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Improved method for computing temperature programmed retention indices from isothermal data[1]

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Abstract

Temperature-programmed retention indices can be rigorously calculated under a single gas chromatography run without any co-injected *n*-alkanes, using an extension of a concept originally described by Curvers et al. [J. High Resolut. Chromatogr. Chromatogr. Commun., 8 (1985) 607–610 and 611–617] and by Akhporhonor et al. [J. Chromatogr. 405 (1987) 67–76]. The indices are computed from both thermodynamical data, namely Kováts indices, and experimental data (the column gas hold-up time t_0 and the phase ratio β). The two experimental terms can be easily derived, without the need for their exact measurement, if two peaks of the chromatogram are identified. The method was tested to predict the programmed retention indices of a series of monoterpenes. The precision of this procedure is consistent with previously published results.

Keywords: Retention indices; Thermodynamic parameters; Monoterpenes; Terpenes

1. Introduction

The search for reliable retention parameters is a long standing objective in gas chromatography (GC) research, and many efforts have been made to find universally accepted retention data for qualitative studies of solutes or stationary phases. It is now well accepted that relative retention parameters, especially Kováts retention indices I, offer the best compromise between confidence, universality and ease of experimental determination. The isothermal retention index I_i is characteristic of the solute retention at a given temperature as long as its retention is governed by a pure partition mechanism over the stationary phase, without competitive adsorption on active column sites. Major improvements in column manufacturing over recent years has led to the production of stable apolar capillary columns that guarantee good reproducibility of experimentally determined I_i, leading to the compilation of retention data for a large number of compounds belonging to various classes [1]. As I_i show an almost linear relationship with the column temperature T for temperature intervals common in GC [2], a particular I_i can be predicted provided that two experimental data are reported for the same stationary phase: either two isothermal retention indices, or the value of the slope dI_i/dT together with one retention index, at a known temperature.

Nevertheless, the isothermal retention index system has not gained widespread acceptance because

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many GC analyses are run under temperature programmed conditions (TPGC), due to its many advantages including shorter analysis time and reduced peak-spreading. The programmed temperature retention index of a solute I_p can be defined in a similar fashion as under isothermal GC, using its retention temperature, T_r (the column temperature at the moment corresponding to solute elution), instead of the logarithm of the adjusted retention time [3]; however, unlike I_i, I_p values are dependent on both the column geometry (through its phase ratio β and the gas hold-up time t_0), and the programme parameters (the initial programme temperature T_0 and the programme rate r). Consequently, I_p parameters are considered to be less compound specific and more difficult to predict.

A number of authors have addressed this problem and reported theories and experimental verifications for I_p prediction, using both thermodynamic data and experimental values [4–12]. As a result, accurate prediction of I_p can be derived from the experimental determination of isothermal I_i under some predefined conditions. The method of Curvers et al. [7,8], can be used to calculate both I_i and I_p from either compiled or experimentally determined thermodynamic parameters, using three possible procedures for obtaining the necessary primary data. It showed excellent accuracy when predicting solute retention temperatures under a given set of temperature programme parameters. This approach has been adopted by several other workers [10–13], and is the basis for a commercially available software package for GC users.

In this paper we have extended the model of Curvers et al. [7,8] to include a situation in which a mixture of a large number of solutes with known retention indices (both I_i and dI_i/dT) from compiled data contains at least two substances that can be well separated by GC. If, in addition, the solution thermodynamic parameters of a minimum of two n-alkanes are determined separately, but once together, using any column coated with the same phase, then it will be shown that all solute I_p values can be computed at the end of the temperature programmed GC run with the analytical column, without the need for any co-injected n-alkane. One of the protocol advantages is that it completely avoids the experimental determination of both the column gas hold-up time, t_0, and the phase volume ratio β. The model has been applied to the prediction of temperature-programmed retention indices of a series of various monoterpenes, as their precise identification is frequently required in many analyses of interest for the cosmetic and food industries.

2. Theory

Temperature programmed retention indices are defined by:

$$I_p = 100z + 100\frac{T_{r,s} - T_{r,z}}{T_{r,z+1} - T_{r,z}} \tag{1}$$

where $T_{r,s}$ is the solute retention temperature, $T_{r,z}$ and $T_{r,z+1}$ are the retention temperatures for the n-alkanes with z and $z+1$ carbon atoms respectively. The retention temperature can be replaced by the corresponding retention time t_r since for linear temperature programming, the relation between time, t, and temperature T is

$$T = T_0 + rt \tag{2}$$

T_0 is the initial column temperature, and r is the programme rate.

