the components of complex environmental and biological samples. A procedure for the screening and verification of chemical defence compounds, based on their temperature-programmed retention indices, has been developed and applied to the analysis of a soil sample.

GC retention data have been used extensively for the identification of compounds in a variety of samples². The correlation of retention times between an unknown component and a reference compound on two or more columns of differing polarities is usually sufficient for identification purposes. Additional spectroscopic data (*e.g.*, mass spectral data) can further substantiate the identity of an unknown component. Retention data relative to an arbitrary standard³⁻⁶, relative to a homologous series of similar compounds^{3,4,7,8} and relative to a homologous series of *n*-alkanes⁷⁻¹¹ have all been used for chemical agents and related compounds. Finland currently uses a homologous series of alkyl dimethylphosphinothionates for organophosphorous compound retention index determinations^{3,4,7,8}. This method works well since the standards are similar to the compounds of interest and may be used with specific detectors. However, this retention index system is limited to compounds of a similar class. Retention indices, based on a homologous series of *n*-alkanes, were

used in this study since they are applicable to a wide variety of compound classes.

The Kováts retention index¹², based on the relative retention times of a particular compound to an *n*-alkane standard under isothermal conditions, has been used extensively for the GC study of many compound classes², including those compounds of interest to the defence community¹¹. This index system is practical only when compounds with similar retention behaviour are to be studied since the column temperature is held constant. More practical is the determination of retention indices during temperature programming since this allows indices to be calculated during one analysis for compounds over a wide range of volatilities. Temperature-programmed GC retention indices were calculated using Van den Dool's equation¹³.

An on-column injector of our own design was used for retention index determinations and the subsequent analysis of a soil sample for chemical warfare agents. On-column injection systems¹⁴⁻²¹ are preferred by many analysts for sample introduction since this method provides excellent quantitative precision and accuracy for samples with a wide range of volatilities^{14,15,22}. Recent studies of priority micro-pollutants²³ and organophosphorous and chlorinated pesticides²⁴ confirmed the superiority of on-column *versus* splitless injection for reliable quantitative analysis of environmental samples.

This paper reports the retention indices of twenty-two compounds determined on fused-silica capillary columns coated with DB-1, DB-5, DB-1701 and DBWAX films. Included in this study were organophosphorous chemical warfare agents, vesicants, irritants and various simulants. In addition a simple, low cost on-column injector of our own design is described.

EXPERIMENTAL

Standards

The *n*-C₇-*n*-C₁₉ and *n*-C₂₀-*n*-C₃₂ (even numbers only) alkanes were purchased from Alltech (Deerfield, IL, U.S.A.). Two 0.1-mg/ml standards containing *n*-C₇-*n*-C₁₉ and *n*-C₇-*n*-C₃₂ were prepared in HPLC grade hexane (Fisher Scientific, Edmonton, Canada) for co-injection with the chemical defence compounds.

Retention indices were determined for twenty-two compounds available in our organic laboratory. Some of the compounds are commercially available. Standards, often containing several compounds in the range 0.04-0.05 mg/ml per component, were prepared in HPLC grade chloroform (Fisher Scientific).

Instrumental analysis

A Varian 3700 gas chromatograph (Varian, Georgetown, Canada) equipped with a flame ionization detector and a Varian 4270 integrator was used for all analyses. Four bonded and cross-linked J&W 15 m × 0.32 mm I.D. capillary columns coated with 0.25- μ m films (J&W Scientific, Rancho Cordova, CA, U.S.A.) were used for retention index determinations. The columns used were: (a) DB-1 (100% dimethyl-polysiloxane); (b) DB-5[(95%)-methyl-(5%)-diphenyl-polysiloxane]; (c) DB-1701 [(86%)-dimethyl-(14%)-cyanopropylphenyl-polysiloxane] and; (d) (100% polyethylene glycol). All injections were on-column at 50°C. Following injection the column temperature was held at 50°C for 2 min and then temperature programmed at 10°C/min to 300°C for columns a-c and to 250°C for column d. The upper temper-

ature was maintained for 5 min. High purity helium at a linear velocity of 35 cm/sec (methane injection at 50°C) was used as the carrier gas.

