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Review

Retention index systems: alternatives to the *n*-alkanes as calibration standards

Gianrico Castello*

Dipartimento di Chimica e Chimica Industriale, Università di Genova, Via Dodecaneso 31, 16146 Genova, Italy

Abstract

The retention index systems based on homologous series or selected compounds differing from the *n*-alkanes series proposed by Kováts are reviewed and discussed. The reference series proposed for the identification of various kinds of organic compounds, on polar and non-polar columns and with universal or specific detectors, are described. Reference systems proposed for the identification of polyaromatic compounds and of polychlorobiphenyls are also compared. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Retention index; Calibration standards; *n*-Alkanes

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1. Introduction

The Kováts retention index [1] is a useful tool for the comparison of retention data obtained by various authors in different conditions, as it is nearly in-

dependent on many of the parameters and conditions of the gas chromatographic analysis. The gross retention time of a substance, t_R , depends on the composition of the stationary phase, on the column diameter and length, on temperature and flow-rate of the carrier gas; the adjusted retention time, t'_R , obtained by subtracting from t_R the gas hold-up time, is better correlated with the solute–solvent inter-

*Fax: +39-010-3536199.

E-mail address: castello@chimica.unige.it (G. Castello)

action and with the partition coefficient, but still depends on the parameters used for the analysis. The use of retention times relative to a reference compound, r , permits to compare chromatograms carried out with different column length and carrier gas flow-rate. However, the choice of the compound to be used as the reference term (n -nonane was suggested in an early application of this method) [2] does not fulfil completely the requirements: when mixtures with a wide range of retention values are analysed, the r values are accurate and reproducible enough for compounds with retention times close to that of the reference standard, but lack their validity when very different retention values cause a great error in the calculation of their ratio. In addition, the relative retention times cannot be applied when programmed temperature analyses are compared.

The use of the retention volumes, V_R and V'_R , and of their ratio, does not solve the problems described above: the influence of the variation of carrier gas flow-rate is eliminated. But the other parameters still influence the results. The specific retention volume, V_g , i.e. the volume of carrier gas which elutes a given compound from an ideal column containing 1 g of liquid phase, is a value independent on the majority of analytical parameters, excluding temperature, and is therefore suitable for the identification of the compounds. However, its calculation is rather complex as the absolute amount of liquid phase in the column must be exactly known, and this is not possible when the packed columns are not prepared with special care in the laboratory itself. Again, only isothermal analyses can be used as the source of data.

2. n -Alkanes as reference series

The use of the homologous series of n -alkanes, proposed by Kováts in 1958 [1], is based on the well-known property of the components of a homologous series to be eluted in isothermal conditions with retention times increasing exponentially. As a consequence, the plot of the logarithm of the retention values follows a straight line as a function of the number of carbon atoms, z . Further accurate measurements have shown that this linearity is not rigorously followed, and a slight curvature is ob-

served [3–5], but when the range of z values is not too great, the straight-line correlation can be accepted. The fundamental equation for the retention index is:

$$I_s^{\text{st.ph.}}(T) = 100 \frac{\log X_s - \log X_z}{\log X_{(z+1)} - \log X_z} + 100z \quad (1)$$

where I is isothermal retention index (i.u.) at temperature T ; s is the compound of interest; st.ph. is stationary phase; z and $z+1$ are n -alkanes with z and $z+1$ carbon numbers, respectively; X is retention value used in the calculation.

The retention indices of the n -alkanes used as reference compounds are by definition taken as equal to $100z$ for any stationary phase and at any column temperature. With this procedure the relative retention of a substance is not referred to a single compound, but to a series of milestones formed by the terms of the reference homologous series, having retention times close enough to that of the compound of interest to minimise the errors in their measurement. Mathematic interpolation of the straight-line whose slope and intercept are obtained by applying least-square procedures to the retention values of the n -alkanes is also used for calculation of I , but the strict application of Eq. (1) reduces the effect of the observed curvature of the $\log X_z$ vs z plot, as this curvature can be considered as negligible within two or three contiguous terms of the homologous series. The retention index summarises the advantages of two quantities mentioned above, i.e. the relative retention and the specific retention volume. However, whereas the possibility of comparing results increases when the gross retention times are replaced by more sophisticated parameters as relative retention or retention indices, some of the information of the original chromatogram is lost. For example, as the value of the retention index depends on the retention of the substance and of the reference alkane series, it is difficult to judge if a greater I value on columns of different polarity corresponds to an increased retention of the substance of interest or to a decreased retention of the members of the reference series. This uncertainty also influences the use of retention indices and of their ratio or differences for the classification of stationary phases [6]