A relation between thermodynamic parameters of the solute-phase system has been derived [7–11] and it can be written as:

$$\int_{T_0}^{T_{r,s}} \frac{dT}{t_0(T)(1 + k_S(T))} = \int_{T_0}^{T_{r,s}} \frac{dT}{t_0(T)\left(1 + \frac{a}{\beta}\exp\left(\frac{-\Delta H}{RT}\right)\right)} = r \tag{3}$$

where $a = \Delta S/R$, R is the gas constant, ΔH and ΔS are the solution molar enthalpy and entropy, respectively. Eq. (3) cannot be solved analytically, thus T_r can be obtained by numerical integration. Several simplifications and procedures have been reported [7,10,12,13] to obtain the required data for the integration, *i.e.*, t_0, ΔH, ΔS and β, as the calculation is not possible if one item is missing. The solution thermodynamic parameters are assumed to be temperature-independent over the range of interest for

GC; the gas flow regime of the mobile phase is assumed to obey the Poiseuille's law, thus under constant inlet and outlet pressures across the GC column, the dead time t_0 is a function of the mobile phase viscosity, according to [14]:

$$\eta(T) = \eta_0\left(\frac{T}{T_0}\right)^p \quad (4)$$

where $p=0.68$ for hydrogen and η_0 = the viscosity at T_0. Therefore, $t_0(T)$ can be expressed as:

$$t_0(T) = CT^p \quad (5)$$

where C is a constant for a given column with a fixed head pressure of hydrogen.

The other parameters are derived from the classical GC equation:

$$\ln k = \ln\frac{a}{\beta} + \frac{-\Delta H}{RT} \quad (6)$$

based on either isothermal GC experiments at two different temperatures, compiled data, or a combination of both approaches. The solute I_p values are finally obtained from Eq. (1) if the retention temperatures of two n-alkanes are known, either from an experimental determination, e.g., by co-injection with the investigated solutes, or by reapplying Eq. (3) to the prediction of $T_{r,z}$ and $T_{r,z+1}$.

This concept has shown excellent results for the prediction of solute I_p values under conditions of single step-linear temperature programming [7,10,12] and multi-step programmes [13], and has validated the initial simplifications.

The experimental determination of solute I_p values in a complex mixture containing a large number of solutes can be simplified if a minimum of two well-separated GC solutes, A and B, are identified (e.g., by either co-injecting the standards, or from their mass spectra in a GC–MS experiment), with known I_i and dI_i/dT values from the literature.

In a preliminary experiment, we could inject two n-alkanes on any column, referred to as u, with an unknown phase ratio β_u, but coated with the same stationary phase as the analytical column, referred to a, with its ratio β_a also a priori unknown (one column could be used, but the situation with two different columns with the same phase is examined as a general example). In a first isothermal experiment at temperature T_1, the dead time t_0 and the retention time $t_{r,z}$ of the the n-alkanes are obtained. The two isothermal indices of A and B at temperature T_1, $I_{i,A}$ (T_1) and $I_{i,B}$ (T_1), can be calculated since both $dI_{i,A}/dT$ and $dI_{i,B}/dT$ are known. As n-alkanes belong to a series of homologues, Thus the retention factors k_z^u (T_1) for the two n-alkanes, at temperature T_1 on column u, can be computed directly from the chromatogram. k_z^u (T_1) can be obtained for any value of z. The retention factors of the two solutes $k_A^u(T_1)$ and $k_B^u(T_1)$ are derived from the basic definition of the Kováts isothermal $I_{i,A}$ and $I_{i,B}$ and solving for the unknown $k_A^u(T_1)$ and $k_B^u(T_1)$. The experiment can be repeated isothermally at a second temperature T_2, yielding the results k_z^u (T_2) for any n-alkane, k_A^u (T_2) and k_B^u (T_2). Then, as a consequence of Eq. (6), the two separated isothermal injections on column u result in the determination of the three functions: $k_z^u(T)$ for any n-alkane, $k_A^u(T)$ and $k_B^u(T)$ at any temperature.

