Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1019 (2003) 3–14
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Methods for generating second dimension retention index data in comprehensive two-dimensional gas chromatography

Robert J. Western, Philip J. Marriott*

Australian Centre for Research on Separation Science, RMIT University, GPO Box 2476V, Melbourne 3001, Australia

Abstract

Two methods of generating transportable second dimension retention data are outlined for comprehensive two-dimensional gas chromatography (GC × GC). They are both refinements of a previously outlined procedure, which adapted 'isovolatile' curves to retention prediction maps developed in our laboratory, extended to a more polar homologous series, the linear primary alcohols. The earlier work investigated maps based on alkanes and methyl ketones and methyl esters; here the method of data collection to generate the retention map has also changed, extending the retention base range. The resulting retention map permits a retention basis to be used for GC × GC data, however, a more polar retention set than alkanes is required. The calculation of 'retention indices' is aided by the generation of 'fractional reference compound' curves, by either direct manipulation of data local to the solute, or generation of a discrete curve coincident with the retention co-ordinates of the target compound.

Keywords: Gas chromatography, comprehensive two-dimensional; Retention correlation; Isovolatility plots; Retention indices; Alkanes; Alcohols; Fatty acids

1. Introduction

Despite the development of confirmatory techniques over the past decades (MS, FTIR) there has always been a need for a gas chromatographic retention-based identification tool. The high repeatability of gas chromatographic retention times is, and has always been, an important identifier. Depending on the stationary phase, information on the relative volatility, polarity or even shape of the resolved components is available. The major identification detectors, MS, FTIR or element-specific detectors, provide varying degrees of confirmation of an eluted component, but none provide an absolute identity. Although MS is regarded as a definitive identification tool, in many instances, particularly in the cases of homologues, structurally similar compounds or isomers (structural or steric) provide mass spectra too similar to provide unambiguous identification. The usual complementary tool for component identification is the chromatographic retention parameter. Petroleum [1–4] drugs [5], pesticides [6] and essential oils [7–9] are all separations where retention data are invaluable. For those techniques where only partial chemical data is provided (FTIR; element

* Corresponding author. Tel.: +61-39-9252632; fax: +61-39-6391321.
E-mail address: philip.marriott@rmit.edu.au (P.J. Marriott).

0021-9673/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2003.09.006

specific detectors), retention data is vital. Organochlorine pesticide analysis using the ECD detector is a good example where sensitive, selective detection is required, but which does not provide absolute identification.

The primary published retention base is the alkane retention index (I) of Kovatś or the linear retention index (LRI) developed by van den Drool and Krantz. In cases especially where the detector does not sense hydrocarbons (AED, ECD or NPD), alternate retention bases have been proposed [10,11]. These are usually based on detector element-sensitive substituted alkane homologous series. Linear saturated fatty acid methyl esters (FAME) [12] are used for identification of derivitised fatty acid and other biological components, as these were deemed more appropriate than the alkane basis. Each of these alternate series can be converted to an alkane-based set by a method outlined below.

Retention prediction, by either the use of determined parameters for each compound or homologous series to be identified [28,30], or through analysis of the molecular shape to determine relative retention parameters [13–15], offers further insight into factors which determine retention.

The development of comprehensive two-dimensional gas chromatography (GC × GC) requires a new method of developing a retention dataset for the second dimension (separation) in the GC × GC analysis space. While the first dimension retention can be characterised by conventional retention index approaches, developing a retention base for the second dimension requires a different approach. The first dimension separation effectively acts as a boiling-point filter which can be considered to remove the non-polar contribution to retention from the second dimension separation, which is a significant factor in controlling retention in single dimension GC [16]. In two-dimensional GC the often-used column set comprises a non-polar followed by polar column; components of differing polarity that have similar first dimension retention times potentially can give widely spaced peaks in the resulting two-dimensional analysis space. As each second dimension chromatogram is a discrete separation, a reference dataset must be superimposed on the second dimension preferably with members that bracket the target solutes, to permit relative retention data to be obtained.

1.1. Retention mapping

A recent paper [17] outlined a relatively simple method of generating isovolatile curves and used these as a basis for forming a two-dimensional retention map. Such curves had been illustrated by Beens et al. [18], based on an earlier observation [19] of unusual tailing phenomenon in GC × GC. Rather than using continuous bleed from a cold injector, mixtures of a homologous series of compounds (linear alkanes, 2-methylketones or linear saturated fatty acid methyl esters (FAME)) were sequentially injected throughout a single temperature programmed chromatographic run. Thus, a given compound will elute into the ${}^2D$ column at increasing temperatures, giving a monotonically decreasing ${}^2D$ retention time. The components of the series then define 'isovolatility' curves in two-dimensional space. Retention co-ordinates for a compound may then be plotted onto a retention map. Determining the retention index for a compound in the first dimension (${}^1I$) is determined from its retention—relative to the ${}^1D$ retention times (${}^1t_R$)—of the bracketing reference compounds, injected at the start of the chromatographic run. The ${}^2I$ value was determined from the position of the compound relative to the reference series isovolatile curves, at the time (and hence temperature) of its introduction into ${}^2D$. Reference series ${}^2t_R$ at this temperature are determined graphically or from the exponential decay formulae of the isovolatility curve. The reference index series used in ${}^2D$ need not be the same as that in ${}^1D$. Thus, alkane linear retention indices (LRI) might be used for ${}^1I$, and a more retentive solute series for ${}^2I$. An alkane reference series cannot be used for ${}^2I$, since they will be the least retained compounds in that dimension; their isovolatility curves will not bracket more polar target solutes.