Another drawback of the Kováts retention index

(isothermal retention index) is related to the temperature of the column. The I values changes with changing temperature; the calculation of a temperature coefficient permits to apply the values obtained at one temperature to analyses carried out at another, but it must be taken into account that the temperature dependence of the index is hyperbolic, and the error in values obtained by linear extrapolation may be high [7]. Moreover, Eq. (1) cannot be used in temperature-programmed gas chromatography (TPGC) analysis. The latter problem was partially solved by the introduction of the Van den Dool and Kratz formula [8]:

$$I_{\text{TPGC},s}^{\text{st.ph.}} = 100n \frac{t_{\text{R}}(s) - t_{\text{R}}(z)}{t_{\text{R}}(z+n) - t_{\text{R}}(z)} + 100z \quad (2)$$

where n is the difference in carbon number of the two n -alkanes taken as references and the other terms have the same meaning as in Eq. (1). This formula is used when linear temperature programs are carried out. In theory, this formula is strictly valid when an 'ideal' temperature program is selected, i.e. when the retention times of the n -alkanes (and not their logarithms) behave linearly as a function of the carbon number z , i.e. when the difference between the retention time of two adjacent homologues is the same in the whole programmed analysis. However, it is commonly used for any PTGC analysis. The gross retention times are used in this formula: this may be an advantage because the measure or calculation of the dead time is not necessary; however, when capillary columns with carrier gas hold-up times, great with respect to the retention of the first eluted peaks, are used, or multi-linear temperature programming is set, this simplified approach may lead to large errors. Golovnya et al. [9] demonstrated that the non-linear behaviour of the retention of n -alkane homologues in TPGC depends on the considerable differences in temperature increments of sorption parameters. They suggested the use of a non-linear fourth parameter equation for extrapolation and interpolation calculations of retention indices, which shows some advantages in comparison with the Van den Dool and Kratz's method, mainly when some members of the n -alkane series are missing. Didauoi et al. [10] tested a multiparametric least-squares regression iterative

method using the adjusted retention times and a cubic interpolation using the uncorrected retention times for the calculation of retention indices at high temperature (200–260°C). The problem of the correct calculation and prediction of PTGC retention indices is still matter of study, because of its importance for the prediction of retention in different temperature programmed analysis.

The possibility of comparing retention data obtained by different authors under many analytical conditions is so attractive that, notwithstanding some not well elucidated problems, thousands of papers were published which use the retention index values as a means for uniform expression of retention data. A series of relationships between the retention indices and structural and physicochemical quantities as carbon number, boiling points, vapour pressure, molecular mass, Van der Waals' volume and connectivity index [11,12], enthalpy and entropy of the solute–solvent interaction of many compounds have been also derived. The retention indices are the base for other applications, e.g. for the prediction of retention on different columns [13], the polarity indicators of Rohrschneider [14], McReynolds [15,16] and Snyder [17,18]. Exhaustive reviews were published with 1392 [19], 490 [20], 363 [21] and 15 [22] references to papers on the applications of the retention index system, and books which contain tables of retention indices for the identification of organic compounds are available [15,23–26]. A discussion on the use of the Sadtler Retention Index Library [23] was published by Sun et al. [27]. A review on the retrieval of structural information on compounds by using the retention index and other molecular descriptors as the retention index increment for atom addition, AZ; and the group retention factor, GRF, measuring the effect of the addition of various functional groups to the molecule was published by Peng [28].

Other logarithmic index schemes similar to the Kováts' retention index have been suggested: molecular retention index [29,30], standard retention index [31], generalised retention index [32,33], invariant retention index [34], homologous index [35], unified retention index [36–40], recently applied by Skrbic for the calculation of retention index at any desired temperature [41,42], universal retention index [43–45], dispersion and selectivity index [46–52]. A

linear retention index was also suggested by Vigdergauz in 1968 [53,54]. All these methods were reviewed and discussed in the reviews listed above [19–21].

The majority of the methods listed above accept the hypothesis of Kováts, by using the *n*-alkanes as the reference compounds. However, other authors suggested alternative reference series. The main reasons of this choice are the irregular behaviour of alkanes due to adsorption phenomena on packed polar columns [5,34,55,56] or glass capillary [57–59], and their undetectability by many element-specific detectors. The use of alternative series was proposed early after the introduction of the retention index and Kováts himself [7], according to the studies of Evans on methyl esters [60], stated that each homologous series can serve as a secondary reference series, provided the respective indices of its members are known in the primary system, the indices of its consecutive members increase by exactly 100 and are not temperature dependent. This rule is not exactly followed in all the methods suggested by various authors, but notwithstanding this, many papers were published on alternative reference series. The validity of the so-called Polar Retention Index Systems was often discussed throughout the years (see for example the Letters to the Editor written in 1971/72 by Grobler and Ackman [61,62]), but nowadays the alternative systems are generally accepted, mainly if their authors verified the possibility of converting the data into the primary Kováts system. This paper is a review of the various alternatives to the use of *n*-alkanes as the reference series for the determination of retention indices. The following paragraphs describe the alternative series proposed in order to avoid the problems encountered with alkanes on polar columns and those suitable for detectors non-sensitive to alkanes. Separate sections are devoted to the series proposed for the identification of polychlorobiphenyls and polyaromatic compounds, as the identification of these toxic compounds received much attention.

3. Alternative series for use with polar columns

Table 1 shows in chronological order the intro-

duction of alternative reference series. Methyl esters of saturated and unsaturated fatty acids were used by lipid chemists as reference series for the determination of the equivalent chain length (ECL) [63,64], carbon number (CN) [65] and modified equivalent chain length (MECL) [66–68]. In these methods the methyl esters of fatty acids perform the same function of hydrocarbons in the Kováts system. Steranes were also suggested by Van den Heuvel and Horning [69]: in the steroid number method, two hydrocarbons with steroidal structure, androstane and cholestane, are used as the fixed points. The steroid number of a given compound can be calculated by the sum of values related to the carbon content of the steroid skeleton and to the functional groups of the molecule. An extension of the CN method to the homologous series of saturated triglycerides was proposed by Podlaha and co-workers [70–72] who called it equivalent carbon number (ECN).