On-column injector design

Fig. 1 illustrates the on-column injector designed for sample introduction into wide bore fused-silica capillary columns. The sample is introduced directly into the capillary column by removing the cap and inserting the syringe needle into the needle guide. Analysis begins with syringe removal and cap replacement. A 5- μ l, 33 gauge Hamilton syringe (Supelco, Oakville, Canada) was used for all injections. The J&W fused-silica capillary syringe can also be used with this injector design.

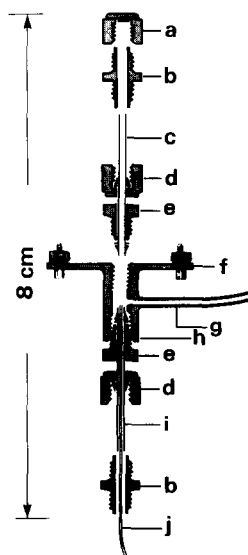


Fig. 1. On-column injector developed for wide bore fused-silica capillary columns. All fittings are Swagelok 1/16 in. stainless steel. a = cap with PTFE seal (1/16-in. hole filled with silver solder); b = union (drilled out to 1/16 in.); c = 1/16 in. \times 0.4 mm I.D. Glass-lined tubing (needle guide); d = nut with graphite ferrule; e = low dead volume nut and ferrule; f = support to attach injector to oven wall (silver soldered to union); g = 1/16 in. \times 0.5 mm I.D. carrier gas line; h = low dead volume union; i = 1/16 in. \times 0.5 mm I.D. stainless-steel tubing (column guide); j = capillary column (attached with 1/16 in. nut and 0.5 mm I.D. graphite ferrule).

Soil sample analysis

A 300-mg soil sample alleged to contain chemical warfare agents was received as part of an international verification exercise. It was transferred into a PTFE-lined screw capped vial and extracted by ultrasonic vibration for 30 min at 25°C with 1 ml of HPLC grade chloroform. The soil material was allowed to settle for 1 h and the chloroform extract was used for GC analysis.

The soil extract, after initial screening by capillary column GC-flame ionization detection (FID) was co-injected with the *n*-C₇-*n*-C₃₂ alkane standards on both the DB-1 and DB-5 capillary columns. Retention indices were then determined for the extract components.

TABLE I

GC RETENTION INDICES FOR SELECTED DEFENCE RELATED COMPOUNDS ON DB-1, DB-5, DB-1701 AND DBWAX FUSED-SILICA CAPILLARY COLUMNS