However, a number of limitations include: the availability of a sufficiently discriminatory (i.e. polar) homologous series; the discontinuous nature of data used to define the isovolatility curve; the difficulty in determining the true position of each peak when a mixture is injected at higher oven temperatures, and; the limitations imposed by using a single temperature program with multiple injections.

The usual geometry GC × GC column set employs a non-polar ${}^1D$ and a polar ${}^2D$ column. Thus, on ${}^2D$ alkanes are the least retained solutes, and an alkane re-

tention map is unsuited for developing a 'universal' ^2D retention index (^2I) map. Recognising this, 2-methyl ketones and FAME were evaluated [17]. While these may be suitable for some compound classes, they were not sufficiently polar to have wide application, since many compounds are still more strongly retained on ^2D than these reference compounds. In this paper two more polar compound set were considered—carboxylic acids and linear 1-alcohols. The first was rejected as being too polar and unlikely to be universally applicable, giving poorly defined peaks. In contrast, the 1-alcohol retention map was found to give a good range of second-dimension retention times and is considered to be an adequate retention base for a range of solutes in GC × GC.

The reference series ^2t_R values for the isovolatility curves which bracket the compound, are used for logarithmic interpolation to derive ^2I. This assumes that the ^2D analysis is essentially isothermal. Fig. 1A shows graphically the raw retention data obtained from multiple timed injections (made at 0, 20, 25, 30, 40 and 45 min) of a saturated FAME sample during a single chromatographic run. A series of isovolatile curves can be generated as in Fig. 1B; the limitations of the method are apparent. To calculate a retention index, an upper and lower bound reference must be available to interpolate the position of a compound. In the case shown here, the available calculation space is limited to the area included in the bounds between the isovolatile lines (solid) and the vertical extremities

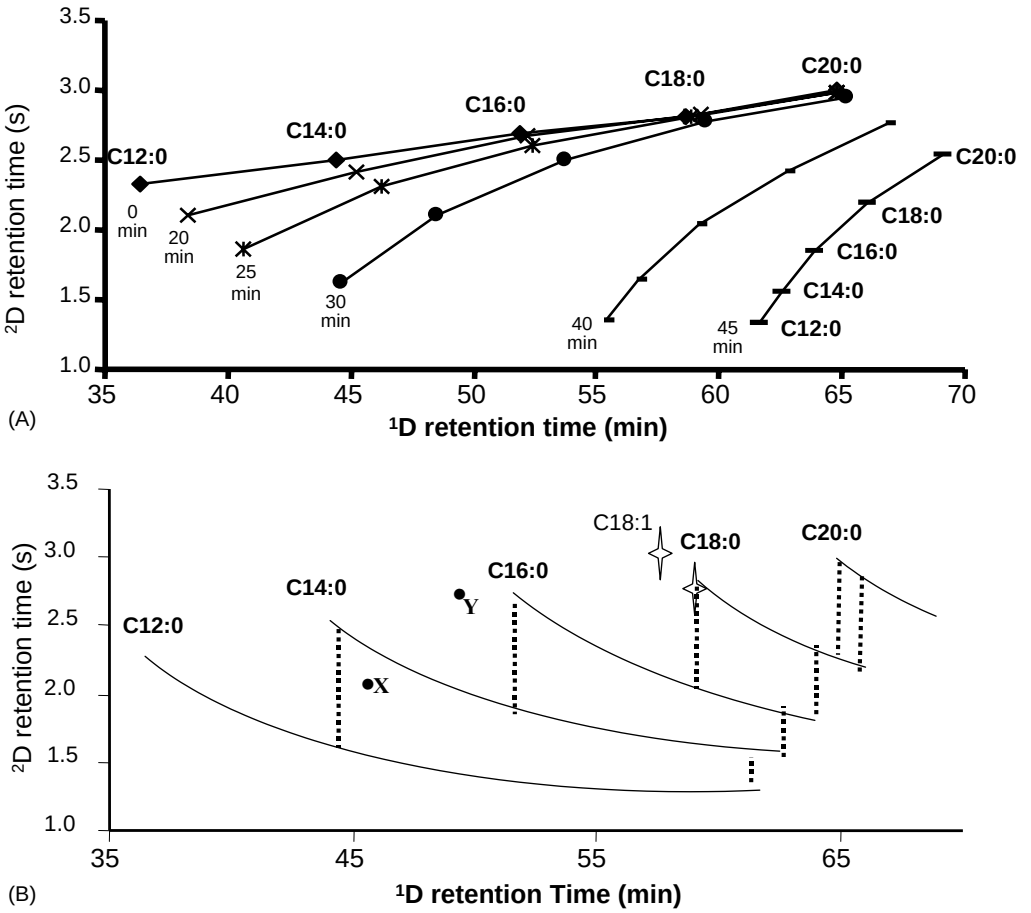

Fig. 1. (A) FAME retention curves for compounds injected at indicated times. (B) Retention map of above, showing retention index calculation limits. The retention index may be calculated for compound X, but not for Y.

of these curves (dotted). Thus, species X is located between C_{14} and C_{12} isovolatile lines; species Y lies above C_{14} but the C_{16} line does not extend to directly above Y. Hence, interpolation between C_{14} and C_{16} to obtain a second dimension retention index for Y is not possible. Without extrapolation of the isovolatile line data, the available space is significantly less than desirable. Using both odd and even FAMEs would extend the range and expand the space to some degree. Fractional "carbon value" isovolatile curves could be generated to increase the analysis range. For example, the position of the $C_{18.5:0}$ curve could be predicted within the $C_{18:0}$ and $C_{20:0}$ isovolatile lines. It was found that all unsaturated FAME still chromatographed outside the limits of the saturated FAME retention map (refer to the position of $C_{18:1}$, Fig. 1B), and second dimension ECL values could not be generated. Alternate methods of extending the isovolatile curves will be discussed later.