The phase volume ratio, β_a, for the analytical column used for TPGC analysis of the solutes is also unknown, however, it can be written that the retention factors of any given solute on the two columns, k_s^u and k_s^a, are linked by the relationship:

$$k_s^a = k_s^u\frac{\beta_u}{\beta_a} \quad (7)$$

From the TPGC analysis of the sample, and provided that the two solutes A and B are correctly identified, their retention temperatures $T_{r,A}$ and $T_{r,B}$ can be read on the chromatogram. These values are introduced into Eq. (3), yielding two non-linear equations of the variable T, whose unknown data are now the two constants C (Eq. (5)) and β_u/β_a (Eq. (7)): this system can be resolved by numerical methods. The first result, β_u/β_a, is combined with the retention factors for the n-alkanes obtained on column u, according to Eq. (7), to compute the retention factors of the n-alkanes on the analytical column. The second result, the constant C, is used to simulate the retention temperatures for the series of n-alkanes. Finally, by applying Eq. (1), the temperature programmed retention temperatures for all solutes can be predicted, based on their experimental retention temperatures.

3. Experimental section

A HP 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA) and either SGE (Scientific Glass Engineering, Villeneuve Saint Georges, France) or HP (Hewlett-Packard, Les Ulis, France) capillary columns were used for this study. The mobile phase was hydrogen and the detector was a HP 5971A mass selective detector (MSD) (Hewlett-Packard, Palo Alto, CA, USA), directly interfaced to the chromatograph. The MSD parameters were set to the values determined by the automatic tuning procedure, and the mass range was 35–225 daltons in 250 ms. This fast-scanning mode allowed sufficient precision in retention time measurements, and the solute retention temperature was identified by the scan number corresponding to the peak maximum on the reconstructed total ion chromatogram. The analytical column for the TPGC analysis of monoterpene mixtures was an apolar SGE column, 25 m×0.10 mm coated with 0.1 μm BP1 silicone gum (a pure methylsilicone phase). Isothermal retention indices were determined using an HP column, 50 m×0.20 mm coated with 0.5 μm of HP1 which is also a 100% methylsilicone phase. The retention factors of *n*-alkanes were measured on both the SGE and on the HP column. The column head pressure was held constant and set to ensure an average carrier-gas velocity of ca. 50 cm/s, as required for optimum column efficiency with vacuum outlet conditions. Syringe injection in the split mode used a split ratio of 1:30 and injector temperature of 250°C. The test sample was a synthetic mixture of various monoterpenes; *n*-alkanes and monoterpenes were purchased from Interchim, Montluçon, France. Programmes for computing the temperature programmed retention indices were written in C language (Microsoft, Redmond, WA, USA), and were run on a PC-compatible machine [15].

4. Results

Three different temperature programmes were then tested for the analysis of a mixture of terpenes and two predictions were performed on each chromatogram. For the first prediction, the retention factor of the alkanes was measured on the SGE column that was used for the analytical programmed temperature run. For the second prediction, the retention factor was obtained on the HP1 column.

Results are presented in Table 1 and Table 2 and Table 3. For easier interpretation, retention temperatures are converted into retention times using equation Eq. (2), and the difference between the predicted and the measured retention time is expressed in units of Van den Dool and Kratz indices, that is, by dividing the difference between the predicted and measured times by the time lag between the two, after running *n*-alkanes on a chromatogram under the same temperature programme and multiplying by 100.

The agreement is quite good in both predictions: the highest differences are within four units of retention index, in the range of the data published for medium polarity molecules: a difference of 4 units for the *o*-xylene or the octanol at 2°C/min; 6 units at 8°C/min [13]. The prediction is somewhat better when the column used for the analysis is identical to the one used to evaluate the alkane capacity factors. This tends to show that HP1 and BP1 phases are slightly different, though both referred to cross-linked methylsilicone. Prediction using alkane capacity factors measured on BP1 are expected to be the more accurate. As the greatest difference between measured and predicted values appears when the heating rate is low (1°C/min), the hypothesis that there is a long time delay between the oven and the inside of the column being heated [16] can be dismissed. Imperfections might come from the model itself, from experimental errors or from the difference between the two stationary phases.

5. Discussion

The expected value for β_u/β_a is 1 when the column used for the *n*-alkane was the same as the one used for the analysis of terpenes (column BP1). Concerning the HP1, as it is twice as wide as the BP1 column and coated with a film five times thicker, the expected value for C should be close to

Table 1
Predicted and measured retention indices of fifteen terpenes.

