



Short communication

Retention index calculation without *n*-alkanes—the virtual carbon number

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Received 30 December 2002; received in revised form 4 February 2003; accepted 4 February 2003

Abstract

For the fast gas chromatographic identification of separated components the retention index is still one of the most often used tools, although mass spectrometry is available in almost all analytical laboratories. For the calculation of the retention indices it is not necessary to use *n*-alkanes or any other homologous series. If the analyte contains some compounds, not necessarily belonging to a homologous series, with well-known retention indices those compounds can be used as index references and the index of the other compounds can be calculated as is done using *n*-alkanes. The only difference is that instead of the carbon number of the *n*-alkanes, virtual carbon numbers of the reference compounds should be used. The method of calculation, and the effect of this method of calculation on the reproducibility are discussed in this paper, and analyses of some halogenated compounds and marjoram oil are used as experimental examples.

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Keywords: Retention indices; Virtual carbon number; Oils; Halogenated compounds

1. Introduction

The retention index in gas chromatography has a long history since its introduction in 1958 by Kovats [1]. There are many theories related to the indices, and their prediction for different stationary phases, for different temperatures, and for programmed temperature from isothermal analysis and vice versa. The number of these publications is uncountable; there are good review articles available to get an overview of the results [2–6]. But from the point of view of daily practice of use with the index, the

identification is the most important. According to the original definition, the use of *n*-alkanes is essential as the peaks of *n*-alkanes mean the scale of the retention index calculation either with injecting them together with the sample or performing the *n*-alkane analysis separately. In the optimal case the *n*-alkanes are injected together with the sample, but if the sample has a lot of components, and the paraffin peaks can overlap with some of the analyte peaks, it is advisable to perform the analysis of the *n*-alkane standard separately. This means, of course, additional experimental error in the calculation of the retention index, and higher uncertainty in the identification. In many cases several components are known, and their identification is not a problem, for example in essential oil analysis the main components, and the other peaks (minor components) can

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be identified with the help of the major components. Sometimes it is simply impossible to detect n -alkanes as the detector is not sensitive to hydrocarbons (halogenated compounds detection by ECD in environmental analysis). In these cases only the detectable components can be used as reference compounds. The alternatives to the n -alkanes as calibration standards were reviewed by Castello [5]. In that review many alternative reference systems are mentioned as secondary reference systems and using them the ‘relative indices’ could be converted to standard retention indices. Also the retention index reference systems for detectors not detecting n -alkanes (for example ECD) are summarised there. The use of alternative series was suggested by Kovats [7] saying that any homologous series can be used as a secondary reference. Later here it will be shown that any component can be used for index reference having known retention index. To be able to use the calculation methods, algorithms or programs a virtual carbon number can be used for any kind of reference compounds and the calculation method used with n -alkanes can be easily adopted for these cases.

There is another method for identification without index calculation using the retention behaviour only, namely retention time locking (RTL) for programmed temperature analysis [8]. This method adjusts the inlet pressure to provide the same retention temperature for the same component. The RTL method applies constant pressure, and tolerates only small deviations in the column size and loading parameters, and cannot be used for constant flow-rate analysis.

2. Theory

The above-mentioned first paper [1] defines the isothermal retention index as follows:

$${}_T I^{\text{st.ph.}} = (Z + \{\log [V'(x)] - \log [V'(P_Z)]\} / b) \cdot 100 \quad (1)$$

where

$$b = \log [V'(P_{Z+1})] - \log [V'(P_Z)] \quad (2)$$

$V'(x)$ is the adjusted retention volume of the com-

ponent of interest, $V'(P_Z)$ and $V'(P_{Z+1})$ are the adjusted retention volumes of the paraffin compounds with Z and $Z + 1$ carbon numbers, and having lower and higher adjusted retention volumes compared to the compound of interest. If the flow-rate is constant, the adjusted retention time can be used instead of adjusted retention volumes. The adjusted retention volume can be calculated by the subtraction of the dead volume from the retention volume, and the same for the retention time.

