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Gas chromatographic system for the identification of halogenated pesticides by retention indices using *n*-alkanes as standards

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ABSTRACT

A gas chromatographic system for the evaluation of linear temperature-programmed retention indices allowing *n*-alkanes to be adopted as the reference retention markers for any type of analyte, irrespective of the atoms present in their molecules, is described. It is based on the simultaneous use of two different detectors (a flame ionization detector and a specific detector suitable for the sample components), both connected (in parallel) to the same column outlet. The performance of this system has been tested by measuring the retention indices of fifteen chlorinated pesticides under conditions of linear programming temperature, by adopting an electron-capture detector as the specific detector. The reliability of the retention indices thus determined has been proven by verifying that they can be reproduced under different chromatographic conditions.

INTRODUCTION

It is a common practice in gas chromatography (GC) to perform qualitative analyses by comparing retention times (RT) or relative retention times (RRT) of unknown analytes with those of standard substances. Nevertheless, this method is adequate only for simple mixtures. As the complexity of the sample increases, analyte identification becomes more doubtful, even though the margin of uncertainty can be reduced by repeating chromatographic runs after suitable changes of either the temperature or the stationary phase or after chemical derivatization of the analytes.

A decided improvement is offered by Kováts indices [1,2] which relate retention times of unknown compounds (RT_x) to those of *n*-alkanes eluting immediately before (RT_n) and after (RT_{n+1}) them. The considerable progress made in analyte identification thanks to the adoption of these indices has been reviewed recently [3,4].

They depend only on the nature of the liquid phase employed and this is the reason why their use for the characterization of stationary phases has been suggested

[5]. Moreover, they present a uniform scale rather than a single fixed point for comparison and can also yield supporting information on the analyte structure [6].

This notwithstanding, their use is limited by two main drawbacks which make Kováts indices inapplicable in some cases. The former is that they are defined for isothermal chromatography, *i.e.* for experimental conditions which are not the most appropriate to separate complex samples containing analytes with quite different chromatographic behaviour. The second drawback comes from the need to employ a flame ionization detector (FID) to gain a satisfactory sensitivity in the evaluation of these indices referred to *n*-alkanes, thus precluding the application of this approach to analytes detectable at trace levels only by specific detectors.

The first limitation can be overcome by using retention indices calculated under conditions of linear programming temperature [7]:

$$I_{px} = 100\left(n + \frac{T_x - T_n}{T_{n+1} - T_n}\right) \tag{1}$$

where T_x, T_n and T_{n+1} are the retention temperatures of the unknown analyte (x) and of the *n*-alkanes eluting immediately before (n) and after it ($n + 1$), respectively.

As a matter of fact, the application of this equation requires careful evaluation of retention temperatures, which is not a problem when retention times are linearly dependent on them. Sometimes, however, the temperature programmes installed into commercially available instruments may deviate from linearity, especially at the beginning and at the end of the temperature programme. In these cases it appears convenient to resort to a cubic spline interpolation procedure [8] of the points describing the plot of the retention temperature *versus* the retention time, by using the following equation:

$$I_{px} = I_{pn} + a(\mathrm{RT}_x - \mathrm{RT}_n) + b(\mathrm{RT}_x - \mathrm{RT}_n)^2 + c(\mathrm{RT}_x - \mathrm{RT}_n)^3 \tag{2}$$

where a, b and c are coefficients calculated by a regression program and other symbols have the meaning reported above, except for n, which indicates in this case a general term of the homologous series. This last relationship offers the additional advantage of allowing temperature-programmed retention indices (I_{px}) to be calculated directly from retention times found under conditions of linear programming temperature even when the dependence of retention times on elution temperatures is not completely linear.

To overcome the second drawback, the use as standards of series of organic compounds suitable for specific detectors, in that they contain atoms such as nitrogen [9–11], sulphur [11,12] or more hetero elements simultaneously [11], has been suggested. Such an expedient does not appear, however, as a remedy since many components of these homologous series are either not commercially available (and hence must be synthesized) or poorly stable with time (and hence cannot be stored). Moreover, these series of standards are usually chosen arbitrarily and this makes an interlaboratory comparison and transfer of the indices thus determined impractical.

