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Journal of Chromatography A, 1071 (2005) 47–53
JOURNAL OF CHROMATOGRAPHY A
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Orthogonality considerations in comprehensive two-dimensional gas chromatography

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Abstract

This study explores separation orthogonality with respect to comprehensive two-dimensional gas chromatography (GC × GC) for a range of different column polarities in the first dimension (^{1}D), with two second dimension (^{2}D) column types. Systematic variation in the net polarity of the first dimension allows the effect of column phase relative polarity on analyte retention in both the first and second dimensions to be evaluated. First dimension polarity manipulation significantly affects elution temperature (T_e) of the analytes. This alters the magnitude of retention on the second dimension, and the extent of utility of separation space. By use of retention factor/temperature data in single column experiments, along with ^{1}D T_e data, retention on the ^{2}D column can be estimated. This allows the two-dimensional separation to be predicted, and compared with experimental data. Predicted GC × GC peak positions corresponded favourably with the experimentally derived chromatograms, yielding a simple approach for predicting two-dimensional separations, using unique column set combinations.

Keywords: Comprehensive two-dimensional gas chromatography (GC × GC); Orthogonality; Correlation; Separation space; Retention prediction; Simulation

1. Introduction

Hyphenated analytical (instrumental) methods offer significant advantages for improved analysis of chemical systems. Hirschfield [1] discussed the role of hyphenation in instrumental analysis with respect to the enhanced differentiating power that can be achieved, and the types of instrumental dimensions that can be effectively coupled. Clearly both dimensions must be compatible towards the analysis of the sample components, and the coupling interface must take cognisance of the possible different nature of the fluids used in each dimension. The general multidimensional analytical method employs two (or more) fundamentally different techniques to provide the information increase desired for the analysis task. Provided that the characteristic measured parameter(s) in one dimension is (are) independent of the measured parameter in the second, then the system can be termed orthogonal, i.e. there is no correlation between the measured properties, and one cannot predict the result on the second dimension based on knowledge of the measured property on the first. Typical examples involving a gas chromatographic first dimension will be those that employ spectroscopic detection in the second dimension, such as GC–FT-IR and the familiar GC–MS techniques.

The importance of orthogonality in multidimensional separations is critical, and determines the magnitude of two-dimensional separation space that is utilized. Retention correlation across dimensions reduces the maximum peak capacity to some fraction of that which is theoretically available. A high degree of retention correlation can reduce a multidimensional separation to an essentially one-dimensional separation, with peaks distributed along a diagonal [2]. Orthogonality in a two-dimensional separation may be achieved when elution times for each dimension can be treated as statistically independent [3]. Venkatramani et al. [2] identified two approaches that can be used to achieve orthogonality; the first involves combining techniques that utilise very different chemistries, or mechanisms [4], for separation. The second approach [2] relates to "creating" orthogonality, by varying or tuning the operation conditions of the second dimension as a function of the progress of the first dimension.

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doi:10.1016/j.chroma.2004.09.020

By necessity, this approach requires that operating conditions be readily tunable. Thus, in comprehensive two-dimensional gas chromatography (GC × GC), it is generally accepted that orthogonality may be achieved by temperature tuning, as demonstrated by Phillips and Beens [5]. By correctly raising the temperature, retention in the second column decreases to compensate (exactly) for the decreasing volatility of substances eluting from the first column.

Conventionally in GC × GC, two dimensions of disparate composition (stationary phase) are used in order to utilise the maximum separation space possible for the 2D separation. Typically, the first phase is non-polar or is of low polarity, whilst the second is more polar (although the reverse geometry has been described in the literature [6]). Consequently, the separation space may be defined based on component boiling point properties in the first dimension, and 'polarity' in the second. Clearly, however, the degree of orthogonality will depend upon how well or poorly correlated are the stationary phases of the two dimensions. It cannot, therefore, be assumed that all GC × GC separations are truly orthogonal but rather what must be considered is the degree of GC × GC system orthogonality, or 'relative' or 'partial' orthogonality.