The multiple injection method outlined [17] depends on identifying the individual series members from the curve pattern of the homologous series. This can be difficult when solvent bands, impurities and components from other injections interfere. A alternative method, and one which overcomes the problems of limited curve range and component identification, is to perform a number of chromatographic analyses at different ramp rates, thus creating a range of ${}^2D$ elution temperatures for each component of the mixture (increasing the ramp rate increases the elution temperature).

From this approach, the curve is generated as an elution temperature (launch temperature onto ${}^2D$)/retention plot, upon which the elution temperature/${}^2D$ retention time of unknown compounds may be placed.

1.2. 'Thermodynamic' predictors

Calculation of retention indices depends on bracketing a solute by a pair of compounds in a reference homologous series. This is rarely a problem in conventional GC. Whilst even the least polar stationary phases exhibit some polarity [20], in single dimension gas chromatography, the net elution is a complex function of a number of concurrent mechanisms. For convenience these can be called polar and non-polar (boiling point) effects. If the primary mechanisms can be effectively isolated on the different columns then it may be possible to obtain orthogonal separations. Since in GC × GC the column set normally comprises a non-polar followed by a polar column, for a pair of compounds (one polar, one non-polar) that co-elute on the non-polar phase, the polar compound must elute later on the polar column. It is not possible to use a non-polar compound retention index set to define a retention map for polar columns on the second column. Alternate descriptors may therefore be required.

The formula:

$$t_R = t_M(1 + e^{a+b/T}) \tag{1}$$

has been used [13] as an alternate method of predicting retention times. These terms have a theoretical basis where:

$$a = \frac{\Delta S^\circ}{R} + n\frac{\delta S}{R} + \beta \tag{2}$$

$$b = \frac{\Delta H^\circ}{R} + n\frac{\delta H}{R} \tag{3}$$

where ΔH° and ΔS° are the standard molar enthalpy and entropy respectively for the 'zeroth' member of a homologous series, and δH and δS are the unit increments of enthalpy and entropy of the components in the homologous series. R is the universal gas constant, T is temperature in Kelvin, and β the phase ratio.

Calculation of these descriptors is usually done from a number of different temperature isothermal chromatograms. For each of these temperatures t_M must also be accurately measured. The above values are determined through a plot of $\ln(t'_R/t_M)$ versus $1/T$. On a single column t_M is readily measured, but where values must be determined for the second of a two-dimension column set, direct measurement is not possible. Marker compounds (methane, butane, etc.) are not retained in the modulating cryotrap, so an indirect method must be used. Previously [17], an estimate was made from the total void time, pressure drop and column dimensions. Although results were obtained which agreed with expected values, the method was considered too dependent on indirect measurement and column dimension data. A method has been suggested [21] using measurements of retention times of homologous series (alkanes) under isothermal conditions, and by plotting carbon number against retention time, obtaining the retention time of the 'zeroth' member of the series. This method

uses the δH and δS values of the above formulae. Although it is not practical to simultaneously trap two adjacent members of a homologous series on the second dimension column, being well separated on the first column, the isovolatile plots generated from the multiple injections technique used in the earlier work [17] should allow calculation of void time by this method. However, a straight-line relationship between either polar (alcohols) or non-polar (*n*-alkane) solutes on the polar column could not be established that gave a reasonable result. Rather, the method of flow calculation outlined earlier [17] was used.

2. Experimental

2.1. Gas chromatograph

An Agilent 6890 GC (Agilent Technologies, Wilmington, DE) fitted with a conventional split/splitless injector and a fast (100 Hz) flame ionisation detector (FID) was used to generate all chromatographic data. Injections were made at the beginning of each run using an Agilent 7683 autoinjector. Hydrogen carrier gas was used, and supplied to the column under constant flow conditions, at an initial pressure (at 50 °C) of 100 kPa. Constant flow characteristics are determined partly by the length and diameter of a single column. A composite column diameter for the column set (see Section 2.2) was determined by applying the void time and the column length to the Agilent flow calculator [22].

Solutions were diluted as required, so that with a GC inlet split of approximately 1:80 peak response signal after modulation was about 100 pA. Temperature program rates of 1, 2, 3, 4, 5, 6 and 8 °C/min were used to generate isovolatile curves, with an initial isothermal hold of 1 min, and a temperature range from 50 to 260 °C. All components were eluted in the linear temperature program region. The injector was set to 280 °C and detector at 260 °C.

Isothermal experiments were carried out on a Shimadzu GC-14A fitted with a split injector, and a flame ionisation detector, with a suitable split ratio injection. Measurements of retention time were made from 80 to 260 °C in increments of 10 °C, with hydrogen carrier gas at a pressure of 75 kPa.