Compound (common name)	Retention index*				C.A. registry number
	DB-1	DB-5	DB-1701	DBWAX	
<i>Organophosphorous compounds</i>					
Isopropyl methylphosphonofluoridate (Sarin)	791.8 ± 0.1	823.8 ± 0.2	965.9 ± 0.2	1278.4 ± 0.4	[107-44-8]
Pinacolyl methylphosphonofluoridate (Soman)**	1008.1 ± 0.3	1045.2 ± 0.2	1187.5 ± 0.1	1458.7 ± 0.2	[96-64-0]
Ethyl N,N-dimethylphosphoroamidocyanidate (Tabun)	1012.9 ± 0.3	1049.3 ± 0.2	1193.3 ± 0.2	1470.4 ± 0.3	[77-81-6]
2-Methylcyclohexyl methylphosphonofluoridate**	1077.9 ± 0.3	1131.6 ± 0.5	1339.8 ± 0.4	1790.4 ± 0.6	
o-Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate	1210.2 ± 0.3	1256.7 ± 0.6	1421.1 ± 0.4	1784.6 ± 0.4	
Triethyl phosphate	1211.7 ± 0.4	1258.5 ± 0.4	1423.3 ± 0.6	1790.2 ± 0.5	
Dimethyl morpholinophosphoramidate	1664.1 ± 0.1	1710.1 ± 0.5	1881.1 ± 1.0	2275.3 ± 0.3	[50782-69-9]
Tributyl phosphate	1090.7 ± 0.3	1137.2 ± 0.4	1306.4 ± 0.7	1672.1 ± 0.6	[78-40-0]
	1296.2 ± 0.3	1353.6 ± 0.2	1562.1 ± 0.1	2145.4 ± 1.2	[597-25-1]
	1615.7 ± 0.5	1658.8 ± 0.5	1830.9 ± 0.6	2157.4 ± 1.1	[126-73-8]
<i>Vesicants and related compounds</i>					
1,4-Dithiane	1018.7 ± 0.3	1060.2 ± 0.1	1156.7 ± 0.3	1575.7 ± 0.8	[505-29-3]
Bis(2-chloroethyl)sulfide (Mustard)	1123.8 ± 0.1	1172.7 ± 0.2	1325.8 ± 0.4	1820.0 ± 0.2	[505-60-2]
2,2'-Thiodiethanol (Thiodiglycol)	1130.9 ± 0.4	1181.5 ± 0.1	1458.2 ± 0.2	2422.6 ± 0.9	[111-48-8]
Bis(2-chloroethyl)disulfide	1336.3 ± 0.7	1391.2 ± 0.1	1561.8 ± 0.5	2134.7 ± 0.4	[1002-41-1]
Bis(2-chloroethyl)trisulfide	1561.7 ± 0.4	1626.3 ± 0.5	1805.4 ± 0.6	2456.2 ± 1.3	
Sesquimustard	1622.7 ± 0.6	1688.8 ± 1.0	1922.7 ± 0.2	2619.9 ± 0.7	[3563-36-8]
1,1'-Oxybis[2-(2-chloroethyl)thio]ethane]	1909.9 ± 2.7	1982.6 ± 1.0	2241.3 ± 0.6	3040.3 ± 0.7	[63918-89-8]
<i>Irritants</i>					
1-Methoxycycloheptatriene	993.8 ± 0.3	1019.0 ± 0.3	1085.1 ± 0.3	1411.9 ± 0.3	
2-Chloroacetophenone (CN)	1230.2 ± 0.1	1283.0 ± 0.3	1471.0 ± 0.1	2114.4 ± 0.5	[532-27-4]
o-Chlorobenzylidenemalonitrile (CS)	1489.6 ± 0.5	1555.1 ± 0.1	1805.6 ± 0.1	2483.6 ± 1.0	[2698-41-1]
Dibenz[b,f]-1,4-oxazepin	1736.0 ± 0.1	1800.0 ± 0.3	1990.8 ± 0.6	2813.4 ± 0.7	[257-07-8]
<i>Simulants</i>					
Dimethyl sulfoxide (DMSO)	786.6 ± 0.1	843.1 ± 0.5	1066.6 ± 0.3	1582.3 ± 0.5	[67-68-5]
Dimethyl sulfone (DMSO ₂)	851.4 ± 0.2	921.6 ± 0.7	1195.0 ± 0.6	1772.8 ± 0.1	[67-71-0]
Methyl salicylate	1163.1 ± 0.1	1195.5 ± 0.4	1302.9 ± 0.1	1897.4 ± 0.1	[119-36-8]

* Retention indices expressed as mean ± S.D. (n = 3).

** Retention indices given for both enantiomer pairs.

RESULTS AND DISCUSSION

Retention indices

Table I summarizes the retention indices of twenty-two compounds on capillary columns coated with DB-1, DB-5, DB-1701 and DBWAX films. Amounts of 20–40 ng of each standard were co-injected with a similar amount of *n*-alkane standard. The retention indices were calculated using eqn. 1¹³.

$$RI_c = 100n \left[\frac{t_{R(c)} - t_{R(z)}}{t_{R(z+n)} - t_{R(z)}} \right] + 100z \quad (1)$$

where: *c* is the compound of interest, *n* is the difference in carbon number between two *n*-alkanes either side of compound *c*, *t_R* is the retention time, *z* is the carbon number of *n*-alkane immediately prior to compound *c*.

Excellent reproducibility (S.D. usually ≤ 1) during the course of this study was attributed to the co-injection technique, since this method minimized retention index variation associated with minor retention time changes. Reproducibility was generally poorest on the more polar columns where the retention behaviour of the non-polar alkanes and the more polar compounds studied vary most. Other parameters that may have influenced the reproducibility of this method include: (a) batch-to-batch stationary phase variability; (b) catalytic decomposition or polymerization of the stationary phase; (c) film thickness; (d) the degree of activation of the stationary phase; (e) thermal degradation of the stationary phase due to repeated column use; (f) amount of analyte; (g) temperature programming rate and; (h) operating temperature.