In this investigation, the concept of orthogonality and its relationship with dimension correlation was explored. The polarity of the first dimension was systematically varied, through the combination of different lengths of a polar and a low polarity column, producing composite ^{1}D columns of constant total length, but variable overall polarity. The composite ^{1}D columns were coupled to both polar and non-polar second dimension columns, in order to study the extent of 'orthogonality' that might be obtained with each column set. It may be proposed that the extent of orthogonality can be determined by the percent usage of the available separation space. This definition depends on how the separation space availability is defined, and then gauging how much of this space is ultimately used with respect to the components in the sample.

1.1. Theory

Orthogonality in GC × GC is dependent upon different separation mechanisms in ^{1}D and ^{2}D, generally achieved using different polarity stationary phase columns in each dimension. Column orthogonality, however, does not necessarily result in optimum sample resolution, but rather offers the best opportunity for maximum use of the available separation space. It is only through correct instrumental tuning that column orthogonality can be fully exploited. Thus, in GC × GC, ^{2}D will be operated under conditions that give the necessary speed to permit compounds to elute in about the timescale of the modulation period. Such conditions are achieved through proper selection of column relative phase ratios (β), column dimensions, carrier gas flow, and temperature.

The GC × GC result comprises a two-dimensional (2D) plot with axes that represent total first dimension (^{1}D) and second dimension (^{2}D) time, respectively. The latter corresponds to the modulation period (P_M), a critical parameter in defining GC × GC operation. According to accepted criteria, P_M should approximate the ^{1}D peak standard deviation time (σ); excessive retention should be avoided since this demands a longer ^{2}D time—and hence longer P_M—which may lead to under-sampling of the peak [7]. Alternatively, if P_M is too small, some peaks may not elute within one modulation cycle, resulting in peak wrap-around. Whilst orthogonality per se is independent of the modulation process, peak wrap-around may be an important criterion when deciding the most suitable conditions for an analysis.

Fig. 1 illustrates the distribution of, or available elution region for, solutes in the 2D space, under conditions of all analytes eluting within one modulation cycle. Accordingly, when compounds are not wrapped-around, some proportion of the possible separation space is redundant, and so theoretical maximum peak capacity cannot be exploited. The avoidance of wrap-around determines the minimum P_M that must be used, and results in the early part of ^{2}D being unused for location of solute peaks, defined by the void time (^2t_M) on this column. The upper retention limit is then defined by the most highly retained components in the sample. Conversely, when wrap-around is permitted, P_M can be reduced (all other conditions constant) or more generally the ratio of ^{2}D retention to P_M increased, and compounds can elute within one or more modulation cycles. The goal of GC × GC in the general sense, therefore, would be to aim for maximum column orthogonality and maximum use of separation space, consistent with achieving resolution of components. The second column in an orthogonal GC × GC separation could lead to different extents of utility of separation space, if ^{2}D is a very short column, little of the space might be used. If ^{2}D is a long column, much more of the space will be used. Hence, to compare different column sets in an experiment to gauge orthogonality, it is imperative that experimental conditions are kept consistent from one experiment to the next.

1.2. Methods for defining and calculating orthogonality

To date, three main approaches have been used to evaluate the extent of system orthogonality in 2D separations. Liu

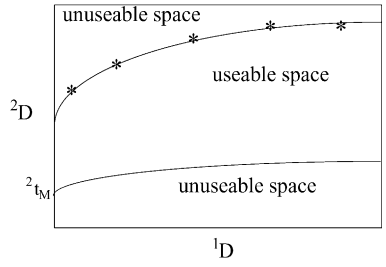

Fig. 1. Diagram illustrating the general use of the two-dimensional separation space. Refer to text for description. The symbol (*) is used to represent the elution of the most retained analytes (e.g. the most polar solutes on a polar column phase).

et al. [8] defined orthogonality using a correlation matrix, with correlation coefficients ranging from 0 (orthogonal) to 1 (perfectly correlated). The matrix was composed of solute retention parameters (including retention times and capacity factors for each dimension), from which a peak spreading angle (β) matrix was calculated, as a measure of the separation space utilisation. A spreading angle of 90° between the two separation dimensions, or vectors, indicates complete use of the separation space, and represents the maximum theoretical peak capacity of the two-dimensional system. Correlation of the dimensions results in a reduced β, and accordingly, a reduced orthogonality coefficient, and so practical peak capacity of the system is also reduced. This approach was described for isothermal conditions and so might not be readily accommodated in a temperature-programmed analysis, especially with samples that would elute over an extended temperature range.