Bemgård and Blomberg [73] compared the performances of three homologous series, i.e. *n*-alkanes, 2-ketones and fatty acid methyl esters, on 60% cyanopropyl-methyl siloxane polar columns and found that *n*-alkanes were adsorbed at the gas–liquid interface causing retention times variations as a function of the area/volume ratio of the stationary phase and therefore a change of the Kováts retention index values, whereas the retention of the polar homologues was rather insensitive to this effect and the alternative retention indices are more constant. Garzó and Alexander [74] suggested the use of dimethylcyclopolysiloxane homologues as they found that a linear relationship exists between the number of Si atoms of the linear and cyclic dimethylsiloxane molecules and their retention indices on non-polar (Apiezon L) medium polar (QF-1) and polar (TWEEN-60) liquid phases. The dimethylsiloxane number (DN) values are correlated with the structure of the solute and are nearly temperature independent. Homologous 2-ketones [75–77], *n*-propyl esters [78], alkanolate esters [79], alkanols and acetates [32] were used in order to minimise the unfavourable behaviour of *n*-alkanes on polar stationary phases.

The generalised retention index, suggested by Novak and Ruzickova [32] also employs standards different from alkanes, which were found not to be suitable reference compounds for high polarity

Table 1

Series proposed for the determination of retention indices and other normalised retention values, year of the introduction, specific detector used

Year	Reference series	Authors	Ref. number	Specific detector ^a	Special names and notes
1958	<i>n</i> -Alkanes	Kováts	[1]		Retention index
1960	Saturated acids methyl esters	Miwa et al.	[63]		Equivalent chain length
1960	Saturated acids methyl esters	Woodford and Van Gent	[65]		Carbon number
1962	Steranes	Van den Heuvel and Horning	[69]		
1963	Monounsaturated acids methyl esters	Ackman	[66]		Modif. equivalent chain length
1963	Saturated acids methyl esters	Evans	[60]		
1966	Methyl <i>n</i> -alkyl ketones	Dymond and Kilburn	[75]		
1969	1-Iodo- <i>n</i> -alkanes	Castello and D'Amato	[82,83]	ECD	
1971	Dimethylcyclopolysiloxanes	Garzó and Alexander	[74]		
1972	<i>n</i> -Propyl esters	Hawkes	[78]		
1973	1-Chloro-, 1-bromo-, 1-iodo- <i>n</i> -alkanes	Castello and D'Amato	[84–87]	ECD	Equivalent methylenes
1974	Alkanoate esters	Ashes and Haken	[79]		
1974	Alcohols, ketones, acetates	Novák and Ruzicková	[32]		Generalized retention index
1977	<i>n</i> -Alkan-1-ols	Castello and D'Amato	[84,85]		
1978	<i>n</i> -Alkyl trichloroacetates	Neu et al.	[99]		
1978	Alkyl-benzenes	Mathiasson et al.	[56]		
1979	<i>n</i> -Alkyl dimethylphosphinothioates <i>n</i> -alkylbis(trifluoromethyl)phosphinothioates	Enqvist et al.	[108,109]	ECD, FPD AFID, FID	P series
1979	Naphthalene, phenanthrene, chrysene, picene	Lee and co-workers	[123,124]		Poly aromatic compounds
1980	<i>n</i> -Alk-1-ene, 1-chloro- <i>n</i> -alkanes, <i>n</i> -aldehydes, <i>n</i> -alkan-1-ol, <i>n</i> -alkan-2-ol, 1-amino- <i>n</i> -alkanes, <i>n</i> -alkylbenzenes, <i>n</i> -alkylcyclohexanes	Heldt and Köser	[80]		
1981	<i>n</i> -Dialkyl-sulphides	Zotov et al.	[120]	FPD	
1981	Sulphur heterocycles	Willey et al.	[121]	FPD	
1982	1-Bromo- <i>n</i> -alkanes	Pacholec and Poole	[95,96]	ECD	
1982	Saturated triglycerides	Podlaha and co-workers	[70–72]		Equivalent carbon number
1983	<i>n</i> -Alkyl trichloroacetates	Schwartz et al.	[100]	ECD	
1984	Alkyl iodides	Fernandez et al.	[98]	ECD	Analysis of elemental iodine
1984	Alcohols, acetate	Berezkin and Returnsky	[34]		
1985	2-Alkanones	Raymer et al.	[76]		
1985	1-Bromo- <i>n</i> -alkanes	Yasuhara et al.	[97]	ECD	
1986	tri- <i>n</i> -alkylamines	Hall et al.	[112]	AFID	
1987	Alkylbis(trifluoromethyl)phosphine sulphides	Manninen et al.	[114]	ECD, FID, AFID, PID, FPD	M series
1988	1-Iodo- <i>n</i> -alkanes	Castello et al.	[90]	ECD	
1988	1-Nitroalkanes	Aderjan and Bogusz	[115]	AFID	
1993	1,4-Benzodiazepines	Rasanen et al.	[116]	AFID	Drugs identification
1993	1-Alkenes, 3-alkenes, <i>n</i> -alkylbenzenes	Orav et al.	[57]		
1990	Alkylbis(trifluoromethyl)phosphine sulphides	Kostiainen and Nokelainen	[122]	GC-MS	
1995	Alkylmethylhydantoin, alkyhydantoin, alkylmaleimides, alkylbis(trifluoromethyl)phosphine sulphides	Rasanen et al.	[116–119]	AFID	Drugs identification
1996	PCB congeners 8,31, 44, 101, 138, 180, 194	Chu et al.	[151]	ECD	PCB identification
1996	PCB congeners 1, 11, 29, 47, 121, 136, 185, 194, 206, 209	Castello and Testini	[140,153]	ECD	PCB identification
1996	PCB congeners 1, 9, 27, 69, 121, 151, 178, 200, 207, 209	Castello and Testini	[140,153]	ECD	PCB identification
1997	<i>n</i> -Alcohols, aromatic hydrocarbons	Berezkin et al.	[81]		
1998	Alkyl 2,2-dichloro-1,1-difluoro ethyl esters	Rodin et al.	[102]	ECD	PCB identification