The columns used in this study ranged from non-polar (e.g., DB-1) to polar (e.g., DBWAX). There was a noticeable increase in retention index value for each compound with increasing column polarity. Retention index changes between two columns (ΔRI) were most pronounced for more polar compounds such as thiodiglycol. The ΔRI values for each compound, although not calculated in this report, could provide information regarding the relative polarities of these compounds.

Column selection is easily made for target compound analysis by consulting the data in Table I. For example, the determination of mustard in the presence of its principle hydrolysis product, thiodiglycol, is more easily attained on a more polar stationary phase such as DB-1701 or DBWAX. The DB-1 or DB-5 columns were found to be more useful for sample screening since analysis was performed more quickly than on the polar columns.

The inter-laboratory applicability of retention indices is best illustrated by comparison of our data with that obtained independently by another laboratory. Temperature-programmed retention indices, recently published in Finland⁷, for four of the compounds investigated were found to be similar to those determined in our study. Table II compares the data obtained using our 15-m DB-1 capillary column with the Finnish results obtained with a 15-m OV-1 capillary column. Retention index differences of 2 to 4 units were observed. We recommend a working window of about ± 4 retention units for identification purposes for long term use.

TABLE II

INTERLABORATORY COMPARISON OF RETENTION INDICES (RI) OBTAINED FOR SEVERAL COMPOUNDS (10°C/min; METHYL SILICONE STATIONARY PHASE)

Compound name	RI	
	This study*	Finnish ⁶
Bis(2-chloroethyl)sulfide	1123.8 ± 0.1	1127
2-Chloroacetophenone	1230.2 ± 0.1	1234
<i>o</i> -Chlorobenzylidenemalonitrile	1489.6 ± 0.5	1493
Dibenz[<i>b,f</i>]-1,4-oxazepin	1736.0 ± 0.1	1734

* Retention indices expressed as a mean ± S.D. ($n = 3$).

Application

The retention indices tabulated in this study were applied to the analysis of a soil sample allegedly containing chemical warfare agents. Fig. 2 illustrates the DB-5 chromatogram obtained by co-injection of the *n*-alkane standard and the soil extract. Table III lists the DB-1 and DB-5 retention indices for the four major sample components. There was a good correlation between the sample data and standard data (refer to Table I) which was obtained about six months earlier. Compound identity was confirmed by capillary column GC-mass spectrometry in the electron-impact mode²⁵. One compound, found to fragment similarly to 1,1'-oxybis[2-((2-chloroethyl)thio)ethane], remains unidentified.

On-column injector

Fig. 1 illustrates the design of the on-column injector used for sample intro-

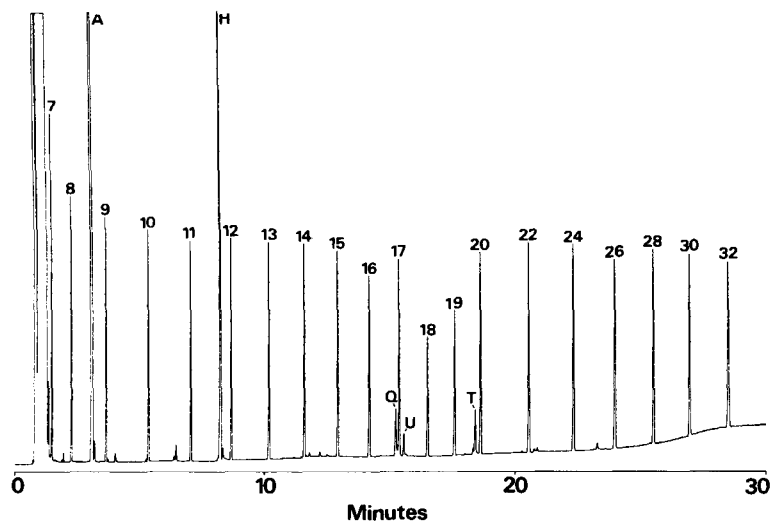


Fig. 2. Determination of DB-5 retention indices for the major components found in the soil extract. The analysis was performed by co-injection of a solution of *n*-alkanes (C₇-C₃₂) with the equivalent of 9 µg of the soil sample. The calculated indices and lettered peak identities are presented in Table III.