Informational theory (IT) has also been used to determine orthogonality, whereby the extent of data overlap or informational similarity, provides a measure of informational orthogonality of the coupled system [9]. Slonecker et al. [10] have applied IT to provide a numeric indicator of informational orthogonality in projected two-dimensional liquid chromatographic analyses by using experimental one-dimensional retention data to calculate the extent of solute crowding in the 2D separation. Normalised retention data from each dimension are plotted on a two-dimensional plane. A high degree of solute crowding results in an informational orthogonality of 1, whilst complete utilisation of the separation space, results in a numeric value of zero.

The third approach to defining orthogonality, called percent synentropy, also uses IT. This provides a measure of the percentage of 2D informational entropy that is contributed equally from each dimension [10]. A percent synentropy of 0% and 100% describe systems with no correlation, and complete correlation (or retention mechanism equivalency) between the dimensions, respectively [9]. An estimation of percent synentropy can be calculated as the percentage of normalised retention data that are diagonally aligned on the normalised retention plane, or more specifically by dividing the informational entropy of the diagonally aligned normalised data by the total two-dimensional informational entropy.

1.3. Prediction of retention

Prediction of 2D separation is a valuable tool, enabling the separation performance of any set of columns to be tested for a particular sample (provided required primary retention data of the target compounds on the columns are available). Although orthogonality implies decoupling of the two separation dimensions, such that the retention of any randomly selected solute cannot be derived solely from its first dimension retention characteristic, this does not mean that for a specific solute, it is not possible to predict its anticipated ^2t_R from its ^1t_R. Previous methods for prediction of two-dimensional separations have been conducted by Beens et al. [11], and most recently Vogt et al. [12]. Western and Marriott proposed a methods for quantifying solute retention based upon retention index concepts [13].

In the present work, the task of predicting two-dimensional separation is based on prior determination of the elution of compounds on the first column directly from experiment. This is because basic thermodynamic retention data may not be readily derived for the novel combined-polarity columns used. Experimentally derived T_e values may then be used to estimate ^2t_R on the particular second column phase, where the relationship between retention factor and temperature is established from the respective, single column isothermal data on the same phase. Two-dimensional co-ordinates for each analyte can then be compiled and used to predict the 2D separations in the GC × GC experiment.

Table 1
Classification of combined-polarity columns used in the first dimension

First dimension	Length of BPX5 column[a]	Length of BP20 column[a]
A	20	0
B	15	5
C	10	10
D	5	15
E	0	20

[a] Each column, 0.25 mm i.d.; 0.25 μm d_f.

2. Experimental

2.1. Standard preparation

A 17-component standard mixture containing alkanes (nonane, decane, undecane, dodecane, tridecane), alcohols (hexanol, heptanol, octanol, nonanol, decanol), terpenes (α-pinene, γ-terpinene, linalool, terpinen-4-ol, bornyl acetate), monoaromatic (propylbenzene) and naphthalene components was prepared in hexane (Merck) at a concentration of 10000 mg/L (per component). This mixture was diluted to 100 mg/L (per component) prior to GC and GC × GC analysis. Standard mixtures of the specific analyte classes were also prepared (100 mg/L per component) for verification of individual component retention time data.

2.2. Instrumentation

Single dimension GC and GC × GC experiments were conducted using an Agilent 6890 gas chromatograph (Agilent Technologies, Burwood, Australia). For GC × GC, the GC was retrofitted with a longitudinally modulated cryogenic system (LMCS) from Chromatography Concepts (Doncaster, Australia). The polarity of the first dimension was progressively altered by coupling different lengths of BPX5 (low polarity 5% phenyl methyl polysilphenylene siloxane phase; dimensions of 0.25 mm i.d.; 0.25 μm d_f) and BP20 (polar polyethylene glycol phase; dimensions, 0.25 mm i.d.; 0.25 μm d_f) columns to a constant total length of 20 m. These are indicated in Table 1, with the five columns denoted A–E for increasing polarity (increasing length of BP20 phase).