2.2. Chromatography columns

The column set used for GC × GC was a BPX5 column (30 m × 0.25 mm i.d. × 0.2 μm film coating d_f) directly coupled to a BP20 column (1.2 m × 0.1 mm i.d. × 0.2 μm d_f) using a zero dead volume union. The single column retention data were obtained on a BP20 column (3.3 m × 0.1 mm i.d. × 0.2 μm d_f). All columns were supplied by SGE International (Ringwood, Vic., Australia).

2.3. Cryogenic modulator

An Everest model longitudinally modulated cryogenic system (LMCS), supplied by Chromatography Concepts (Doncaster, Australia) was used for chromatographic modulation. The moving cryotrap of the modulator was mounted near the end of the first dimension column, shortly before the connecting union. The LMCS principles of use [23], specific operating modes [24] and selected applications [24–26] have been reported. In all data presented here the modulation period was 3.000 s, although to determine the correct second dimension retention time for some slow eluting compounds modulation periods of 5 and 7 s. were used, as was the targeted mode of operation [24]. The Agilent Chemstation control software instructed the modulation to commence at a nominated period after injection, usually exactly 8.000 min after initiation of the chromatographic run.

2.4. Data manipulation

ASCII comma separated variable data (*filename.csv*) were exported from Agilent Chemstation, then converted into two-dimensional array format according to modulation time and data acquisition frequency. A contour map of this two-dimensional data was generated using Transform (Fortner Research, Boulder, CO, USA).

The two-dimensional peak position data were calculated from the map, and entered into an Excel (Microsoft, Redmond, WA, USA) spreadsheet. Correct identification of the two-dimensional data array was confirmed by examining a two-dimensional curve plot of each (alkane, ketone, FAME or alcohol) injection sequence, as outlined in Fig. 1A. From these data, the isovolatile curves were generated (Fig. 1B). Origin 6

(Microcal Software Inc. Northampton, MA, USA) was used for exponential decay formula fitting of each of these isovolatile datasets. The constants generated by this method were of the form:

$$y = y_0 + A_1 e^{(-x/t_1)} \quad (4)$$

where $y = t_R$ and $x = T$.Values of second dimension retention time were measured for each compound at a series of temperatures, either by varying ramp rate on a two dimensional column set or isothermal injection onto a single column coated with the polar (BP20) stationary phase.

For estimation of the empirical 'thermodynamic' constants of formula (1), plots of $\ln(t'/t_M)$ versus $1/T$ (in K) were generated, and the intercept (A) and slope (B) were estimated on the individual compound datasets. The fit of the data was measured as R^2. Although these formulae gave nearly identical graphical results over the chromatographic data range, the mathematical forms are not interchangeable.

2.5. Reagents

A solution of the linear alkanes (C_{12}, C_{14}–C_{22}) was prepared in pesticide grade hexane (Merck, Kilsyth, Vic., Australia) using a standard kit supplied by Alltech Associates (Baulkham Hills, NSW, Australia). The FAME mixture was supplied premixed by Nuchek (Elysian, MN, USA) and diluted with A.R. acetone (Merck, Kilsyth, Vic., Australia). Hexanol, heptanol, octanol, nonanol and decanol were purchased from Sigma–Aldrich (Castle Hill, NSW) and were diluted with A.R. acetone. The SGE Test Mix B1 (2-octanone, tetradecane, 1-octanol, hexadecane, naphthalene, 2,4-dimethylaniline (2,4-DMA) and 2,6-dimethylphenol (2,6-DMP)), supplied as a column test solution, was used as a test sample. Void times were estimated using the laboratory natural gas supply.

3. Results

3.1. Reference data

To evaluate the retention parameters of the second dimension column, a series of isothermal chromatograms were run using alkane, alcohol and test mixes from 50 to 250 °C at 10 °C intervals, on a column with the same diameter and stationary phase as the second dimension column (BP20) used for GC × GC studies. The void time at each temperature was measured. This provides equivalent retention data under elution conditions that arise for the BP20 column of the GC × GC two column set, across the total range for temperature ramp rates from an effective 0 °C/min to greater than the 8 °C/min used for the retention map experiments. The retention indices for all compounds in the SGE B1 mix were also estimated across a range of temperatures, and the alcohol-based retention index was determined for a number of compounds in the test mixture (Table 1A).

3.2. Retention map

The linear primary alcohol homologous series were investigated as reference sets in preference to those used previously [17]. The dataset was generated on the C_6 to C_{10} primary linear alcohols by the multiple temperature ramp method outlined above. The homologous series plots, as well as the retention points of the components in the test mix for a 4 °C/min temperature ramp, are shown in Fig. 2. As indicated above, the abscissa is plotted as second dimension launch temperature rather than first dimension retention time. As a check on the timing of these compounds, particularly those with considerable wraparound, the uncorrected retention time plots for all of these compounds were generated, and the curve formulae for each compound was estimated. Limitations of the *n*-alcohols as a retention set are apparent (Fig. 2) from the positions of octanone, the two alkanes and the highly polar 2,6-DMP. The first three compounds fall below the lower bound (faster ramp rate) of the reference set, and although faster ramp rates may allow these compounds to be included in the space, the decrease in retention set resolution resulting from the ramp rate increase may give rise to data too inaccurate to be of any practical value. It may be necessary to use a less polar retention set (such as the ketone set reported earlier [17]) to properly encompass these compound classes. 2,6-DMP (and other significantly polar compounds) present a different problem—the lack of a sufficiently polar homologous reference series. Examination of the position of this compound on the isovolatile plot of Fig. 2 shows it to have a lower, but no