Solute	Measured t_r (min)	Column used for the analysis of *n*-alkanes			
		BP1		HP1	
		Predicted t_r (min)	Difference (index units)	Predicted t_r (min)	Difference (index units)
α-Pinene (*A*)	3.975	3.981	0.41	3.981	0.43
D-Camphene	4.167	4.181	1.01	4.169	0.15
Sabinene	4.476	4.494	1.21	4.469	−0.53
β-Pinene	4.563	4.556	−0.49	4.531	−2.32
Δ^3-Carene	5.087	5.094	0.39	5.056	−1.82
p-Cymene	5.200	5.206	0.34	5.169	−1.88
1,8-Cineol	5.365	5.381	0.94	5.331	−2.00
γ-Terpinene	5.813	5.831	1.06	5.769	−2.62
Camphor	7.129	7.169	2.20	7.094	−1.97
Lavandulyl acetate	9.753	9.769	0.92	9.731	−1.20
Longicyclene	11.524	11.556	1.91	11.519	−0.31
Longifolene	12.081	12.094	0.84	12.081	0.05
Calarene	12.490	12.519	1.80	12.506	0.98
Aromadendren	12.601	12.606	0.31	12.606	0.31
α-Humulene (*B*)	12.804	12.806	0.12	12.806	0.12

Initial temperature T_0=60°C; heating rate r=8°C/min.

Table 2
Predicted and measured retention indices of fifteen terpenes

Solute	Measured t_r (min)	Column used for the analysis of *n*-alkanes			
		BP1		HP1	
		Predicted t_r (min)	Difference (index units)	predicted t_r (min)	Difference (index units)
α-Pinene (*A*)	3.183	3.184	0.05	3.184	0.05
D-Camphene	3.377	3.385	0.60	3.360	−1.22
Sabinene	3.667	3.662	−0.35	3.637	−2.22
β-Pinene	3.768	3.763	−0.35	3.738	−2.22
Δ^3-Carene	4.325	4.316	−0.39	4.266	−2.73
p-Cymene	4.449	4.442	−0.29	4.392	−2.62
1,8-Cineol	4.649	4.669	0.91	4.593	−2.55
γ-Terpinene	5.208	5.197	−0.50	5.122	−4.00
Camphor	7.106	7.110	0.14	6.984	−3.99
Lavandulyl acetate	12.181	12.144	−1.00	12.043	−3.67
Longicyclene	15.815	15.794	−0.52	15.718	−2.34
Longifolene	17.119	17.102	−0.41	17.052	−1.63
Calarene	18.222	18.235	0.32	18.210	−0.28
Aromadendren	18.485	18.461	−0.57	18.461	−0.56
α-Humulene (*B*)	18.991	18.990	−0.02	18.990	−0.02

Initial temperature T_0=80°C; heating rate r=2.8°C/min.

$2^2/(5{\cdot}2)$ i.e. 0.4. However, we got the values reported in Table 4.

In fact, C and β_u/β_a are linked by the non-linear equation Eq. (3). It is not yet guaranteed that their calculated value is exact. We then decided to test the resolution of Eq. (3) for the unknowns C and β_u/β_a.

Table 3
Predicted and measured retention indices of fifteen terpenes

Solute	Measured t_r (min)	Column used for the analysis of *n*-alkanes			
		BP1		HP1	
		Predicted t_r (min)	Difference (index units)	Predicted t_r (min)	Difference (index units)
α-Pinene (*A*)	2.345	2.325	-2.34	2.375	4.01
D-Camphene	2.468	2.475	0.84	2.475	0.95
Sabinene	2.622	2.625	0.32	2.625	0.36
β-Pinene	2.700	2.725	2.99	2.725	3.39
Δ^3-Carene	3.018	3.025	0.47	3.025	0.47
1,8-Cineol	3.228	3.275	3.21	3.225	−0.24
γ-Terpinene	3.557	3.575	1.24	3.525	−2.20
Camphor	4.953	5.025	2.94	4.925	−1.17
Lavandulyl acetate	9.448	9.475	0.71	9.375	−1.89
Longicyclene	14.101	14.125	0.43	14.025	−1.36
Longifolene	15.978	15.975	−0.06	15.925	−0.95
Calarene	17.685	17.675	−0.14	17.675	−0.14
Aromadendren	18.100	18.075	−0.34	18.075	−0.33
α-Humulene (*B*)	18.939	18.925	−0.19	18.925	-0.18

Initial temperature T_0=100°C; heating rate r=1°C/min.