This paper describes the evaluation of I_{px} by adopting *n*-alkanes as the retention standards for any type of unknown analytes, irrespective of the atoms contained in their molecules. These indices are acquired using a gas chromatograph equipped with a single column but two different detectors, one of which is a FID while the other is a specific detector. The suitability of this system for the retention index evaluation under conditions of linear programming temperature has been proven for a standard mixture of fifteen chlorinated pesticides. Moreover, the possibility of reproducing the temperature-programmed retention indices obtained by this approach under different chromatographic conditions has also been considered.

EXPERIMENTAL

All samples were analyzed under temperature-programmed conditions by a Mega Series 5300 gas chromatograph (Carlo Erba, Milan, Italy) equipped with both a Model 40 FID (Carlo Erba) for standard *n*-alkanes and a Model HT25 electron-capture detector (ECD) (Carlo Erba), for chlorinated pesticides. Simultaneous detection of these two classes of compounds was accomplished by clamping an Y-shaped glass press-fit (Carlo Erba) at the end of the chromatographic column so as to split the column outlet into two equivalent channels connected to the mentioned detectors by equal 10-cm pieces of silanized fused-silica, as shown in Fig. 1.

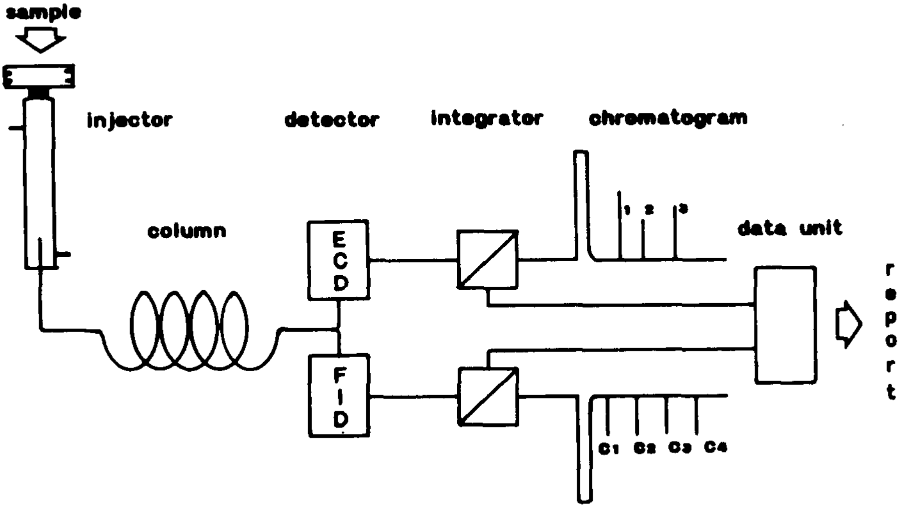


Fig. 1. Schematic view of the gas chromatographic apparatus employed.

Two 25 m × 0.32 mm I.D. methyl silicone (OV-1) on fused-silica capillary columns (Mega, Legnano, Italy) coated with different film thicknesses were employed for sample separations. Both these columns were used under different flow-rates of the carrier gas and different conditions of programming temperature. Operation parameters together with column characteristics are given in Table I.

Samples (1 μl) were introduced with a split injector maintained at 250°C (split ratio 1:20). A constant current of 200 μA with pulse duration of 0.5 μs was used for the ECD whose temperature was set at 275°C, while the temperature of the FID block was

TABLE I

OPERATION PARAMETERS AND CHARACTERISTICS OF THE TWO OV-1 CAPILLARY COLUMNS EMPLOYED, BOTH HAVING DIMENSIONS OF 25 m × 0.32 mm I.D.

Column	Operation condition	ft^a (μm)	β^b	fr^c (ml/min)	$t_0{}^d$ (min)	r^e (°C/min)	rt_0/β (°C)
A	A-a	0.10	800	1	1.6	1.5	0.0030
A	A-b	0.10	800	1	1.6	2.0	0.0040
A	A-c	0.10	800	2	0.8	1.5	0.0015
A	A-d	0.10	800	2	0.8	2.0	0.0020
B	B-a	0.15	530	1	1.6	1.0	0.0030
B	B-b	0.15	530	1	1.6	1.3	0.0040
B	B-c	0.15	530	2	0.8	1.0	0.0015
B	B-d	0.15	530	2	0.8	1.3	0.0020

[a] Film thickness of the stationary phase.