^aThe acronym AFID (alkali flame ionisation detector) is used as a general term for the various names of the detectors specific for nitrogen and phosphor (NPD, TSD, TID, thermionic detector). Other terms: FID, flame ionisation; ECD, electron capture; FPD, flame photometry; PID, photoionisation. When no symbol is reported, non-specific detectors (FID or thermal conductivity detectors) were used.

stationary phases. Nine homologous series (*n*-alk-1-ene, 1-chloro-*n*-alkanes, *n*-aldehydes, *n*-alkan-1-ol, *n*-alkan-2-ol, 1-amino-*n*-alkanes, *n*-alkylbenzenes, *n*-alkylcyclohexanes) were analysed by Heldt and Köser [80] on squalane, di(2-ethylhexyl)sebacate, Carbowax 20M, 1,2,3-tris(2-cyanoethoxy) propane. It has been found that compounds of medium polarity, such as the aldehydes, give more precise values for phases of both low and high polarity than those obtained with alkanes. A polarity scale should therefore be based on these more reproducible indices. The comparison of values obtained by injecting different homologous series can also be used to establish a polarity scale of many stationary phases. If aldehydes are not available or their use is precluded for any reason (e.g. because they react with the sample or with the column), retention data can be quoted by reference to homologous series similar to the solute of interest. The same conclusion that it is preferable to use standards similar to the compounds examined was also drawn by Luo et al., who introduced in 1985 the homologous index [35] suggesting to use the homologous series of the substances to be characterised as the reference series. This concept was previously applied to halogenated compounds by Castello et al. [82] (see below), mainly because of the impossibility of using *n*-alkanes as the reference series with the electron capture detector.

Kersten et al. [77] found that many ambiguities in the determination of the polarity with the McReynolds stationary phase constants are due to the use of alkanes as reference series, and evaluated on 15 columns spanning a wide polarity range the 2-alkanones as the universal retention index markers to replace the alkanes which do not partition with polar phases. Ketones were suggested by many authors as a good alternative series to alkanes; however, Mathiasson et al. [56] found that, because of the variation in retention volume with amount injected, alkanols and 2-alkanones are unsuitable on polar and, respectively, non-polar columns, and suggested the use of alkylbenzenes as reference compounds, as these compounds seem to behave almost ideally on liquid phases of different polarity, such as 3,3'-oxydipropionitrile, alkyl esters, phthalate esters, haloalkanes, squalane and octadecane. Alkylbenzenes and unsaturated hydrocarbons (1-alk-

kenes and 3-alkenes) were also proposed as reference series showing better reproducibility and less temperature dependence on polyglycol columns [81].

4. Alternative reference series for specific detectors

With the introduction of specific detectors capable of detecting traces of organic compounds containing halogens, nitrogen, phosphorus, sulphur, but insensitive to hydrocarbons, the possibility of using the Kováts method for identification purposes was strongly reduced because the reference series could not be injected with the same analytical conditions used for the unknown sample. The possible solutions of this problem, e.g. the injection of very large amounts of alkanes or the replacement of the specific detector with an universal flame ionisation or thermal conductivity detector, are time-consuming or could cause modifications of the analytical system leading to intolerable changes in the retention values.

4.1. Electron capture detector

In order to obtain the retention indices of alkyl-iodides and other compounds detected in very small amounts by the electron capture detector (ECD), which is not sensitive to alkanes, Castello and co-workers [82,83] suggested the use of the homologous series of 1-iodo-*n*-alkanes as a reference. A flame ionisation detector (FID) was previously connected in parallel with the ECD in order to detect the reference Kováts' series, but this arrangement was inadequate for quantitative analyses because of the possible variation of the splitting ratio between the two detector with changing temperature and flow-rate in the column. The retention index relative to normal iodoalkanes, I_{ni} , was therefore used and its values could be easily converted to standard Kováts indices as the lines of $\log t'_R$ of iodoalkanes and *n*-alkanes are parallel as a function of the number of carbon atoms in the molecule, independent on the polarity of the column (squalane, Apiezon L, tricresylphosphate, polyglycol Carbowax 20M columns were used) as shown by analysis of larger amounts of 1-iodo-*n*-alkanes and *n*-alkanes with FID. The same behaviour was observed with 1-chloro- and