In all experiments reported here, the BPX5 column was connected to the GC injector. Whilst a small difference arises in relative retentions and hence T_e values, when the order of the columns was swapped, the primary interest in this study was in the effect on T_e when different columns were used, and data for the swapped column arrangement will not be included. Two different ^{2}D columns were used in combination with columns A–E, namely a BPX5 (0.8 m × 0.1 mm i.d.; 0.1 μm d_f), or a BP20 phase column (0.8 m × 0.1 mm i.d.; 0.1 μm d_f). For GC × GC experiments, the LMCS was operated at a modulation period of either 3.5 or 8 s, for the BP20 and BPX5 second dimension, respectively, and the flame ionization detection (FID) system was operated at 270 °C with a data acquisition rate of 100 Hz (or 20 Hz for single dimension GC). Injections (1 μL) were performed at 250 °C using split (10:1) conditions and a constant flow rate of 1 mL/min using hydrogen carrier gas. The GC oven was ramped from 50 to 240 °C at 5 °C, and held at 240 °C for 3 min. Second dimension retention times were ascertained using an in-house Matlab program (L. Xie). Injections of un-retained butane under the particular GC condition were used to determine GC hold up times, and the resulting t_M values were approximately equivalent to the t_M of the first dimension.

3. Results and discussion

3.1. Prediction of the two-dimensional separation

In order to test a method's orthogonality, the test sample must be properly selected to contain substances distributed over the whole range of properties relevant to the method [2], since orthogonality is dependent not only upon the instrumental separation mechanisms, but also on the properties of the solutes and separation conditions [8]. Thus, the 17-component mixture was chosen to contain a range of chemical classes, covering an adequate range of analyte polarities. Wrap-around was avoided, yielding direct comparisons between different column sets. Since all conditions were kept consistent, the use of the separation space can be directly related to the degree of orthogonality between the first and second dimensions.

Isothermal analyses conducted at temperature intervals from 70 to 160 °C using pure BPX5 and BP20 stationary phase (first dimension) columns yielded retention time (t_R) and retention factor (k) data for each compound, at each particular temperature. As the temperature increased, the retention of analytes on each column decreased accordingly, yielding the expected linear plots of log k versus inverse temperature ($1/T$) for each analyte (data not shown here). From these data, the retention of a particular compound at any given temperature can, therefore, be calculated. With respect to the GC × GC experiment, the only parameters that influence the second dimension separation are the carrier gas flow rate and the temperature at which the compounds enter the column. The latter is equivalent to the respective elution temperature (T_e) of the analyte on the first column, since the ^{2}D column operates essentially isothermally [11]. Thus, from ^{1}D elution temperatures, retention of the analytes on the BPX5 and/or BP20 stationary phase second dimension columns can be determined. Given that k is dependent essentially only on temperature, the different column dimensions used to calculate reference k values will still produce data which can be applied to the short ^{2}D columns used in GC × GC.

Temperature-programmed analyses using columns A–E in the first dimension were conducted, and the temperature of elution for each analyte was determined. From isothermal k data, these T_e data may then be used to determine the equivalent second dimension retention for each component on either the BPX5 or BP20 phase, as described above. First dimension retention times, combined with the second dimension retention factors can then be used to predict retention co-ordinates in the two-dimensional separation space.

Accurate prediction of the two-dimensional separation will depend upon the determination of second dimension hold up time, ^2t_M. Whilst the first dimension hold up time can be easily determined (for example by the injection of un-retained butane into the GC system), it is impossible to directly measure the hold up time of the second column directly in GC × GC, when the two columns are connected in series [11]. Beens et al. [11] identified three indirect means to determine ^2t_M. Alternatively, Poiseuille's law can be used to calculate the second dimension inlet pressure, and when combined with the second column dimensions, can be used to calculate ^2t_M. In this investigation, ^2t_M was estimated using experimental two-dimensional separations, and fitting of the predicted retention times on the second column. The estimated ^2t_M value represents an approximation, since ^2t_M will decrease as the GC temperature program progresses. Retention factors on ^{2}D at the prevailing T_e using the single column reference k values permits a common ^2t_M estimate to be obtained from k and ^2t_R values. The ^2t_M values for the BP20 and BPX5 second dimension columns were taken to be 0.55 and 0.50 s, respectively.