[b] Phase ratio defined as the ratio between I.D. and 2 *ft*.

[c] Carrier gas flow-rate.

[d] Gas hold-up time of the column measured as the methane elution time at 160°C.

[e] Temperature programme rate from 160°C (hold for 1 min) to 240°C.

set at 250°C. Data handling was performed by a dual-channel Spectra-Physics SP4270 (Santa Clara, CA, USA) integrator connected to an IBM XT computer (Valhalla, NY, USA).

Pure chlorinated pesticides were obtained from Riedel de-Häen (Seelze, Germany); their formulae, as well as their basic characteristics, are reported in Table II. Stock solutions of these test compounds were prepared at a concentration of 0.1 ppm in *n*-hexane by diluting successively the pure products, while stock mixtures of C_{11}–C_{24} n-alkanes at a concentration of 20 ppm in *n*-hexane were prepared by diluting the pure compounds obtained from Fluka Chemie (Buchs, Switzerland). These last mixtures of reference retention markers were mixed with the pesticide samples prior to chromatographic analysis.

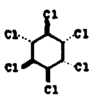
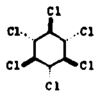
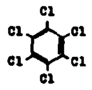
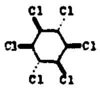
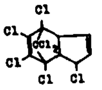
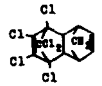
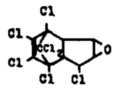
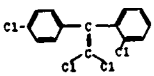
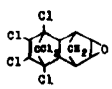
RESULTS AND DISCUSSION

ECD and FID responses relative to each injection were simultaneously recorded by the dual-channel integrator and then compared by the connected computer so as to obtain the relevant difference, as shown in Fig. 2. This last step allowed us to show the possible coincidence of retention times for pesticides with those for the reference retention markers (see the coincidence in Fig. 2 between retention time for the alkane C_{21} and that for heptachlorepoxid). Retention indices (I_{px}) for all pesticides employed were then calculated by inserting the corresponding retention times, evaluated from chromatograms like those in Fig. 1, in a computation program written on the basis of eqn. 2 which is available on request.

In order to test the reliability of these indices, their independence from the particular chromatographic conditions employed has been verified following the criterion reported recently by Yin and Sun [13]. These authors point out that capillary columns of different sizes (length and inner diameter), working under different heating

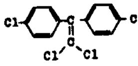
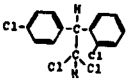
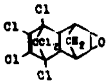
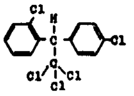
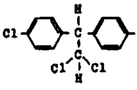
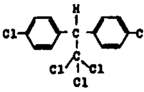
TABLE II

CHLORINATED PESTICIDES EMPLOYED

No.	Compound	Structure	Characteristics
1.	α-1,2,3,4,5,6-Hexachlorocyclohexane (α-HCH)		Insecticide $C_6H_6Cl_6$ M = 290.83 g/mol CAS 319-84-6
2.	β-1,2,3,4,5,6-Hexachlorocyclohexane (β-HCH)		Insecticide $C_6H_6Cl_6$ M = 290.83 g/mol CAS 319-85-7
3.	Hexachlorobenzene (HCB)		Fungicide C_6Cl_6 M = 284.78 g/mol CAS 118-74-1
4.	γ-1,2,3,4,5,6-Hexachlorocyclohexane (lindane)		Insecticide $C_6H_6Cl_6$ M = 290.83 g/mol CAS 58-89-9
5.	1,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (heptachlor)		Insecticide $C_{10}H_5Cl_7$ M = 373.32 g/mol CAS 76-44-8
6.	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*-*exo*-5,8-dimethanonnaphthalene (aldrin)		Insecticide $C_{12}H_8Cl_6$ M = 364.91 g/mol CAS 309-00-2
7.	1,4,5,6,7,8,8-Heptachloro-2,3- epoxy-4,7-endomethano-3a,4,7,7a-tetrahydroindene (Heptachlorepoxid)		Insecticide $C_{10}H_5Cl_7O$ M = 389.32 g/mol CAS 1024-57-3
8.	2-(2-Chlorophenyl)-2-(4-chlorophenyl)-1,1-dichloroethene (2,4′-DDE)		Metabolite $C_{14}H_8Cl_4$ M = 318.03 g/ml CAS 3424-82-6
9.	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*-5,8-*exo*-dimethanonaphthalene (dieldrin)		Insecticide $C_{12}H_8Cl_6O$ M = 380.91 g/mol CAS 60-57-1