1-bromo-*n*-alkanes [84–87] and *n*-alkan-1-ols [88,89] and the indices I_{nc} , I_{nb} , I_{OH} , were used for studying the behaviour of secondary, tertiary or branched chain halo-alkanes and alcohols. In general, when the $\log t'_R$ of the terms of the homologous series X used as alternative reference behave linearly as a function of the number of carbon atoms, z , an index I_X can be calculated and two equations can be written:

$$y_X = q_X + (m_X I_X / 100) \quad (3)$$

for the reference series, and

$$y_P = q_P + (m_P I / 100) \quad (4)$$

for the *n*-alkanes. The Kováts retention index I can therefore be calculated:

$$I = \frac{(q_X - q_P)100}{m_P} + \frac{m_X - I_X}{m_P} = A_0 - A_1 I_X \quad (5)$$

and the constants A_0 and A_1 permit a quick conversion of the I_X into I values for the column and reference series considered. As the straight lines for the X reference series and for the *n*-alkanes are often parallel, it is also possible a graphical determination of the I values, with horizontal lines drawn from the position of the substance on the X series line to cross the *n*-alkane line [90]. As a consequence, all the used alternative indices, I_{ni} , I_{nc} , I_{nb} , I_{OH} , could be converted into I values. The researches carried out to elucidate the gas chromatographic behaviour of branched-chain compounds with respect of the linear ones and to correlate their retention with the structure and some physical parameters permitted to predict the retention, the boiling point, the molecular volume and hence the density of compounds not available as standards; they have also shown the utility of the 'equivalent methylene' concept, i.e. the number of methylene groups that must be added to a linear paraffinic chain to have the same effect on its properties (boiling point, retention, molecular volume) of the addition of a polar group or an etheroatom [84–88]. A further extension of this concept, applied by using as reference the *n*-alkane and the *n*-alkanols homologous series, lead to the introduction of a method for the classification of the polarity of gas chromatographic columns, based on the difference in apparent carbon number of linear alkanes and *n*-alkanols with the same retention time,

ΔC . This polarity indicator evaluates the relative contribute to the retention of the dispersion forces and of the hydrogen bonds and dipole moment [91–94].

1-Bromoalkanes were also used by Pacholec and Poole, both as identification and quantitation standards, using two calibration methods which arise from the observation that the ECD response is proportional to the concentration of bromine atoms and is nearly independent on the length of the alkyl chain almost for the highest members of the series [95,96]. Yasuhara et al. [97] used the same reference series for the determination of retention indices (I') of 221 halogenated compounds on a methyl silicone capillary column for analysis with ECD and identification of water pollutants. Fernandez et al. determined elemental iodine in toluene and cyclohexane by using as reference the methyl-, ethyl-, 1-propyl and 2-propyl-iodides [98].

The use of *n*-alkyl-trichloroacetates, CCl_3-CO_2R , as standards detectable by ECD was suggested by Neu et al. [99] and further implemented by Schwartz et al. [100] which synthesised these compounds up to octadecyl trichloroacetate and used them as reference for the identification of polychlorobiphenyls. Alkyl trichloroacetates of from eight to 20 carbon atoms were used for the same purpose by Wegman and Hofstee [101]. Rodin et al. [102] proposed to replace the trichloroacetates with the 10 first normal linear homologues of alkyl-2,2-dichloro-1,1-difluoro ethyl esters, $RO-CF_2-CHCl_2$, whose first member show a smaller value of the Kováts retention index with respect of the first member of the trichloroacetate series (708 ± 8 instead of 902 ± 15 on non-polar siloxanes). The indices with respect to the proposed series of substances detected by ECD can be converted into Kováts indices or precalculated with a lin–log relationship [103]:

$$\log RI(\text{class I}) = a \log RI(\text{class II}) + bA + c \quad (6)$$

where A is equal to the number of carbon atoms in the alkane radical, and corresponds to the position of the compound within the homologous series. A similar equation is also proposed to convert the retention indices measured on a non-polar column into indices on a polar one, if the compounds being characterised have non-specific interactions with this

polar phase (i.e. absence of active hydrogen atoms). Dichlorobenzyl alkyl ethers were proposed by Wells et al. [104].

4.2. Thermionic detector

The nitrogen- and phosphorus-sensitive thermionic detector or alkali flame ionisation detector (AFID, indicated by different authors and producers as TID, NPD or TSD), when set with the proper alkali bead temperature and hydrogen and air flow-rates to enhance the sensitivity to phosphorus or nitrogen, does not detect the hydrocarbons (average selectivity ratios 10^5 for P/C and 5×10^4 for N/C). A temporary change of its properties can be made for measuring the retention of the *n*-alkanes reference series by switching off the electric heating current to the alkali salt bead [105], or increasing the hydrogen flow-rate [106,107]. The splitting of the column effluent to two or three detectors (FID–ECD–TSD) was also used for identification purposes and sensitivity studies, and this arrangement also allows to measure the retention indices with respect to alkanes [107,108] or to the phosphorus-labelled homologous series *n*-alkyl dimethylphosphinothioates and *n*-alkyl bis(trifluoromethyl)phosphinothioates [109]. In studies on the research of drugs, the identification was carried out by deducting the retention indices of unknown substances by comparison with those of known nitrogen-containing drugs, used as internal standards [110,111]. In this instance no specific reference series of homologous compounds was used. The *n*-trialkylamines are detected both with FID and thermionic detectors [112,113] and an I_N retention index based on the series from tri-*n*-propyl- to tri-*n*-decylamine was used for the identification of many pesticides on 100% methyl-, 5% phenyl- and 50% phenyl-methyl-polysiloxane capillary columns.