The programmed temperature retention index can be calculated in a similar way [9]

$$I^{\text{st.ph.}} = \{Z + [V(x) - V(P_Z)] / [V(P_{Z+1}) - V(P_Z)]\} \cdot 100 \quad (3)$$

where $V(x)$ is the retention volume of the component of interest (x) and $V(P_Z)$ and $V(P_{Z+1})$ are the retention volumes of paraffin compounds with Z and $Z + 1$ carbon numbers, and having lower and higher retention volumes compared to the compound of interest assuming that the flow-rate is constant. If the flow-rate and temperature program rate are constant, the retention times can be used instead of the retention volumes.

For use of the definition Eq. (1) for the index calculation with any kind of compound, the virtual carbon number could be defined:

$$ZV(A) = I(A) / 100 \quad (4)$$

where $I(A)$ is the retention index (isothermal or programmed) of component A.

For the further calculation the Eq. (1) can be modified as follows:

$${}_T I^{\text{st.ph.}} = (ZV(A) + \{\log [V'(x)] - \log [V'(P_{ZV(A)})]\} / b) \cdot 100 \quad (5)$$

where

$$b = \log [V'(P_{ZV(B)})] - \log [V'(P_{ZV(A)})] \quad (6)$$

and $V'(P_{ZV(B)})$ and $V'(P_{ZV(A)})$ are the adjusted retention volumes of the A and B components having known retention indices (and having a virtual carbon number) at the given stationary phase and temperature. At the same time A and B have lower and higher retention volume than that of the compound

of interest (x). Again, if the flow-rate is constant, the adjusted retention times can be used instead of the adjusted retention volumes. The value of b from Eq. (6) should be equal to the value obtained from (2). This means, if the value of b is known, only one reference compound (virtual carbon number) is necessary for the calculation of the retention indices of the other components.

For programmed temperature calculation:

$$I^{\text{st.ph}} = \{[V(x) - V(P_{ZV(A)})]/[V(P_{ZV(B)}) - V(P_{ZV(A)})] + ZV(A)\} \cdot 100 \quad (7)$$

where $V(x)$, $V(P_{ZV(A)})$ and $V(P_{ZV(B)})$ are the retention volumes of x, A and B assuming, that the flow and temperature program rates are constant.

The method of the retention index calculation detailed here has several advantages and disadvantages. Using the virtual carbon numbers for reference compounds makes it possible to use the retention index for identification also for the detectors not detecting paraffin compounds (ECD, NPD), and the retention index databases can be used. Any component can be used, they should not belong to a homologous series. The retention index can be transferred from a system equipped with a flame ionisation detection (FID) system to any other system. Unfortunately, the isotherm retention index of a certain compound is temperature dependent and the programmed temperature index depends on the temperature program rate. But at the same time, the retention index tolerates the flow changes and the column size deviations.

3. Experimental

There were two instruments used for the measurements. The analyses using FID and n -alkanes for the index determination were performed out on a 6850 gas chromatograph of Agilent Technologies. The gas chromatograph was equipped with split/splitless inlet and FID. A 6850 series autosampler was used for sample injection.

The analyses using ECD were carried out on an Agilent 6890N gas chromatograph. That instrument

was equipped with split/splitless inlet and ECD. A 7683 autosampler was used for injection.

Common operation parameters

Inlet temperature	200 °C
Detector temperature	300 °C
FID operation with constant column flow + make up	
ECD operation with constant make up flow (N ₂)	
Carrier gas (He)	35 cm/min

Constant carrier gas flow was applied in both isotherm and programmed temperature analyses.

For the analysis of the volatile organic compound mixture the column temperature for isotherm analysis was 100 or 120 °C, for programmed temperature analysis the column temperature was kept at 60 °C for 2.8 min, and then heated at 3 or 4 °C/min up to 180 °C.

In the case of the analysis of marjoram oil the isothermal measurements were carried out at 120 and 140 °C.

The sample for comparison of FID and ECD retention data was the standard mixture for the EPA 8021 method.

The marjoram oil sample for inter-laboratory comparison was obtained from the Research Institute for Medical Plants, Budakalász, Hungary.

The analyses were carried out on a J&W DB-5 column (part no. 123-5063) 60 m × 0.32 mm × 1 μm or J&W DB-1 column (part no. 123-1035) 30 m × 0.32 mm × 5 μm.

Chromatograms of the volatile organic compound standard mixture obtained under isothermal conditions at 120 and 100 °C, and using FID are shown in Fig. 1. The component identification with ECD was based on retention times of the components. The chromatograms obtained at 120 °C with FID and ECD are shown in Fig. 2.