(*Continued on p. 360*)

TABLE II (*continued*)

No.	Compound	Structure	Characteristics
10.	2,2-Bis-(4-chlorophenyl)-1,1-dichloroethene (4,4′-DDE)		Metabolite $C_{14}H_8Cl_4$ M = 318.03 g/mol CAS 72-55-9
11.	2,(2-Chlorophenyl)-2-(4-chlorophenyl)- 1,1-dichloroethane (2,4′-DDD)		Insecticide $C_{14}H_{10}Cl_4$ M = 320.05 g/mol CAS 53-19-0
12.	1,2,3,4,10,10-Hexachloro-6,7-epoxy- 1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*- *endo*-5,8-dimethanonaphthalene (endrin)		Insecticide $C_{12}H_8Cl_6O$ M = 380.91 g/mol CAS 72-20-8
13.	2-(2-Chlorophenyl)-2-(4-chlorophenyl)- 1,1,1-trichloroethane (2,4′-DDT)		Insecticide $C_{14}H_9Cl_5$ M = 354.49 g/mol CAS 784-02-6
14.	2,2-Bis-(4-chlorophenyl)-1,1-dichloroethane (4,4′-DDD)		Insecticide $C_{14}H_{10}Cl_4$ M = 320.05 g/mol CAS 72-54-8
15.	2,2-Bis-(4-chlorophenyl)-1,1,1-trichloroethane (4,4′-DDT)		Insecticide $C_{14}H_9Cl_5$ M = 354.51 g/mol CAS 50-29-3

rates and/or carrier gas flow-rates and characterized by different phase ratios β are able to reproduce the same values of retention indices (within $\pm$ 2 index units), provided that their ratios rt_0/β are kept unchanged (the meaning of the symbols is defined in Table I).

Consequently, retention indices for the fifteen chlorinated pesticides here analyzed have been determined on two different columns (A and B in Table I) having the same size but different phase ratios β. They have been used under the different experimental conditions (*fr* and *r*) reported in Table I. Both flow-rate and temperature programme rate were chosen in such a way that same values of the ratio rt_0/β could be attained for both columns A and B.