In order to eliminate errors not due to computation and to determine the true solution, the chromatograms used for the tests were obtained on the very column used for the measurement of the capacity-factor of the *n*-alkanes. The true value of β_u/β_a was then known to be equal to one. Mathematical independence of C and $\beta u/\beta a$ was first studied. A value of β_u/β_a was set and C calculated using Brent's method of minimization of a single variable function [15]. Results are plotted in Fig. 1. The variation of β_u/β_a implies a variation of C, following a non-linear curve which demonstrates a complex relationship between these two parameters that are therefore neither independent, nor linearly linked. It should be noted that the error is minimum for the true value of the parameters, and because a relationship exists, though not analytically expressed, between C and β_u/β_a, one can consider that there is a set of solutions that are close to the true solution. Within this set, the error remains low. Thus, even if computing is not perfect, the proposed solution should lead to retention index values close to the exact ones. Even when the value of β_u/β_a alone is significantly different fromthe true value, for instance 0.911 instead of 1 for the column BP1 used at 8°C/min, the couple (C, β_u/β_a) is an acceptable solution to the set of non-linear equations like Eq. (3). Actually, Fig. 1 shows that between β_u/β_a=0.8 and β_u/β_a=1.25 the error remains below 4 index units. This explains why results reported in Table 4

Table 4
Coefficients C and β_u/β_a determined for two columns and three temperature programmes

Temperature programme	BP1		HP1	
	C	β_u/β_a	C	β_u/β_a
T_0=60°C; r=8°C/min	0.0195064	0.9115499	0.0255575	0.4068105
T_0=80°C; r=2.8°C/min	0.0173482	1.0051733	0.0208478	0.5083683
T_0=100°C; r=1°C/min	0.0172713	0.9989998	0.0195123	0.5406678

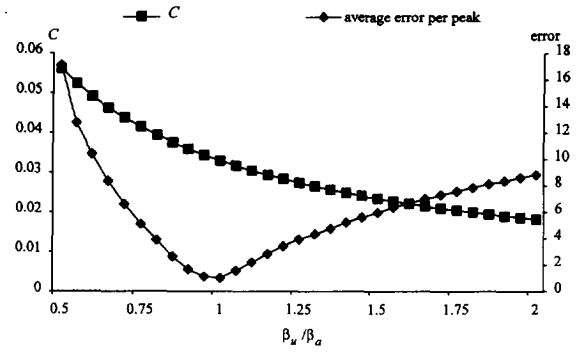

Fig. 1. Predicted value of C and average error per peak for the calculation of fifteen retention times as functions of β_u/β_a. The error is expressed as retention indices and is minimum for the true value of β_u/β_a i.e., unity.

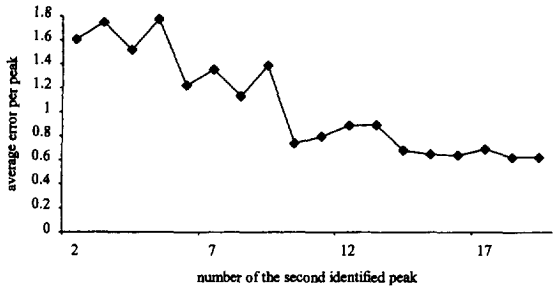

Fig. 2. Error per peak in the prediction of the retention times on a chromatogram as a function of the distance between the two reference peaks. The results show the average value for the whole chromatogram. The error is expressed as retention indices. The reference solutes, A and B, are the first peak of the chromatogram and a variable one.

lead to the good prediction of Table 1 and Table 2 and Table 3.

Another aspect of the roughness of the method was investigated. In regression, the variance of the estimator, i.e. the error in C and β_u/β_a, is inversely proportional to the variance of the controlled variable (the difference between the retention times of A and B). To check if a similar phenomenon exists in our method, C and β_u/β_a were calculated using successively different pairs of peaks as identified compounds. As expected, and as can be seen in Fig. 2, the longer the time between the two references, the better the precision. However, the error for a peak eluted between the two identified ones is always less than 1.5 index units. This has practical consequences: the prediction method should be used when the identified solutes A and B are located at the beginning and the end of the chromatogram respectively.

6. Conclusions

The prediction of the programmed temperature retention time is of great interest, for instance to confirm the identity of a peak. The procedure proposed here uses the Kováts retention index, which is one of the most common forms of chromatographic information in the literature. It also requires the capacity ratio of n-alkanes, but, contrary to other published methods, it might be measured on any column with the same stationary phase.

The identity of two peaks of the chromatogram is required. Only reproducible characteristics of the stationary phases can lead to accuracy as low as those obtained from a classical calculation when n-alkanes are co-injected with the analytical mixture. No information is needed about the gas hold-up time. This is an advantage when the detector is a mass spectrometer which is usually switched off during solvent elution. The method can also be applied to any chromatogram, even when the value of the column head pressure is unknown.

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