3.2. Separations using combined-polarity first dimension columns

The GC × GC 2D contour plots obtained from the coupling of columns A–E with the polar BP20 and low-polarity BPX5 ^{2}D columns are given in Figs. 2 and 3, respectively. Fig. 2 shows a progressive reduction in the utilisation of the separation space as the polarity of the ^{1}D phase increased, and so became more similar to the ^{2}D phase. Coupling of column E (100% BP20) with the BP20 second dimension effectively resulted in a "one-dimensional" separation of the analytes under the specific instrumental conditions used, with compounds distributed along a diagonal (Fig. 2E(i)). A similar result was achieved when column A (100% BPX5) was coupled to the BPX5 second dimension column (Fig. 3A(i)),

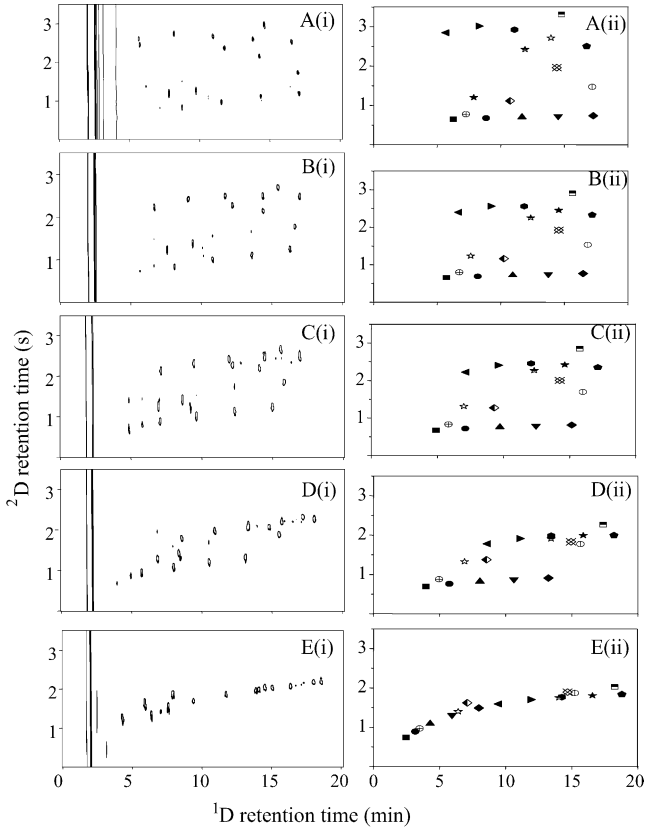

Fig. 2. 2D plots for different first dimension columns, coupled with a BP20 ^{2}D column. Parts (A–E) correspond to column designation according to ^{1}D columns shown in Table 1; (i) refers to experimental data, whilst (ii) refers to simulated 2D result. Key: (■) nonane; (●) decane; (▲) undecane; (▼) dodecane; (♦) tridecane; (◄) hexanol; (►) heptanol; (⬢) octanol; (⋆) nonanol; (⬟) decanol; (⊕) α-pinene; (⬖) γ-terpinene; (★) linalool; (▩) terpinen-4-ol; (Φ) bornyl acetate; (▬) naphthalene; (☆) propylbenzene.