Tables III–VI list the temperature-programmed retention indices found for all

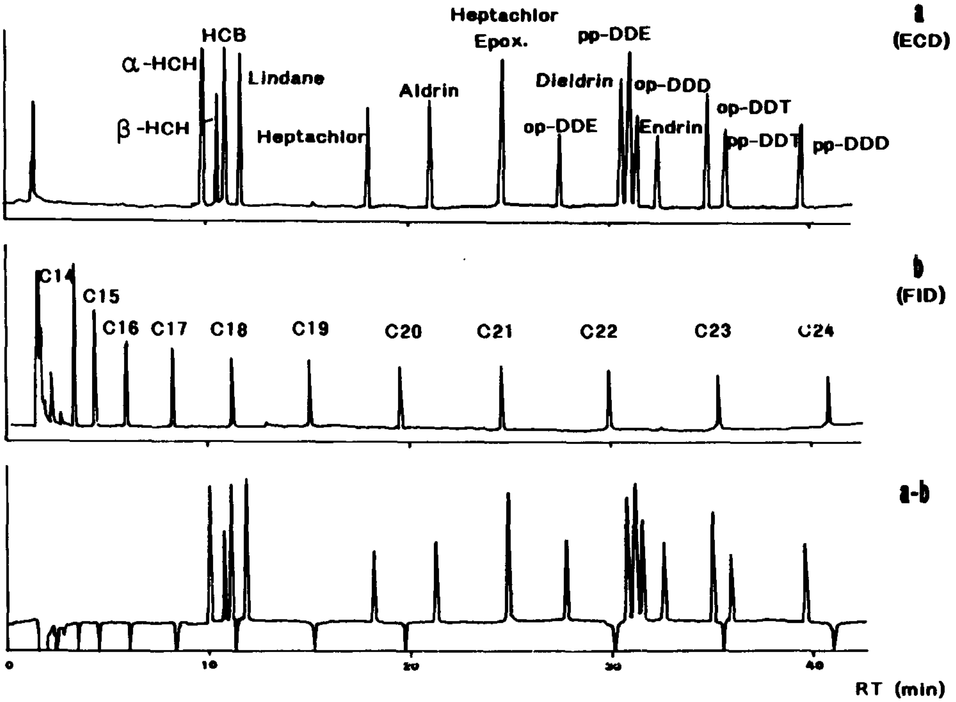


Fig. 2. Analysis of a mixture containing the fifteen chlorinated pesticides listed in Table II and the C_{11}–C_{24} reference retention markers. Gas chromatograms were recorded on the capillary column A under the experimental conditions A-c in Table I.

TABLE III

TEMPERATURE-PROGRAMMED RETENTION INDICES (I_{px}) FOUND WITH A RATIO $rt_0/\beta = 0.0030$ (OPERATION CONDITIONS A-a AND B-a IN TABLE I)

Pesticide	Column A[a] (A-a)	Column B[a] (B-a)	Difference $(I_{A\text{-}a}-I_{B\text{-}a})$
1. α-HCH	1657.62 (0.14)	1657.16 (0.25)	0.46
2. β-HCH	1678.72 (0.17)	1678.74 (0.32)	−0.02
3. HCB	1690.43 (0.23)	1690.58 (0.21)	−0.15
4. Lindane	1710.92 (0.09)	1710.78 (0.07)	0.14
5. Heptachlor	1863.41 (0.27)	1862.70 (0.18)	0.71
6. Aldrin	1928.13 (0.25)	1927.87 (0.26)	0.26
7. Heptachlorepoxid	1997.42 (0.32)	1997.03 (0.09)	0.39
8. 2,4′-DDE	2053.43 (0.20)	2053.68 (0.29)	−0.25
9. Dieldrin	2107.58 (0.12)	2107.37 (0.06)	0.21
10. 4,4′-DDE	2116.56 (0.07)	2116.04 (0.05)	0.52
11. 2,4′-DDD	2122.95 (0.17)	2123.07 (0.11)	−0.12
12. Endrin	2140.77 (0.17)	2140.55 (0.09)	0.22
13. 2,4′-DDT	2186.01 (0.23)	2186.57 (0.06)	−0.56
14. 4,4′-DDD (TDE)	2202.69 (0.30)	2202.72 (0.10)	−0.03
15. 4,4′-DDT	2270.20 (0.29)	2269.95 (0.08)	0.25

[a] Mean values of five replicate measurements; standard deviation in parentheses.

TABLE IV

TEMPERATURE-PROGRAMMED RETENTION INDICES (I_{px}) FOUND WITH A RATIO rt_0/β = 0.0040 (OPERATION CONDITIONS A-b AND B-b IN TABLE I)

Pesticide	Column A[a] (A-b)	Column B[a] (B-b)	Difference ($I_{A\text{-}b}$–$I_{B\text{-}b}$)
1. α-HCH	1659.47 (0.67)	1658.95 (0.05)	0.52
2. β-HCH	1681.68 (0.52)	1681.66 (0.73)	0.02
3. HCB	1692.68 (0.53)	1692.71 (0.11)	−0.03
4. Lindane	1713.18 (0.43)	1713.27 (0.07)	−0.09
5. Heptachlor	1867.26 (0.35)	1866.69 (0.17)	0.57
6. Aldrin	1932.91 (0.24)	1932.73 (0.16)	0.18
7. Heptachlorepoxid	2002.67 (0.36)	2002.27 (0.19)	0.40
8. 2,4′-DDE	2057.70 (0.13)	2058.02 (0.25)	−0.32
9. Dieldrin	2114.37 (0.17)	2114.33 (0.11)	0.04
10. 4,4′-DDE	2120.93 (0.14)	2120.24 (0.06)	0.69
11. 2,4′-DDD	2127.97 (0.12)	2128.09 (0.15)	−0.12
12. Endrin	2148.33 (0.31)	2148.35 (0.13)	−0.02
13. 2,4′-DDT	2191.38 (0.28)	2191.69 (0.16)	−0.31
14. 4,4′-DDD (TDE)	2208.38 (0.36)	2208.27 (0.08)	0.11
15. 4,4′-DDT	2276.91 (0.49)	2276.81 (0.28)	0.10

[a] Mean values of five replicate measurements; standard deviation in parentheses.

