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Influence of chromatographic conditions on separation in comprehensive gas chromatography

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Abstract

Comprehensive gas chromatography (GC×GC) is now established as a powerful technique, which offers unprecedented separation power. For complex samples, the distribution of peaks in the two-dimensional (2D) space still may need to be optimised. Since temperature (T) is a critical variable, and compounds can be shifted in relative positions on column 1 arising from temperature program rate (r_T) changes, and since retention in the second dimension, D2 (^2t_R) is likewise affected by the prevailing T (elution temperature; T_e), then any factors which alter T_e will affect the extent of separation in D2. Since temperature program and carrier gas velocity rate will affect the T_e of the solutes, these two factors are considered in this paper. Apart from these two parameters, results of different stationary phase choice for the second dimension column as well as the second dimension column length are reported. r_T is found to have the most profound impact on the T_e of solutes and will be more likely to cause an inversion of elution order if such behaviour can occur. Peak widths and ^2t_R increase with a decrease in T_e. On the other hand, flow-rate has less impact on peak widths and ^2t_R although T_e is affected by a change in flow-rate. Specific solute–stationary phase interactions will cause the elution order of certain solutes to be altered, and may be observed when a different stationary phase is employed as the second column, depending on the solute–stationary phase separation mechanism. Experiments conducted on different second dimension column length showed that although a longer column will lead to better separations, wrap-around may confound the separation process and may cause the solutes from sequential modulation events to co-elute. Thus a suitable second dimension column phase and length must be employed in order to obtain good separation. The factors investigated in this study will cause different extents of changes in the solute elution order and solute separations, and will affect the 2D contour presentation. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Gas chromatography, comprehensive two-dimensional; Elution order; Temperature programming; Flow rate; Stationary phases, GC; Column length; Pesticides; Organochlorine compounds; Tridecane; Benzenes; Phenols; Naphthalenes; Amines; Phthalates

1. Introduction

The comprehensive two-dimensional gas chromatography (GC×GC) technique is just over a decade old [1], and in this time has demonstrated its relevance to high resolution separation capabilities.

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The majority of GC×GC studies have focused on practical applications. The fundamental concepts of comprehensive gas chromatography are based on familiar GC principles, and it should be instructive to extend such understanding to the appreciation of relationships between experimental conditions and the resulting separation in GC×GC. Rather than attempt to reduce the observations to a theoretical model on the prediction of the elution order of components, the present interpretation will identify the trends and develop considerations that will be readily appreciated by users of GC×GC and aid in understanding of how experimental settings affect the resultant two-dimensional (2D) separation.

A study representative of earlier reports on the effect of temperature on elution order of solutes in GC was by Yabumoto et al. in 1977 [2]. Subsequent work discussed the influence and effects of analytical conditions on the elution order of analytes [3–8]. Thus it is well recognised that relative component elution may be affected by temperature, and temperature program used. The temperature dependency of relative retention is one of the reasons that accounts for irreproducibility of chromatograms, especially in fingerprinting [9] and in the determination of ''column equivalency'' [5].

In gas chromatography, the elution order of analytes is governed by several factors [5] such as latent vapour pressures, solubilities in stationary phase, and propensities for molecular interaction in the stationary phase. All of these effects change with temperature and their concerted effect ultimately determine the equilibrium distribution of solute molecules between the mobile and stationary phase. In comprehensive gas chromatography, the retention principles are the same as for one-dimensional gas chromatography. Bertsch published two reviews on multidimensional and comprehensive gas chromatography, which provides an excellent reference to the principles of GC×GC [10,11]. The control of relative retention by temperature variation in serially coupled columns was reported as early as 1978 in a short communication paper, as a simplified method to improve the resolution of a multi-component mixture [12]. Comprehensive two-dimensional GC utilises columns coupled in series, where a common phase selection strategy uses a first dimension separation of analytes according to boiling point, whilst the second dimension separates analytes according to polarity. The difference between the two serially-coupled columns is intended to provide separation orthogonality, which leads to increased component resolution where two unresolved compounds in the first dimension have sufficiently different retention factors on column 2 phase at the given oven temperature to lead to their resolution. An orthogonal separation in GC×GC connotes that solutes are subjected to two separation mechanisms that are independent of each other. Orthogonality in GC×GC is critical because it efficiently utilises the separation space. The total peak capacity is the product of capacities on each column, which is greater than the peak capacity for a one-dimensional separation system. Furthermore an added separation dimension will aid in the identification of the solutes. If the analytical conditions change, in particular the oven temperature, solutes will redistribute within the retention plane. A detailed discussion on separation orthogonality in GC×GC was reported by Venkatramani et al. [13]. A review by Phillips and Beens presented an example on the effects of temperature program on the order or structure of the 2D chromatogram [14]. Most sample analyses in GC×GC are performed under linear temperature program condition, and so ''peak shifting'' resulting from the program rate is expected to occur primarily on column 1; the column 2 temperature is essentially isothermal for a given single modulation elution due to fast elution on column 2. Hence if the temperature dependency of relative retentions of components are known from prior isothermal studies, it would be possible to make a good estimation of how changes in temperature programming may affect second dimension absolute and relative retention.