Manninen et al. [114] compared the properties of the so-called P series, i.e. the alkyl dimethylphosphinothioates $\text{CH}_3\text{-P(S)(CH}_3\text{)-O-(CH}_2\text{)}_n\text{-CH}_3$, detectable by AFID, FID and flame photometric detector (FPD), with the series alkylbis(trifluoromethyl)phosphinothioates $\text{CF}_3\text{-P(S)(CF}_3\text{)-O-(CH}_2\text{)}_n\text{-CH}_3$, detected by the ECD also but rather unstable on polar columns, and with the ‘universal retention index standard series’ (M series): alkylbis(trifluoromethyl)phosphine sulphides $\text{CF}_3\text{-P(S)(CF}_3\text{)-}$

$\text{(CH}_2\text{)}_n\text{-CH}_3$. The various groups of the molecules of this series permit the use of different detection systems: ECD is sensitive to the six fluorine atoms, alkaline thermionic detector and flame photometric detector (FPD) to the P atom, FPD and photoionization detector (PID) respond to sulphur, and the hydrocarbon chain is detected by FID. Aderjan and Bogusz [115] used synthesised 1-nitroalkanes of up to 26 carbon atoms as a reference series, which can be detected by FID, ECD and TID. Rasanen et al. used a homologous 1,4-benzodiazepine index standard for benzodiazepine drugs screening with NPD [116] and four homologous series, the alkylmethylhydantoin, alkyhydantoin, alkylmaleimides and alkylbis(trifluoromethyl)phosphine sulphides (M series) for identification of acidic and neutral drugs in blood samples on capillary 5% phenylmethylsiloxane and 14% cyanopropylphenylmethylsiloxane columns [117,118]. The specific indices obtained, I^* , can be converted to Kováts indices I by calibration graphs as the correlation between I^* and I was linear on both columns. It was found that alkane derivatives could not compete with index standards formed by compounds similar to the drugs of interest in terms of high precision of identification in drug screening [119].

4.3. Other detection methods

The specific detection of sulphur-containing compounds can be carried out by microcoulometric detector or by flame photometric detector (FPD). The latter detector is sensitive to sulphur atoms and does not respond to hydrocarbons (selectivity ratio $\text{S/C} = 10^6$) independent on the organic molecule where the sulphur is contained, and as a consequence the quantitative calibration does not require standard samples of all the detected compounds (often reactive and difficult to obtain with adequate purity) but can be carried out with any stable sulphur-containing compound easily available. However, some problems arise because the response of the detector is not linear as a function of the sulphur amount. With dual flame or pulsed-type FPD, the dependence is parabolic with a good approximation, and the square root of the peak area or height can be plotted as a function of the absolute sulphur amount and applied to external standard quantitative methods. Any sul-

phur-containing molecule, e.g. the alkyl dimethylphosphinothioates (P series) and alkylbis (trifluoromethyl)phosphine sulphides (M series) [114], can therefore be used for qualitative identification. The FPD is also sensitive to phosphor-containing molecules, with a linear correlation between the signal and the amount of P in the sample; therefore, the same reference series used for the AFID can be employed.

The symmetrical *n*-dialkyl-sulphides with four to 12 carbon atoms in the chain were suggested by Zotov et al. for the identification both in isothermal and programmed temperature analysis of sulphur-containing analytes. [120]. An alternative method was suggested by Willey et al. for the determination of sulphur heterocycles in coal liquids and shale oils [121]. They used as the reference 32 aromatic compounds and sulphur heterocycles, some of them synthesised, whose Kováts retention indices were carefully measured on the used column (5% phenylmethyl polysiloxane SE-52 capillary) and ranged between 200 for naphthalene to 500 for picene and triphenyleno[2,3-*b*]thiophene. Although the retention values of the selected standards did not follow a regular behaviour, their knowledge permitted to interpolate the retention of the analytes for identification purposes.

The alkylbis(trifluoromethyl)phosphine sulphides were also found to be useful for identification by mass spectrometry with electron impact ionisation (GC–EI–MS). All compounds in the M series produce two characteristic and common ions at m/z 147 ($C_2H_3SPF_3$) and at m/z 229 ($C_4H_4SPF_3$) which can be selected for monitoring with characteristic ions of the analyte [122].