The peak identification was checked on an Agilent 6890N gas chromatograph-5973 mass spectrometry system using the same column with the same nominal linear carrier gas velocity.

The retention index calculations were performed with the *PCI* software [10], which is able to calculate the retention indices using either n -alkanes or any

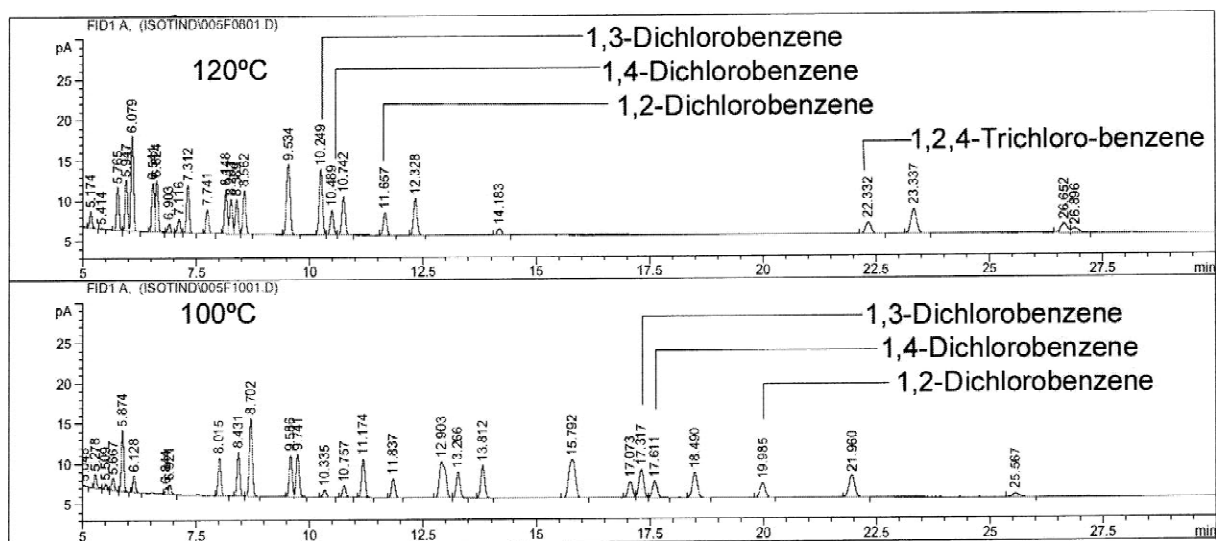


Fig. 1. Isotherm analysis (120 and 100 °C) of the volatile organic compound standard mixture on DB-5 capillary column using FID.

other reference compounds. For the calculations the CHEMSTATION report was used as input data. For isotherm indexes the hold-up time calculation the algorithm of Domingues [11] was used.

4. Results

To demonstrate the use of the virtual carbon number of nonhomologous reference compounds