Table 1
Prediction of alcohol based retention indices of selected components in column test mix for analysis conditions of 0 and 100 °C

	1-Octanol	Hexadecane	Naphthalene	2,4-DMA	2,6-DMP
A					
I_{OH}; 0 °C	812	862	969	1080	1116
I_{OH}; 100 °C	804	812	1062	1130	1132
dI/dT	−0.075	−0.52	0.93	0.53	0.194
T range (°C)	140–165	140–165	140–175	140–170	140–175
B					
I_{OH}; 0 °C	804		976		
I_{OH}; 100 °C	798		1085		
dI/dT	0.059		1.09		
T range (°C)	140–165		140–175		
C					
I_{OH}; 0 °C	808	1282	952	1001	1147
I_{OH}; 100 °C	802	1222	1075	1025	1083
dI/dT	−0.0063	−0.899	1.23	0.059	−0.59
T range (°C)	71–110	132–198	84–133	83–146	76–120

A: from isothermal measurements; B: from empirical curve fitting; C: from "infinite series" calculations. Experimental data were obtained over ranges shown.

upper bound reference. The dataset used does not contain a compound member to provide an upper bound position, but even if such a curve was available, no upper bound value could be obtained by this method (the longest second dimension time arises when using the slowest ramp rate of 1 °C/min). For any polar homologous set that can be generated, there will always be compounds, which will exceed its bounds. A possible method of generating retention index values for compounds which just fall outside the reference

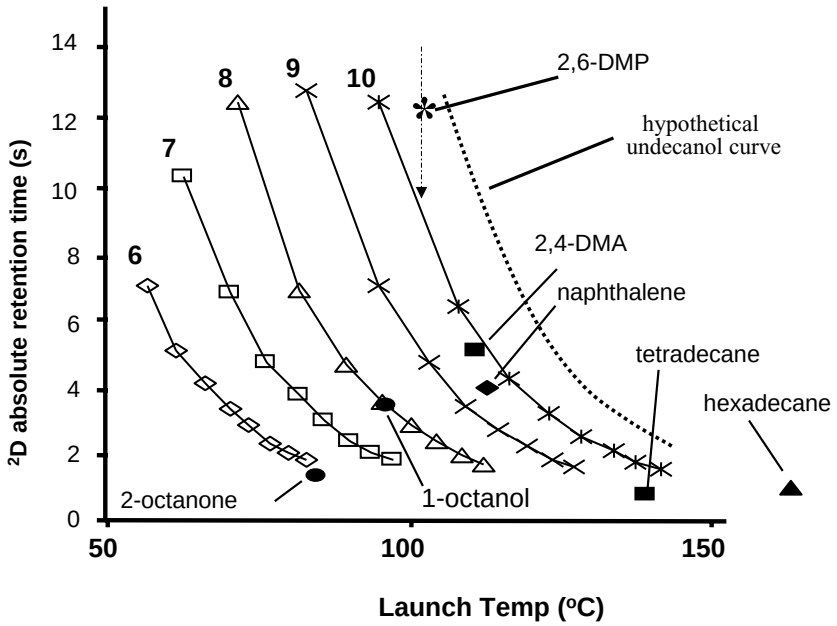

Fig. 2. Alcohol based retention map showing two-dimensional positions of test mix components at 4 °C/min.

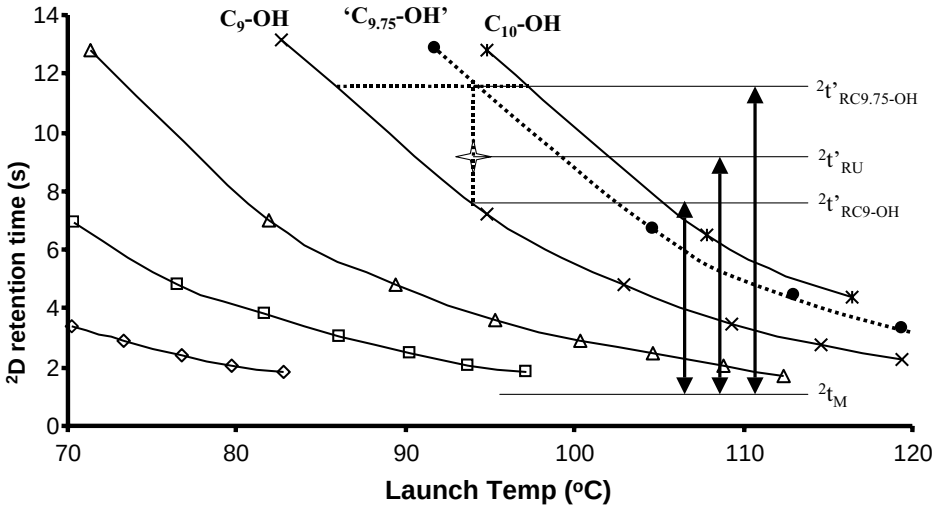

Fig. 3. Method of 'pseudo' isovolatile curve generation method to overcome upper bound limitation.

compound isovolatile retention map may be to use limited extrapolation, but with any form of mathematical extension, the results must be treated with some caution.