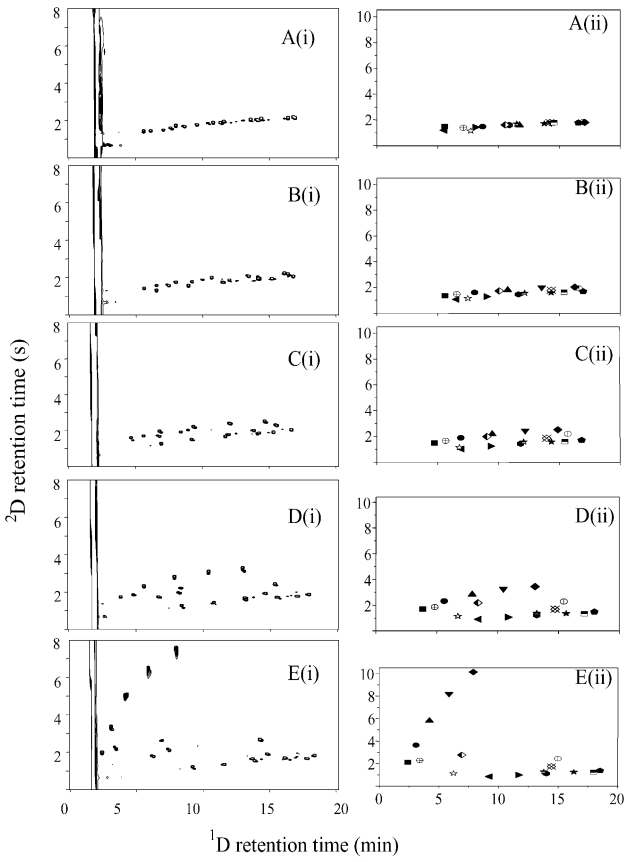

Fig. 3. 2D plots for different first dimension columns, coupled with a BPX5 ^{2}D column. A–E correspond to column designation according to ^{1}D columns shown in Table 1; (i) refers to experimental data, whilst (ii) refers to simulated 2D result. Compound symbols are the same as that used for Fig. 2.

with all analytes falling almost exactly along a single horizon in the 2D space.

Conversely, maximal use of the separation plane was achieved when the first and second dimensions were most disparate, namely column A (lowest polarity ^{1}D) coupled with a BP20 second dimension (highest polarity ^{2}D; Fig. 2A(i)), and column E (highest polarity ^{1}D) coupled with a BPX5 second dimension (lowest polarity ^{2}D; Fig. 3E(i)). The latter combination ensured the longest retention of compounds in the ^{2}D column (Fig. 3E(i)), being that of the alkanes, since alkanes are not strongly retained on the polar first dimension, which results in low analyte elution temperatures (T_e). Because of this, alkanes are then strongly retained on ^{2}D at the prevailing low oven temperature at which they are delivered to ^{2}D. Conversely, alkanes are the earliest eluting solutes in ^{2}D for the low-polarity, polar column set combination (Fig. 2A(i)). This is because on the non-polar first dimension column, alkanes are well retained, so they have a relatively high elution temperature (T_e) and a corresponding low retention on the ^{2}D column.

Figs. 2 and 3 also provide the corresponding simulated 2D chromatograms for each of the respective column set combinations. These plots were prepared by plotting the retention times of the solutes, with their predicted ^2t_R values, based on k data generated from single column isothermal experiments and estimated ^2t_M. In general, the simulated 2D plots bear close similarity to the experimental data, and Table 2 compares the simulated and experimental second dimension retention data for column A coupled to the BP20 second dimension. Predicted second dimension retention times were within 0.47 s, respectively, of the experimental values, which compares favourably with differences reported by Vogt et al. [12] for prediction of retention data in GC × GC separations. In the present simulation strategy, actual ^{1}D elution time is used to plot simulated peak position. As a more general approach, linear temperature-program retention index calculation may be used along with temperature-dependent retention time for prediction of a solute's elution time/temperature, followed by reference to k values as above for ^2t_R estimation. The value of such prediction is readily apparent, since with a suitably large database it will be possible to simulate any column set combination (for which reference data are available) to test various column sets for the ability to provide required GC × GC separation performance. Combined with efficiency estimation for each column, not only can peak position be derived, but also solute resolution in the 2D plot.