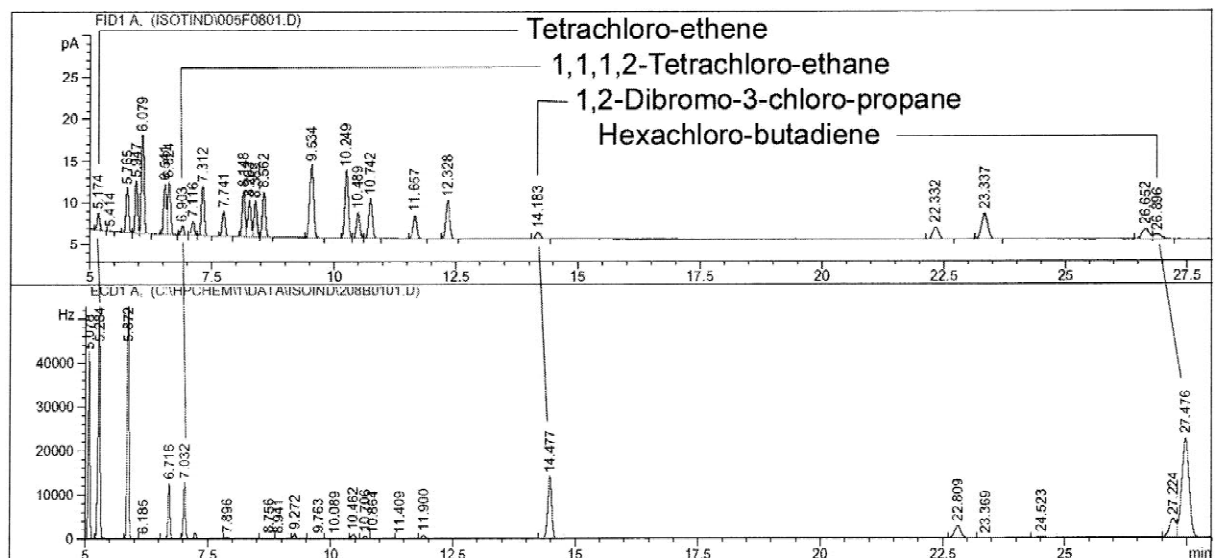


Fig. 2. Isotherm analysis (120 °C) of volatile organic compound standard mixture on DB-5 capillary column detected by FID and ECD.

here are the analysis data of the same mixture on gas chromatographs equipped with FID and ECD. The component identification was made by GC–MS analysis. The sample to be analysed is a standard mixture of volatile organic compounds for environmental analysis, consisting of halogenated and aromatic hydrocarbons (see Experimental).

4.1. Isotherm retention indices

The isotherm retention data and indices of some selected (and in another later experiment targeted) compounds determined on a gas chromatographs equipped with FID and ECD systems, both equipped with DB-5 capillary column are shown in Table 1. The retention time values used as references are in italics. The columns (b)–(f) and (d)–(h) include the corresponding retention index data. The retention indices listed in columns (b) and (d) were calculated using *n*-alkanes as references, while the retention indices in column (f) and (h) were calculated using selected non *n*-alkane reference compounds.

The results obtained with ECD and non *n*-alkane reference compounds are very similar to those obtained with FID using *n*-alkanes for retention index calculation. There are small differences in the index values, which are in the range of the experimental error. At the same time it can be seen that there is a systematic deviation: the index values obtained with the instrument equipped with ECD are higher. This can be caused by a systematic small difference in the oven temperature values of the two instruments. In the case of 1,3-dichlorobenzene at 100 °C the difference is higher (2.7) than in any other case (0.1–0.2). This is caused by a coeluting (aromatic hydrocarbon derivative) peak, which is not detected by the ECD. The results show that the method using non *n*-alkane components as reference is reliable.

4.2. Programmed temperature retention indices

For the same compounds programmed retention indices were determined with FID, and using the same reference compounds as were used in the isothermal analyses, the retention indices were determined using ECD. The programmed temperature

retention data and indices of the chlorobenzenes determined with FID and ECD are shown in Table 2.

Similar to the isotherm analyses, the columns (b)–(f) and (d)–(h) include the corresponding retention index data. The retention indices listed in columns (b) and (d) were calculated using *n*-alkanes as references, while the retention indices in column (f) and (h) were calculated using selected non *n*-alkane reference compounds.

The retention indices obtained with *n*-alkane references are in a good agreement with those obtained with non *n*-alkane references, although the differences are higher (up to 1 index unit) than in the case of isothermal analyses. The reason for this could be that the retention index scale of the reference compound is not as dense as the paraffin scale. The index is calculated with the linear function of the retention time (7), while the retention times of *n*-alkanes are not a linear function of the carbon number. The more reference compounds the higher confidence in the identification based on retention indices.

4.3. Inter-laboratory application

To demonstrate the use of the virtual carbon number of nonhomologous reference components the reanalysis of marjoram oil was performed, and the components were identified using the virtual carbon numbers of some of them. The component identification of marjoram oil was published by Tarján [12], programmed temperature retention indices as well as isothermal retention indices of the components on several temperatures were determined. Table 3 presents the isothermal retention data of the components of marjoram oil measured at 120 °C, and a comparison of the published retention indices and the calculated retention indices using three components of marjoram oil for the index calculation shows very similar values. The index differences are less than 1 I.U., which gives the possibility of the component identification. Almost the same good results were obtained at 140 °C (Table 4). Only in a case of one component (α -terpineol) was the index difference higher than 1 I.U. In both tables are the results of the calculation of retention indices using just one reference component using the hold-up time and *b* values for the calculation according to the Eq. (5). The