TABLE V

TEMPERATURE-PROGRAMMED RETENTION INDICES (I_{px}) FOUND WITH A RATIO rt_0/β = 0.0015 (OPERATION CONDITIONS A-c AND B-c IN TABLE I)

Pesticide	Column A[a] (A-c)	Column B[a] (B-c)	Difference ($I_{A\text{-}c}$–$I_{B\text{-}c}$)
1. α-HCH	1655.40 (0.24)	1654.61 (0.75)	0.79
2. β-HCH	1676.31 (0.12)	1676.70 (0.17)	−0.39
3. HCB	1687.93 (0.22)	1688.22 (0.07)	−0.29
4. Lindane	1709.35 (0.05)	1709.46 (0.07)	−0.11
5. Heptachlor	1861.10 (0.33)	1860.62 (0.05)	0.48
6. Aldrin	1925.83 (0.08)	1925.43 (0.12)	0.40
7. Heptachlorepoxid	1993.13 (0.18)	1992.64 (0.11)	0.49
8. 2,4′-DDE	2050.46 (0.13)	2051.22 (0.21)	−0.76
9. Dieldrin	2104.43 (0.12)	2104.24 (0.12)	0.19
10. 4,4′-DDE	2113.18 (0.11)	2112.76 (0.09)	0.42
11. 2,4′-DDD	2118.56 (0.10)	2118.78 (0.29)	−0.22
12. Endrin	2135.27 (0.11)	2135.17 (0.06)	0.10
13. 2,4′-DDT	2180.51 (0.09)	2181.05 (0.10)	−0.54
14. 4,4′-DDD (TDE)	2196.31 (0.52)	2195.92 (0.12)	0.39
15. 4,4′-DDT	2263.77 (0.07)	2263.43 (0.45)	0.34

[a] Mean values of five replicate measurements; standard deviation in parentheses.

TABLE VI

TEMPERATURE-PROGRAMMED RETENTION INDICES (I_{px}) FOUND WITH A RATIO rt_0/β = 0.0020 (OPERATION CONDITIONS A-d AND B-d IN TABLE I)

Pesticide	Column A[a] (A-d)	Column B[a] (B-d)	Difference ($I_{A\text{-}d}$–$I_{B\text{-}d}$)
1. α-HCH	1657.15 (0.28)	1656.85 (0.24)	0.30
2. β-HCH	1679.10 (0.22)	1679.50 (0.60)	−0.40
3. HCB	1690.81 (0.43)	1691.04 (0.09)	−0.23
4. Lindane	1711.33 (0.30)	1711.20 (0.05)	0.13
5. Heptachlor	1865.81 (0.34)	1865.45 (0.44)	0.36
6. Aldrin	1929.71 (0.34)	1929.41 (0.46)	0.30
7. Heptachlorepoxid	1999.40 (0.44)	1998.95 (0.10)	0.45
8. 2,4′-DDE	2054.26 (0.33)	2054.76 (0.10)	−0.50
9. Dieldrin	2111.28 (0.15)	2111.04 (0.07)	0.24
10. 4,4′-DDE	2116.40 (0.09)	2115.83 (0.08)	0.57
11. 2,4′-DDD	2124.24 (0.15)	2124.26 (0.05)	−0.02
12. Endrin	2142.85 (0.16)	2143.09 (0.06)	−0.24
13. 2,4′-DDT	2186.33 (0.09)	2186.87 (0.35)	−0.54
14. 4,4′-DDD (TDE)	2202.22 (0.25)	2201.93 (0.06)	0.29
15. 4,4′-DDT	2269.69 (0.17)	2269.23 (0.51)	0.46

[a] Mean values of five replicate measurements; standard deviation in parentheses.