Table 2
Comparison of simulated and experimental retention data for column A ^{1}D and a BP20 phase ^{2}D

Compound	Experimental ^1t_R (min)	Simulated ^2t_R (s)	Experimental ^2t_R (s)	Difference ^2t_R(s)[a]
Nonane	6.18	0.65	0.73	−0.08
Decane	8.75	0.68	0.84	−0.16
Undecane	11.55	0.70	0.98	−0.28
Dodecane	14.35	0.72	1.14	−0.42
Tridecane	17.04	0.74	1.21	−0.47
Hexanol	5.72	2.85	2.46	0.39
Heptanol	8.13	3.02	2.73	0.29
Octanol	10.91	2.92	2.68	0.24
Nonanol	13.75	2.72	2.60	0.12
Decanol	16.52	2.50	2.54	−0.04
α-Pinene	7.18	0.78	0.80	−0.02
γ-Terpinene	10.62	1.11	1.10	0.01
Linalool	11.74	2.43	2.38	0.05
Terpinen-4-ol	14.21	1.96	2.15	−0.19
Bornyl acetate	16.96	1.47	1.73	−0.26
Naphthalene	14.58	3.32	2.98	0.34
Propylbenzene	7.81	1.20	1.19	0.01

[a] Difference ^2t_R values were generated by subtracting experimental ^2t_R values from respective simulated ^2t_R values.

It is interesting to note that both experimental and simulated retention data for the low-polarity BPX5 ^{2}D column show the most dramatic change when ^{1}D is changed from column D to column E (refer to Table 1), resulting in rather large ^2t_R values in the latter. Whilst not tried here, it may be that a combined-polarity ^{1}D column arrangement of, for example, 18 m BP20 + 2 m BPX5 could be useful for certain applications where non-polar compounds are of interest, so that their ^{1}D elution temperatures are not so low that they have excessively long ^2t_R values. Clearly, many strategies can be proposed in GC × GC to obtain optimum analysis of a given sample type.

Fig. 4 shows the resultant specific component shifts with different ^{1}D phase compositions A–E, with a BP20 (Fig. 4A) and BPX5 (Fig. 4B) ^{2}D phase, respectively. As the polarity of the first dimension was increased (from column A to E), the first dimension retention, and so the temperature of elution, of bornyl acetate and tridecane was found to decrease, whilst that of hexanol increased. Thus, because ^2t_R is inversely related to T_e, the ^2t_R of bornyl acetate and tridecane increased, whilst that of hexanol decreased from column A to E. Polarity of the first dimension was found to greatly affect analyte elution temperatures, which ultimately determines second dimension retention and the overall use of the two-dimensional separation space. Nevertheless, the choice of second dimension stationary phase was critical. The BP20 ^{2}D phase ensures the elution of all analytes within 3.5 s, whilst that of the BPX5 phase required a significantly longer modulation period due to the large ^2t_R elution time of 10.5 s for tridecane, when column E was used. It is unlikely that such a long modulation period would be used for routine analysis, since this risks under-sampling of the peaks, although faster sampling then would exacerbate wrap-around. Therefore, use of a column set that leads to excessive ^2t_R elution time would be unadvisable. The range of ^{2}D retention times for the three different polarity analytes on ^{1}D columns A–E were 0.75, 0.39 and 1.26 s for tridecane, bornyl acetate and hexanol, respectively, for the BP20 ^{2}D column, whilst a range of 8.33, 0.62 and 0.34 s, respectively, was found with the BPX5 ^{2}D column. Clearly the polarity of the analyte will play a major role in