Table 1
Isotherm retention times and indices measured using FID and ECD

	Retention time at 100 °C (min) FID (a)	Retention index at 100 °C FID (b)	Retention time at 120 °C (min) FID (c)	Retention index at 120 °C FID (d)	Retention time at 100 °C (min) ECD (e)	Retention index at 100 °C ECD (f)	Retention time at 120 °C (min) ECD (g)	Retention index at 120 °C ECD (h)	Retention index difference at 100 °C FID–ECD (b)–(f)	Retention index difference at 120 °C FID–ECD (d)–(h)
<i>n</i> -Octane	<i>6.288</i>		<i>4.764</i>							
Tetrachloroethene	6.919	825.59	5.174	832.3	<i>7.086</i>					
<i>n</i> -Nonane	<i>9.473</i>		<i>6.344</i>							
1,1,1,2-Tetrachloroethane	10.333	918.70	6.904	924.94	<i>10.574</i>		<i>7.032</i>			
<i>n</i> -Decane	<i>15.552</i>		<i>9.164</i>							
1,3-Dichlorobenzene	17.312	1020.06	<i>10.249</i>	1027.05	<i>17.488</i>	1017.40	10.468	1027.35	2.66	–0.30
1,4-Dichlorobenzene	17.608	1023.18	<i>10.489</i>	1032.51	18.040	1023.15	10.711	1032.76	0.03	–0.25
1,2-Dichlorobenzene	19.980	1046.17	11.658	1056.93	20.472	1046.22	11.906	1057.07	–0.05	–0.14
<i>n</i> -Undecane	<i>27.119</i>		<i>14.175</i>							
1,2-Dibromo-3-chloropropane	25.562	1089.72	14.183	1100.14	<i>26.184</i>		<i>14.477</i>			
1,2,4-Trichlorobenzene			22.333	1193.56			22.821	1193.67		–0.11
<i>n</i> -Dodecane			<i>23.071</i>							
Hexachlorobutadiene			26.900	1229.98			<i>27.477</i>			

The retention time values shown in italics were used for the retention index calculations as references.

Table 2
 Programmed temperature retention times and indices measured with FID and ECD

	Retention time at 3 °C/min (min) FID (a)	Retention index at 3 °C/min FID (b)	Retention time at 4 °C/min (min) FID (c)	Retention index at 4 °C/min FID (d)	Retention time at 3 °C/min (min) ECD (e)	Retention index at 3 °C/min ECD (f)	Retention time at 4 °C/min (min) ECD (g)	Retention index at 4 °C/min ECD (h)	Retention index difference at 3 °C/min FID–ECD (b)–(f)	Retention index difference at 4 °C/min FID–ECD (d)–(h)
<i>n</i> -Octane	<i>11.895</i>		<i>11.190</i>							
Tetrachloroethene	12.662	815.45	11.879	817.10	<i>12.925</i>		<i>12.080</i>			
<i>n</i> -Nonane	<i>16.817</i>		<i>15.218</i>							
1,1,1,2-Tetrachloroethane	17.553	913.87	15.857	915.34	<i>17.814</i>		<i>16.056</i>			
<i>n</i> -Decane	<i>22.109</i>		<i>19.379</i>							
1,3-Dichlorobenzene	22.896	1014.97	20.097	1017.77	23.155	1015.34	20.299	1018.92	–0.38	–1.15
1,4-Dichlorobenzene	23.226	1021.24	20.348	1024.00	23.482	1021.56	20.549	1025.03	–0.32	–1.03
1,2-Dichlorobenzene	24.512	1045.72	21.359	1049.02	24.769	1046.00	21.560	1049.74	–0.28	–0.72
1,2-Dibromo-3-chloropropane	26.965	1092.41	23.242	1095.64	27.211		23.441			
<i>n</i> -Undecane	<i>27.364</i>		<i>23.418</i>							
1,2,4-Trichlorobenzene	32.041	1192.67	27.133	1196.78	32.273	1193.06	27.332	1196.72	–0.39	0.06
<i>n</i> -Dodecane	<i>32.411</i>		<i>27.257</i>							
Hexachlorobutadiene	33.902	1231.20	28.565	1233.94	<i>34.190</i>		<i>28.764</i>			
<i>n</i> -Tridecane	<i>37.192</i>									

The retention time values shown in italics were used for the retention index calculations as references.