Alternately the concept of partial retention reference compounds could be explored. As retention in the first dimension is (usually) due to use of linear temperature programming, a horizontal line drawn on the retention map, would intersect the isovolatile curves of the reference compounds at approximately equal intervals. Thus, at any one point along this line, between two consecutive reference compound isovolatile curve intersections, 'partial' reference curves could be drawn. The simplest way of obtaining points along this generated curve would be to use the weighted averages along the first dimension to define the ${}^2t'_R$ curve. As an example, in Fig. 3, a compound in the two dimensional analysis space, represented by the star, has a lower bound alcohol (nonanol) but no upper bound, as it exceeds the normally usable space enclosed by the solid isovolatile lines and the dashed vertical limits. A 'pseudo-isovolatile' curve, generated from the nonanol and decanol isovolatile curve for a theoretical compound "$C_{9.75}H_{20.5}OH$" would allow the estimation of a value for the compound whose position is represented by the star. Thus, the vertical intersection between this curve and the isovolatile curve of nonanol, would give the retention data required for calculation of the retention index. The second dimension retention index for this unknown compound (U) would be:

$$I_{OH} = 9 \times 100 + 100 \times 0.75 \times \frac{\log({}^2t'_{RU}/{}^2t'_{RC9\text{-}OH})}{\log({}^2t'_{RC9.75\text{-}OH}/{}^2t'_{RC9\text{-}OH})}$$

This method of retention index estimation has been partially tested but will require further investigation. Paradoxically, although this method was developed to increase the available area of the retention map, no compound in the test set met the criteria for determination by this method, although the retention indices of two compounds (octanol and naphthalene) could be determined using the basic retention map (Table 1B).

An extension of this method is to assume an infinite number of isovolatile curves can be generated by pseudo-partial reference compounds, and that the compound whose second dimension retention index is required to be determined, will lie on one of these. Eq. (1) can be rewritten as:

$$\ln\left(\frac{t'_R}{t_M}\right) = a + \frac{b}{T} \quad (5)$$

Using data generated for the alcohol reference compounds, a series of linear relationships between $\ln(t'_R/t_M)$ and $1/T$ (in K) can be generated. From these

the individual values of a (intercept), and b (slope) can be obtained. Both of these factors show a linear relationship to the number of carbons (Cn) in the reference series, corresponding to Eqs. (2) and (3), respectively. The intercept and slope of Cn versus A are $(\Delta S^\circ/R + \beta)$ and $n\delta S/R$ respectively, and for Cn versus B are $\Delta H^\circ/R$ and $n\delta H/R$. Since these data are to be used for generation of data for an individual system and not directly as transportable numbers, the phase ratio (β) can be ignored. A simplified form can be written as:

$$\ln\left(\frac{t'_R}{t_M}\right) = A^0 + Cn\delta A + \frac{B^0}{T} + \frac{Cn\delta B}{T} \quad (6)$$

or

$$Cn = \frac{\ln(t'_R/t_M) - (A^0 + B^0/T)}{\delta A + \delta B/T} \quad (7)$$

Substituting measured values of t'_R, t_M and T (in K) for a compound will give the second dimension index ${}^2I_{OH}$ as $100 \times Cn$.

A representative curve in the alcohol isovolatile plots is shown in Fig. 4. From Eqs. (6) and (7), only a single value would be generated, the 'pseudo-isovolatile' plot (dotted line) has been generated to emphasise its position. Retention index values for test mix components, estimated by this method, are shown in Table 1C. For comparison alcohol based retention indices, calculated from single dimension chromatography on the 3.0 m × 0.1 mm BP20 column are given in Table 1A. From the results, those compounds within, or near, the bounds of the retention map bear good correlation to the retention indices obtained on the single column (3.0 m × 0.1 mm i.d. BP20). For those compounds well outside the bounds, correlation is significantly worse due to uncertainties in extrapolating data.

As an alternative to Eq. (4):

$$\ln(t'_R) = \ln(A_1) - \frac{T}{a_1}$$

or

$$t'_R = Z + YT$$

may be used, where $Z = Z^0 + Cn\delta Z$ and $Y = Y^0 + Cn\delta Y$.

Here,

$$Cn = \frac{\ln(t'_R) - (Z^0 + Y^0T)}{\delta Z + \delta YT} \quad (8)$$

For this column set, the formula was

$$Cn = \frac{\ln(t'_R) - (2.037642 + 0.061407T)}{0.483120 + 0.483120T}$$

Each method was found to give comparable results.

The concept of partial carbon numbers is also dealt with by Harangi [27] (virtual carbon number)

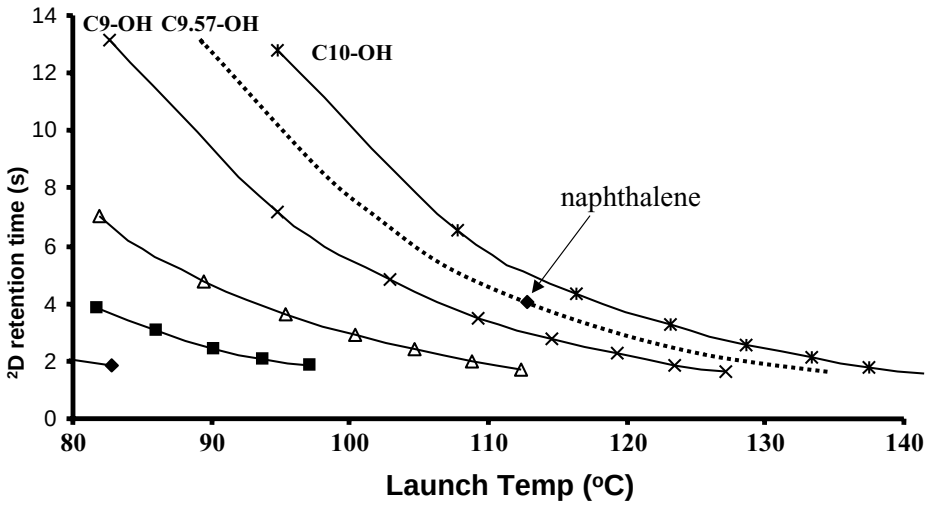

Fig. 4. Method of 'thermodynamic' calculation of second dimension retention index of naphthalene chromatographed at 4 °C/min. The dotted line is not a result of calculation but is plotted to emphasise point position.

in a recent paper for single dimension chromatography.