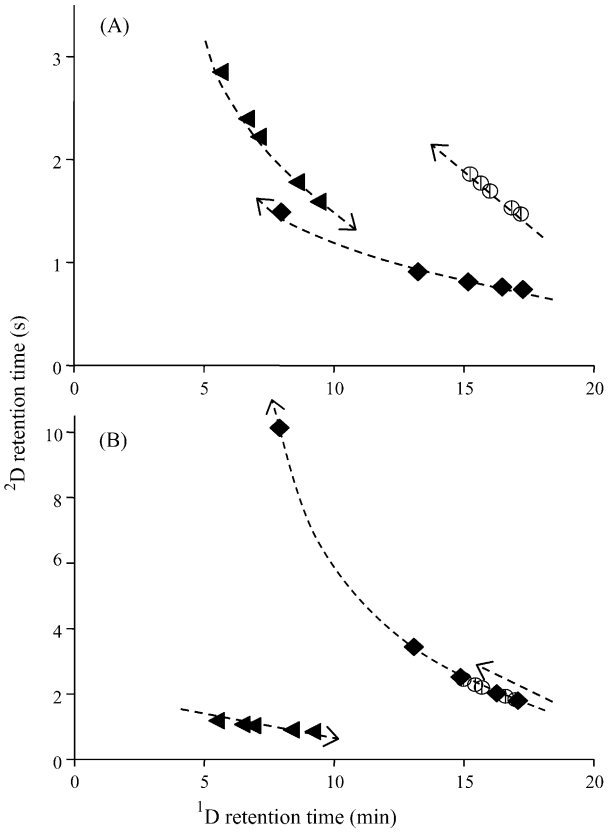

Fig. 4. Effect of specific relative component shifts for different first dimension phase compositions, with ^{2}D columns of (A) BP20 and (B) BPX5. Arrows show the trend in position of solutes progressing from column A to E. Triangles, hexanol; circles, bornyl acetate; diamonds, tridecane.

determining its retention in the second dimension, and so the GC × GC column set must be carefully selected with respect to sample composition.

3.3. Use of separation space

Figs. 2 and 3 demonstrate the changing spread of solutes within the separation space. In terms of orthogonality, it is the vertical differentiation that is of interest. Normalisation of data based on ^2t_M should be considered. Alternatively, this could also be done based on the ^{2}D elution of the least retained solute in the sample. For a polar ^{2}D phase (see Fig. 2), this will be alkanes; for a low-polarity ^{2}D phase (see Fig. 3), this will be a polar solute class; and in this example, the alcohols are least retained. As demonstrated in Fig. 1, the amount of separation space used can be calculated based on the area between the boundaries imposed by ^2t_M and the most retained species. Taking the example of Fig. 2A–E, the amount of separation space used, defined by the least-to-most retained solutes, as a ratio of the area under the void time will be 1.6, 1.2, 1.1, 0.7, 0.1. For Fig. 3A–E, the respective data are 0.15, 0.31, 0.50, 1.2 and 4.1. Because a ratio value has been calculated, the different column sets can be directly compared, regardless of P_M.

From this, we may, therefore, deduce that the column set used for Fig. 3E is the most orthogonal. Examination of Fig. 3E shows the upper boundary defined by the elution of the non-polar alkanes. The more polar analytes elute over a significantly smaller ^{2}D retention range, equivalent to a separation space usage of 1.0, when the upper boundary is attributable to terpene elution. This indicates that utilisation of the separation space is not as significant as the alkane retention suggests. The described method used to calculate the utilisation of the two-dimensional space is an open-ended scale, since it is simply a measure of two areas. Once the maximum retention is defined, however, for a given series of columns, it is possible to normalise the data to this maximum, for instance where the maximum area ratio is set to 1.00. This measure also accommodates wrap-around, provided that the extent of wrap-around and the proper separation space is measured.

4. Conclusion

The systematic variation in ^{1}D polarity, through use of binary coupled columns of widely differing nature, permitted demonstration of the extent of separation achieved for a test sample on different column sets. Reference data of retention factor, *k*, versus temperature on pure phase single columns allowed reasonable prediction of the GC × GC result, where solute elution temperature from ^{1}D is used as the primary variable. This method should allow simulation of GC × GC separations, including for novel column sets, provided the requisite primary retention data on each column are available.

This investigation concluded that ^2t_R elution time in the GC × GC experiment is controlled by both the polarity of the ^{2}D phase and the temperature at which the solute is delivered to ^{2}D (equivalent to the solute ^{1}D T_e). Correlated phases, arising most clearly for similar ^{1}D and ^{2}D polarity phases, can be considered to be those where the elution of solutes in the first dimension results in a very limited elution time range in the second dimension, as illustrated in Figs. 2E and 3A. Conversely, most differentiation in the second dimension for compounds of different chemical classes can be achieved when ^{1}D and ^{2}D column phases are most dissimilar. Utilisation of the 2D separation space can, therefore, be interpreted with respect to column orthogonality, with the latter instance representing the most ‘orthogonal’ separation. In this investigation, orthogonality was contrasted for a range of column sets by estimation of the amount of separation space used for retained solutes, compared with the void space. This is a relatively simple estimator, independent of ^{2}D length, which needs to be tested for a wider range of applications.

Acknowledgement

The authors would like to thank Robert Shellie for valuable input into the initial planning of the investigations.

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