TABLE III

RETENTION INDICES OF COMPOUNDS IDENTIFIED IN THE SOIL SAMPLE

Ethylbenzene (A): present in the hydrocarbon kit used to prepare the *n*-alkane standard.

Compound name (abbreviation)	Retention index*	
	DB-1	DB-5
Bis(2-chloroethyl)sulfide (H)	1124.8 ± 0.1	1174.1 ± 0.1
Sesquimustard (Q)	1623.9 ± 0.1	1688.2 ± 0.1
Unknown (U)	1660.3 ± 0.2	1716.1 ± 0.1
1,1'-Oxybis[2-((2-chloroethyl)thio)ethane] (T)	1908.0 ± 0.4	1979.6 ± 0.1

* Retention indices expressed as a mean ± S.D. (*n* = 3).

duction in all analyses. This injector was modified from an existing design which had to be used earlier to successfully minimize higher molecular weight discrimination in a number of chromatographic applications at McMaster University²⁶.

The reproducibility of this injector was compared to the commercially available J&W on-column injector for the analysis of *n*-alkanes. The data obtained after four analyses, under identical chromatographic conditions, are listed in Table IV. Percentage area contributions by each *n*-alkane are similar for both injectors and generally within the standard deviation associated with the area measurements. Discrimi-

TABLE IV

n-ALKANE ANALYSIS RESULTS OBTAINED WITH THE DESCRIBED ON-COLUMN INJECTOR AND A COMMERCIAL INJECTOR

Area percentages are the mean values of 4 analyses.

<i>n</i> -Alkane	Described injector			Commercial injector		
	Area	S.D.	R.S.D.	Area	S.D.	R.S.D.
C ₈	4.45	0.11	2.5	4.42	0.14	3.2
C ₉	4.80	0.13	2.7	4.93	0.22	4.5
C ₁₀	5.08	0.10	2.0	5.22	0.28	5.4
C ₁₁	5.22	0.12	2.3	5.21	0.10	1.9
C ₁₂	5.29	0.14	2.6	5.26	0.09	1.7
C ₁₃	5.34	0.14	2.6	5.42	0.18	3.3
C ₁₄	5.37	0.15	2.8	5.38	0.15	2.8
C ₁₅	5.31	0.17	3.2	5.35	0.22	4.1
C ₁₆	5.16	0.08	1.5	5.02	0.04	0.8
C ₁₇	5.04	0.16	3.2	5.08	0.11	2.2
C ₁₈	3.39	0.07	2.1	3.36	0.13	3.9
C ₁₉	4.28	0.10	2.3	4.35	0.25	5.7
C ₂₀	6.09	0.17	2.8	6.45	0.41	6.4
C ₂₂	5.72	0.08	1.4	5.57	0.22	3.9
C ₂₄	6.08	0.10	1.6	6.03	0.43	7.1
C ₂₆	6.44	0.31	4.8	5.92	0.15	2.5
C ₂₈	5.83	0.23	3.9	5.74	0.52	9.1
C ₃₀	5.40	0.14	2.6	5.59	0.28	5.0
C ₃₂	5.76	0.23	4.0	5.69	0.31	5.4

nation of higher molecular weight *n*-alkanes, a problem often encountered during splitless injection, was not observed for either injector.

Measurement precision was acceptable for both injectors except in the area measurements of *n*-C₂₄ and *n*-C₂₈ with the commercial injector. The reasons for this discrepancy are not known. Precision was generally in the 2-4% range for analysis with our on-column injector.

CONCLUSIONS

Temperature-programmed retention indices, using a homologous series of *n*-alkanes, were determined for twenty-two chemical warfare agents and simulants using capillary columns coated with DB-1, DB-5, DB-1701 and DBWAX films. These data were applied to a soil sample found to contain several vesicants.

An on-column injector suitable for wide bore fused-silica capillary columns has been described. This low cost unit can be easily fabricated from 1/16-in. Swagelok fittings and may be mounted in any conventional gas chromatograph.

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