3.3. Retention index interconversion

A substantial historical data record on linear alkane based retention indices has been collected, both in-house, or published in the literature. By estimation of the *n*-alkane empirical thermodynamic characteristics outlined above, and knowing the void time (t_M), then from Eqs. (6) and (7), the adjusted retention time can be estimated for any reported compound:

$$\ln\left(\frac{t'_R}{t_M}\right) = A^0_{HC} + \frac{B^0_{HC}}{T} + \left(\frac{I}{100}\right)\left(\delta A_{HC} + \frac{\delta B_{HC}}{T}\right)$$

where I is the alkane based retention index, and HC refers to constants for the linear alkane series.

Similarly for the alcohol based dataset:

$$\ln\left(\frac{t'_R}{t_M}\right) = A^0_{OH} + \frac{B^0_{OH}}{T} + \mathrm{C}n_{OH}\left(\delta A_{OH} + \frac{\delta B_{OH}}{T}\right)$$

and

$$I_{OH} = 100 \times \mathrm{C}n_{OH}$$

where I_{OH} is the alcohol based retention index for the same compound, and subscript 'OH' refers to constants for the linear saturated alcohols.

As $\ln(t'_R/t_M)$ is common to both formulae, the alcohol based retention value can be directly calculated from:

$$I_{OH} = \mathrm{C}n_{OH} \times 100 = \frac{[A^0_{HC} - A^0_{OH} + (B^0_{HC} - B^0_{OH})/T + (I/100)(\delta A_{HC} + \delta B_{HC}/T)]}{\delta A_{OH} + \delta B_{OH}/T}$$

where $\mathrm{C}n_{OH}$ has been previously defined as the alcohol equivalent chain length of the compound being considered. As the majority of terms in the formula are constants derived from experimental methods described above, the only values required are the

Table 2
Conversion of single dimension derived alkane-based isothermal retention indices (I_{HC}) to alcohol based isothermal retention indices (I_{OH}), with estimated I_{OH} differences (ΔI_{OH}) by use of the exponential decay (Eqs. (6) and (7))

T (°C)	1-Octanol (I_{HC})	Hexadecane[a] (I_{HC})	Naphthalene (I_{HC})	2,4-DMA (I_{HC})	2,6-DMP (I_{HC})
Alkane base indices					
140	1539	1600	1735	1852	1881
145	1540	1602	1746	1859	1886
150	1540	1598	1760	1869	1895
155	1544	1604	1764	1870	1895
160	1543	1597	1767	1875	1898
165	1545	1597	1768	1872	1895
170			1783	1883	1903
175			1784		1902

	1-Octanol[b]		Hexadecane		Naphthalene		2,4-DMA		2,6-DMP	
	I_{OH}	ΔI_{OH}	I_{OH}	ΔI_{OH}	I_{OH}	ΔI_{OH}	I_{OH}	ΔI_{OH}	I_{OH}	ΔI_{OH}
Alcohol base indices										
140	799	−3.25	860	0.15	994	−5.28	1111	−7.58	1140	−2.50
145	803	−1.72	860	−0.75	1004	−7.48	1117	1.33	1143	0.95
150	800	0.80	854	0.02	1016	−2.50	1125	−7.43	1151	−1.73
155	802	−3.19	858	−1.54	1018	1.79	1124	−2.84	1148	−5.62
160	799	0.48	849	1.03	1019	0.46	1127	1.56	1150	0.07
165	798	−4.18	848	−5.20	1018	−2.27	1122	−4.97	1145	−1.91
170					1031	−1.03	1131	−8.85	1151	−8.35
175					1030				1148	−5.04

[a] Whilst this value should equal 1600, uncertainty arises from application of standard alkane data to retention values for the injected test mix.

[b] Ideally, these values would equal 800 for the C_8-alcohol.

alkane retention index and the temperature at which it was measured. Comparative results are given in Table 2. The upper set are alkane based isothermal retention indices (from single dimension GC analysis). Integer values in the lower part of the table are alcohol based retention indices calculated from the alkane index set, with the difference (ΔI_{OH}) between these values and those directly calculated from the alcohol homologous series adjusted retention times.

3.4. 'Thermodynamic' reference

The retention index of compounds with high polarity such as 2,4-DMP, will in all probability, not be accurately measured by any retention map plot. An alternate approach may be based on that developed by Cavalli and Guinchard [28,29] with prediction of retention times from experimental data, using an extension of formula (1). By this method, the column was divided into x number of equal lengths (L); for each of these the exit temperature was calculated from the void time, and A and B predetermined compound characteristics:

$$dt_R = \frac{t_M\, dx}{L(1 + e^{A+B/T})}$$

from this

$$t_R = \sum \frac{t_M}{x(e^{A+B/T(I-1)})} \quad \text{from } I = 1 \text{ to } x$$

where the column was 'divided' into 50–100 mm lengths.