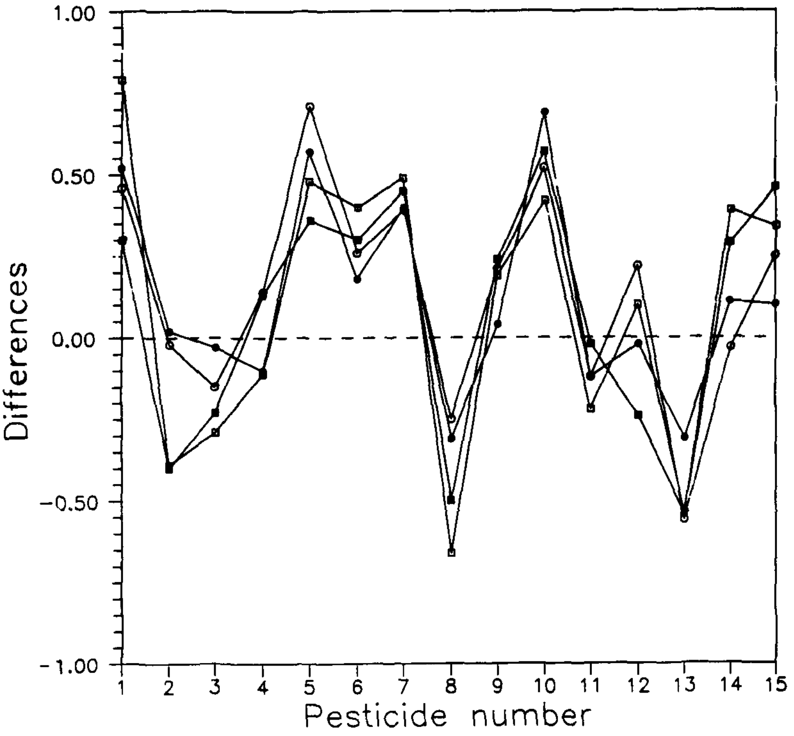


Fig. 3. Differences found between retention indices, evaluated under different operation conditions (see Table I) but with the same value of rt_0/β, plotted against the number attributed to each chlorinated pesticide in Table II. (○) $I_{A\text{-}a} - I_{B\text{-}a}$; (●) $I_{A\text{-}b} - I_{B\text{-}b}$; (□) $I_{A\text{-}c} - I_{B\text{-}c}$; (■) $I_{A\text{-}d} - I_{B\text{-}d}$.

the pesticides analyzed under the experimental conditions entailing the values of the rt_0/β parameter listed in Table I. Each of these tables reports retention indices, always calculated as the means of five replicate measurements, obtained on the two columns employed when they are characterized by the same value of rt_0/β. The quite negligible standard deviation affecting these indices shows that reproducibility is virtually the same for all pesticides analyzed.

Comparison between the indices obtained on the two chromatographic columns shows that a very small discrepancy is observed in any case which is comparable with the standard deviation peculiar to our measurements and that the I_{px} values obtained are reproduced well, within ± 1 index unit, as also shown by the plot reported in Fig. 3, giving a comprehensive view of this finding.

Such a result confirms the possibility of transferring these indices from one set of conditions to another, thus giving an indirect proof of the reliability of our approach for the evaluation of temperature-programmed retention indices.

CONCLUSIONS

The results obtained in this investigation indicate that the method of calculating retention indices under conditions of linear programming temperature based on the simultaneous and parallel use of an FID and a specific detector is an effective and profitable approach suitable for rather general applications. It allows *n*-alkanes to be adopted as the reference retention markers for any type of unknown analyte, irrespective of the atoms present in their molecules, provided that the specific detector to be coupled with an FID is chosen appropriately.

It allows retention indices of eluting components undetectable with an FID to be calculated from a single injection, without resorting to retention index standards containing specific elements. Moreover, it offers the quite important advantage over most currently employed procedures of avoiding any possible and undesired overlap of analyte peaks with peaks due to the reference markers, thus preventing the collection of unreliable responses for both identification and quantitative determination purposes.

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