5. Reference series for the identification of polycyclic aromatic hydrocarbons

The analysis of polycyclic aromatic hydrocarbons (PAH) has received great attention due to their carcinogenic, mutagenic and teratogenic effects. The number of possible isomers and congeners makes it difficult the resolution of all peaks, and very efficient capillary columns only allow a satisfactory analysis. The identification between several possible isomers may be assisted by structure–retention correlation

and by comparison of the retention of the unknown and that of selected reference compounds on two columns of different polarity. Therefore, simple and reproducible retention parameters that can be used by many laboratories are needed. Lee et al. evaluated the *n*-alkanes as reference standards for temperature-programmed analysis on SE-52 capillary columns and found that, due to difference in the film thickness of various columns, surface effects and ageing of the stationary phase after several weeks, a maximum variation of almost 20 index units was possible for some compounds (e.g. benzo[*e*]pyrene). They suggested a parameter named PAH index [123] and further PAC *I* (poly aromatic compounds index) [124], using as standards the compounds naphthalene, phenanthrene, chrysene and picene, with fixed retention indices of 200, 300, 400 and 500, respectively, correlated to the number of aromatic rings in the molecule. A plot of the elution temperature of the four standards as a function of retention index approaches linearity. The proposed equation was similar to that of Van den Dool and Kratz:

$$I = 100 \frac{T_{R(\text{substance})} - T_{R(C_z)}}{T_{R(C_{z+1})} - T_{R(C_z)}} + 100z \quad (7)$$

where $T_{R(C_z)}$ and $T_{R(C_{z+1})}$ are the retention times for the PAH internal standards which bracket the substance of interest and z is the number of rings in the PAH standard that elutes prior to this substance. A series of PAH with linear arrangement of aromatic rings (naphthalene, anthracene, naphthacene and pentacene) was also tested, but the poor solubility of the heaviest terms of this series in the solvents commonly used encouraged the use of the other reference compounds. The PAH retention indices of over 200 compounds was measured and it was found that the largest difference due to the film thickness was 0.14 index units and that the PAH system maintained excellent reproducibility after several months of heavy column usage. The PAH or PAC index was used for the identification of many compounds, including 115 sulphur polycyclic aromatic and 117 nitrogen polycyclic aromatic compounds [124] in synthetic fuels [123,125,126], coal extracts [127], combustion effluents [128], fish tissues and sediments [129,130], air particulate matters [131] organic adsorbates on Diesel particulate mat-

ters [132,133]. The use of slightly different liquid phases (100% methyl-polysiloxane OV-1 [126] and 5% phenyl–1% vinyl–94% methyl-polysiloxane SE-54 [133] did not change appreciably the elution order and the index values.

Both the Kováts and the PAC indices were used for the identification of polyaromatic compounds by many authors, with FID and GC–MS detection [134]. In an article with 93 references, Elizalde-González et al. [135] listed a great number of columns used for the analysis of these substances in many kinds of samples and pointed out that the availability of Kováts indices on the commonly used methylsilicone columns under temperature programming is reduced, whereas under isothermal conditions a larger set of compounds is considered. Therefore they measured on a methylsilicone capillary column the Kováts indices of 71 PAH. The PAC indices calculated with Eq. (7) from the retention times reported in 1996 by Elizalde-González et al. show a linear correlation with the Kováts indices, I (see Eq. (8)) and fairly correspond with those listed in 1976 by Lee et al. for the 5%-phenyl-methylsilicone column [123], which again has a straight-line correlation with the Kováts indices of Ref. [135] (Eq. (9)).

$$\text{PAC } I \text{ (Ref. [135])} = 0.1453I + 40.842$$

$$R^2 = 0.9949 \quad (8)$$

$$\text{PAC } I \text{ (Ref. [123])} = 0.1476I + 36.484$$

$$R^2 = 0.9954 \quad (9)$$

It is therefore possible to convert one kind of indices into the other and to correlate data reported by different authors when the retention times are measured on modern bonded-phase columns where the surface effects are reduced. As an example, the following linear correlation between the data of Lee and that of Elizalde-González permits one to calculate the retention values of compounds not analysed on one of the column by using the data measured on the other, enhancing the possibility of identification of many polyaromatic compounds.

$$\text{PAC } I \text{ (Ref. [135])} = 0.9844 \text{ PAC } I \text{ (Ref. [123])}$$

$$+ 4.9361 \quad R^2 = 0.9994 \quad (10)$$

6. Reference series for the identification of polychlorobiphenyls

Polychlorobiphenyls (PCB) were widely used before their toxicity was fully understood and therefore their residues and metabolites are ubiquitous in the environment. Their separation was carried out on many capillary columns, some of them especially suited for this separation [136–139], but the identification of all of the 209 congeners is still matter of discussion. It has been found that small changes in the polarity of the column cause the inversion of the elution of many peaks [140]. Temperature effects were also observed, and therefore the possibility of correlating each congener with a retention index value less dependent on analytical condition is of paramount importance for further structure–retention studies. The retention indices referred to n -alkanes were measured on packed and capillary columns in isothermal and programmed temperature analyses [111,141–146]. In order to cover the range of retention times of all PCB on polar and non-polar columns, n -alkanes with 14–29 carbon atoms are necessary, and their use is only possible when the FID is used. As the determination of small amounts of PCB in environmental samples requires the use of the ECD, alternative reference series are necessary. The n -alkyl iodides can be used, but homologous with nine to 26 carbon atoms are necessary to cover the retention time span of PCB and, the n -octadecyl-iodide being the heaviest iodoalkane commercially available, the retention indices of PCB containing only up to four chlorine atoms can be measured with this method.