This work was further investigated [30,13] to develop single dimension retention values for FAME.

Two problems outlined by the authors of this method, were the required accuracy of determination of the void time (t_M) and of the two characteristic values of the compounds. Any minor error in measuring these becomes a significant error after a number of exponential calculations have been summed. Attempts were made to estimate ${}^2t'_R$ for 2,4-DMP by this method, but the estimates varied significantly from the measured value and the results are not included in this paper.

4. Conclusion

The method of data generation employing single injections at different temperature ramp rates, was found to be a useful alternative to the previous method of multiple injections during the chromatographic run. This extends the range of the isovolatile curves. This extended range is still not sufficient to encompass all compounds, although limited extrapolation may be sufficiently accurate to accomplish this.

Of the two methods of generating data for the calculation of retention indices the creation of 'pseudo-compound' isovolatile curves, is the simplest, but requires more operator intervention, both in determining the position of the compound whose index is to be calculated, and in manipulation of the estimated data. The second method, pre-calculation of 'thermodynamic' constants requires a greater degree of prior information, but once the required reference data has been generated, is both simpler to use, and has a greater operating range. It is probably more amenable to extrapolation to include compounds falling outside the bounds of the isovolatile map. Available retention index data can be readily converted into a form usable for second dimension databases. Further work will be required on these methods to both determine the accuracy of the methods, within the curve boundaries, and the degree of inaccuracy inherent in estimations where retention index values exceed the map boundary limits.

By reducing the two-dimensional plane to reliable retention index (or possibly retention factor) plot, it should be possible to predict the positions of any compound in the GC × GC experiment, provided adequate reference data are available.

References

[1] D. Bonechev, O. Mekenjan, G. Protic, N. Triajstic, J. Chromatogr. 176 (1979) 149.
[2] N.G. Johanssen, L.S. Ettre, R.L. Miller, J. Chromatogr. 256 (1983) 393.
[3] J.M. Levy, L.E. Wolfram, J.A. Yancey, J. Chromatogr. 279 (1983) 133.
[4] L. Sojak, G. Addova, R. Kubinec, A. Kraus, G. Hu, J. Chromatogr. A 947 (2002) 103.
[5] A. Stowell, L. Wilson, J. Forensic Sci. 1214 (1986).
[6] M.M. Mossoba, R.A. Niemann, J.-Y.T. Chen, Anal. Chem. 61 (1989) 1678.

[7] R.P. Adams, Identification of Essential Oil Components by Gas Chromatographic/Quadrupole Mass Spectroscopy, Allured Publishing Corporation, IL, USA, 2001.
[8] E. Turdor, J. Chromatogr. A 779 (1997) 287.
[9] T.J. Betts, J. Chromatogr. A 936 (2001) 33.
[10] G.L. Hall, W.E. Whitehead, C.R. Mourer, T. Shibamoto, J. High Resol. Chromatogr. Chromatogr. Commun. 9 (1986) 266.
[11] G. Castello, J. Chromatogr. A 842 (1999) 51.
[12] R.G. Ackeman, J.C. Sipos, J. Am. Oil Chem. Soc. 41 (1964) 377.
[13] M. Randic, J. Chromatogr. 161 (1978) 1.
[14] D. Bonechev, O. Mekenjan, G. Protic, N. Triajstic, J. Chromatogr. 176 (1979) 149.
[15] V.E.F. Heinzen, M.F. Soares, R.A. Yunes, J. Chromatogr. A 894 (1999) 495.
[16] G. Tarjan, A. Kiss, G. Koksis, S. Meszaros, J.M. Takacs, J. Chromatogr. 119 (1976) 327.
[17] R.J. Western, P.J. Marriott, J. Sep. Sci. 25 (2002) 832.
[18] J. Beens, R. Tijssen, J. Blomberg, J. Chromatogr. A 822 (1998) 233.
[19] J. Beens, R. Tijssen, J. Blomberg, J. High Resol. Chromatogr. 21 (1998) 63.
[20] J.B. Phillips, J. Beens, J. Chromatogr. A 856 (1999) 331.
[21] J.E. Quintanilla-Lopez, R. Lebron-Aguillar, J.A. Garcia-Dominguez, J. Chromatogr. A 767 (1999) 127.
[22] http://www.chem.agilent.com/cag/servsup/usersoft/main.html#flowcalc205.
[23] P.J. Marriott, R.M. Kinghorn, Anal. Chem. 69 (1997) 2582.
[24] P.J. Marriott, R.C.Y. Ong, R.M. Kinghorn, P.D. Morrison, J. Chromatogr. A 892 (2000) 25.
[25] R.M. Kinghorn, P.J. Marriott, P.A. Dawes, J. Microcol. Sep. 10 (1998) 611.
[26] P. Marriott, R. Shellie, J. Fergeus, R. Ong, P. Morrison, Flavour Fragr. J. 15 (2000) 225.
[27] J. Harangi, J. Chromatogr. A 993 (2003) 187.
[28] E.J. Cavalli, C. Guinchard, J. Chromatogr. Sci. 33 (1995) 370.
[29] E.J. Cavalli, C. Guinchard, J. Chromatogr. Sci. 34 (1996) 597.
[30] K. Kittiratanapiboon, N. Jeyashoke, K. Krisnankura, J. Chromatogr. Sci. 36 (1998) 541.