Table 3
Isotherm retention times and indices of some component of marjoram oil measured at 120 °C on DB-1 stationary phase

	Retention index at 120 °C [12] (a)	Measured retention time (min) (b)	Calculated retention index (c)	Retention index difference (d)	Calculated retention index (e)	Retention index difference (f)
α -Pinene	949.00	14.114	948.81	−0.19	948.55	−0.45
Sabinene	980.42	<i>16.637</i>	Reference		980.38	−0.04
Limonene	1033.38	21.973	1033.04	−0.34	1033.21	−0.17
<i>trans</i> -Sabinenehydrate	1064.72	<i>26.035</i>	Reference		1064.92	0.20
Linalool	1087.00	29.264	1086.41	−0.59	1086.6	−0.40
<i>cis</i> -Sabinenehydrate	1094.00	30.477	1093.92	−0.08	1094.1	0.10
Terpineol-4	1170.68	<i>46.317</i>	Reference		Reference	
α -Terpineol	1179.82	48.597	1179.43	−0.39	1179.4	−0.42

The retention time values in italics were used for the retention index calculations as references. The retention indices shown in the column (e) were calculated with using $t_M = 1.367$ and $b = 0.2464$ values determined separately and using one reference compound.

results are very similar to those obtained using three reference components.

5. Discussion and conclusions

The retention index calculation can be performed based on non *n*-alkane reference compounds with known retention indices. These reference compounds should not belong to a homologous series. Virtual carbon number can be assigned for the reference compounds (the theoretical carbon number of a paraffin having the same retention time under the given chromatographic conditions), that reference carbon number can be used for the retention index calculation.

For isothermal analyses the retention index calculation using reference compounds, and their virtual carbon numbers, results in very similar data compared to the calculation using *n*-alkanes. The differences are in the range of 0.1 index unit (see Table 1).

In the case of programmed temperature analysis the differences of the calculated indices are higher (0.4–1.0 index unit). The reason can be that the index is calculated by linear approximation between the reference compounds and the retention time of *n*-alkanes is not a linear function of the carbon number. The other quite possible reason might be, that using FID detection the peaks of the target and reference compounds might overlap with peaks of other components of the used standard mixture creating a shift of the retention time, while in the

Table 4
Isotherm retention times and indices of some component of marjoram oil measured at 140 °C on DB-1 stationary phase

Component	Retention index at 140 °C [12] (a)	Measured retention time (min) (b)	Calculated retention index (c)	Retention index difference (d)	Calculated retention index (e)	Retention index difference (f)
α -Pinene	956.00	9.062	956.48	0.48	955.92	−0.08
Sabinene	986.93	<i>10.370</i>	Reference		986.91	−0.03
Limonene	1040.32	13.127	1039.19	−1.13	1039.68	−0.64
<i>trans</i> -Sabinenehydrate	1072.09	<i>15.264</i>	Reference		1072.66	0.57
Linalool	1089.00	16.491	1088.82	−0.18	1089.38	0.38
<i>cis</i> -Sabinenehydrate	1101.89	17.520	1101.86	−0.03	1102.39	0.50
Terpineol-4	1180.15	<i>25.305</i>	Reference		Reference	
α -Terpineol	1185.85	26.296	1188.26	2.41	1188.17	2.32

The retention time values in italics were used for the retention index calculations as references. The retention indices shown in the column (e) were calculated with using $t_M = 1.349$ and $b = 0.2195$ values determined separately and using one reference compound.

case of ECD detection only the halogenated components are detected. But even with that experimental error the identification of the target components using the reference compounds for the index calculation can be performed easily.

The only disadvantage of the use of virtual carbon number calculation method is, that for all used analysis temperature (isothermal analysis), or for all temperature program rates (programmed temperature analysis) the indices of a certain component are different. Consequently the virtual carbon numbers are different, and should be determined beforehand.

The results in Tables 1 and 2 demonstrate very well how the virtual carbon number method can be used for retention index calculation and component identification with a detector, which does not detect *n*-alkanes. The indices calculated using reference compounds are in a good agreement with those calculated using *n*-alkanes and measured with FID. With this method the existing retention index databases could be used for component identification also for ECD or NPD (for example in environmental analysis or food analysis).

The results in the Tables 3 and 4 show the application of the indices measured in a different

laboratory on a different instrument using the same stationary phase.

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