Ballschmitter and co-workers [99,147,148] and Schwartz et al. [100] proposed the use of n -alkyl-trichloroacetates; the retention indices of 79 PCB isomers were measured with respect to this series by Driss et al. [149] on a CP-Sil 5 column. The alkylbis(trifluoromethyl)phosphine sulphides were also used [114]. Rodin et al. [101] proposed the use as reference series of the 10 first normal linear homologues of alkyl-2,2-dichloro-1,1-difluoroethyl esters, RO-CF₂-CHCl₂, the indices measured with respect to them can be converted into Kováts indices with use of a lin–log relationship [102]. However, it must be pointed out that the number of congeners is so great (up to 839 compounds if the hydroxylated

possible metabolic derivatives are considered) that the different chemical properties of the reference series, and of the analytes, introduces into the determination of retention indices errors greater than the differences between contiguous congeners. The ideal reference and calibration mixture should therefore be formed by some selected PCB whose retention mechanism is the same of that of the unknown analytes. All the 209 PCB congeners were synthesised and separated on capillary columns, but the use as a standard of a mixture of so many compounds is unpractical. Kozloski [146] obtained various PCB by partial catalytic dechlorination of other PCB and used them as reference for the identification of unknown commercial mixtures such as the insulating oil, Aroclor. The dechlorination of a given PCB yielded a series of products with decreasing number of chlorine atoms, covering all the expected PCB congeners when the starting compound has four or less chlorine atoms. Reaction mixtures with a reduced number of known compounds were obtained and could be used as identification standard. However, this method did not propose a reference series with a linear behaviour, which could be used for determination of alternative retention indices. Octachloronaphthalene was found to have, with non-polar or low-polar phases, an interaction similar to that of PCB molecules, and the retention times relative to this compound were used for identification purposes [137]. The retention times relative to the PCB congeners with IUPAC numbers 52 and 180 (respectively, 2,2',5,5'-tetrachloro- and 2,2',3,4,4',5,5'-heptachloro-biphenyl) were also measured for all the 209 congeners on a CP-Sil 5 column [150].

Chu et al. [151] proposed a reference series formed by seven congeners with two to eight chlorine atoms: the PCB congeners with the following IUPAC numbers (the position of the substituent chlorine atoms is reported in brackets): 8 (2,4'), 31 (2,4',5), 44 (2,2',3,5'), 101 (2,2',4,5,5'), 138 (2,2',3,4,4',5'), 180 (2,2',3,4,4',5') and 194 (2,2',3,3',4,4',5,5'). They calculated the coefficients of a first-order equation by plotting the retention times of the reference PCB as a function of the number of chlorine atoms. With these coefficients, the retention index of any PCB congener analysed under the same conditions can be calculated by using

its retention time. Castello and Testini [140,152,153] used various series detectable by ECD as reference, and suggested as internal reference series two groups of 10 PCB congeners with one to 10 chlorine atoms: the first series tested was the so-called DCMA series (Dry Colours Manufacturer's Association) which is readily available and being used as the reference in the analysis of inks and paints containing PCB. The IUPAC numbers and the chlorination pattern of the PCB members of the DCMA series are the following: 1 (subst. in 2); 11 (3,3'); 29 (2,4,5); 47 (2,2',4,4'); 121 (2,3',4,5',6); 136 (2,2',3,3',6,6'), 185 (2,2',3,4,5,5',6); 194 (2,2',3,3',4,4',5,5'); 206 (2,2',3,3',4,4',5,5',6); 209 (2,2',3,3',4,4',5,5',6,6'). However, the retention times of the components of the DCMA series do not show a linear behaviour as a function of the number of chlorine atoms in programmed temperature analyses; another series of congeners which behave linearly was therefore suggested, by using the PCB with IUPAC numbers 1 (subst. in 2); 9 (2,5); 27 (2,3',6); 69 (2,3',4,6); 121 (2,3',4,5',6); 151 (2,2',3,5,5',6); 178 (2,2',3,3',5,5',6); 200 (2,2',3,3',4,5,6,6'); 207 (2,2',3,3',4,4',5,6,6'); 209 (2,2',3,3',4,4',5,5',6,6'). These compounds are commercially available as pure samples, have low toxicity and show linear behaviour on many capillary columns as a function of the number of chlorine atoms independent on the retention values used: retention times and volumes, retention relative to octachloronaphthalene, index with respect to *n*-alkanes. The Van den Dool–Kratz formula can be used for the calculation of the I_{PCB} retention index in programmed temperature analysis when the retention vs chlorine number plot is linear. When the plots show appreciable curvature due to high initial temperature of the programmed run, the determination of I_{PCB} values can be done with exponential formulas.

7. Conclusions

After the introduction of the retention index system based on *n*-alkanes, thousands of papers were published which used this parameter as an identification method and correlated it to many structural, physical and chemical properties of organic substances. The problems of reproducibility, due to the

interactions of alkanes with polar and non-polar stationary phases, and of detectability by specific detectors, induced many authors to suggest other homologous series or groups of selected compounds as reference terms, many of them briefly reviewed in this paper. An important characteristic of some of these alternative index values is their convertibility into the *n*-alkanes system, which permits general catalogues to be implemented. Other systems which are not readily converted offer advantages in specific application fields, such as the analysis of fatty acids, steroids, polyaromatic compounds; however, as it can be predicted that other reference series suitable for use with new stationary phases and new detectors will be proposed in the future, researchers should make an effort to investigate and clearly explain the procedures of conversion between new and old retention parameters.

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