It should be noted that maximum utility of the separation space depends on the chemical differences between compounds and the ability of the columns to effectively discriminate components (i.e., give sufficiently different retentions) based on these chemical properties. It is possible, however, that no available capillary GC column phase possesses the requisite interaction mechanism that would provide ''ideal'' orthogonality.

1.1. Effect of analytical conditions on the elution order of analytes

The retention or elution temperature T_e in tem-

perature programmed analysis of a compound is defined as the oven temperature at the elution time of the peak maximum under a particular set of conditions [3]. For a given column it is influenced by both the program rate and the carrier gas velocity, and parameters such as column dimensions, stationary phase type and film thickness will cause T_e to vary from column to column. Column temperature has a profound influence on component retention and separation. At a given isothermal temperature operation compounds will not change their elution order if other GC conditions are altered. For temperature programmed operation, analytes may change their relative positions as the temperature changes while they traverse the column. Reversal in elution order of compounds will more likely be observed for solute mixtures of chemically different compounds, and would be less likely, for instance, when mixtures of homologues are studied.

Temperature programming conditions will cause a continuous, monotonic change in the distribution constant for each analyte (refer to Eq. (1)):

$$\log K_D = A/T + B \tag{1}$$

where A and B are experimental constants. Because the distribution constant is directly proportional to the capacity factor, retention time will decrease with increasing temperature.

As the temperature increases, the distribution constants ($K_D = C_S/C_M$; ratio of component concentrations in stationary and mobile phases, respectively) of the analytes become smaller (increase in the relative C_M of analytes), and their rates of movement through the column are accelerated. The time spent in the column will be affected by the rate of temperature programming r_T, average carrier gas velocity $\bar{u}$, column length L and phase ratio β. Changes in any of these parameters may cause changes in the elution order of any susceptible components of a sample. Obviously the dispersion and resolution of those components will also be affected in a manner that might be unpredictable in the absence of chemical information which allows component–stationary phase interaction energies to be modelled. Compounds with equal K_D at one temperature may have inequal K_D values at other temperatures, since individual interactions that yield the global partitioning behaviour will not necessarily be the same.

The distribution constant K_D relates retention and column phase ratio:

$$K_D = k\beta \tag{2}$$

where $\beta = r/2d_f$ (r = column radius, d_f = film thickness), and k = retention factor (t'_R/t_M).

Thus this becomes Eq. (3):

$$K_D = \left[\frac{r}{2d_f}\right] \cdot \left[\frac{t'_R}{t_M}\right] \tag{3}$$

and since $t_M = L/\bar{u}$ ($\bar{u}$ = average linear carrier gas velocity) Eq. (3) can be rearranged as below:

$$t'_R = K_D \cdot \left[\frac{2d_f L}{r\bar{u}}\right] \tag{4}$$

From Eq. (4), the factors that affect T_e and t_R can be summarised as follows: K_D changes with temperature and thus is affected by the initial (T_i) and final temperature (T_f) as well as the r_T. During temperature programming, K_D changes in a reproducible manner, according to Eq. (4). It is possible to make the following statements (keeping other factors constant): d_f; a thinner stationary phase film thickness, reduces t_R and T_e. L; a shorter column will reduce t_R and T_e. r; if all other factors are constant, r should not affect T_e. $\bar{u}$; is inversely proportional to the analysis time. If $\bar{u}$ is increased, T_e will decrease. r_T; as the temperature program rate increases, T_e will increase. Thus compounds will be delivered to D2 at higher temperature, which reduces their ${}^2t_R$ value, and may affect resolution either due to dispersion effects or to changes in relative partitioning mechanisms.

Eq. (5) reports the optimum temperature program rate with respect to L, $\bar{u}$ and β. These are derived for a single column analysis, and will need to be re-evaluated for GC×GC analysis.

$$r_{T,opt} \propto \frac{\bar{u}\beta}{L} \tag{5}$$

Consider now the interplay of settings in the GC×GC experiment: a narrower column has higher $\bar{u}$ if the same carrier pressure is used, this reduces ${}^2t_R$; higher $\bar{u}$ may give lower efficiency if it exceeds greatly the minimum in the van Deemter plot even though narrower columns have inherently greater efficiency (N is inverse to column inner diameter).

Using hydrogen minimises the efficiency loss arising from higher $\bar{u}$. If constant pressure operation is used, at higher temperatures in temperature program analysis the flow-rate will decrease, which in turn may cause related compounds to elute with greater ${}^{2}t_{R}$ in the 2D space.

A change in the temperature will affect the solute–stationary phase interactions which in turn will influence solute volatility and more importantly in the case of comprehensive gas chromatography (because of the need to invoke orthogonal conditions), apparent solute polarity. Most analyses in GC×GC use temperature program operation. If compounds are chromatographed isothermally, or if the temperature program reaches its upper limit and is then held isothermal, any solutes eluted during this period will have an increasingly greater ${}^{2}t_{R}$ value compared with what they would have under temperature programmed conditions. This can cause a dramatic increase in peak widths in first and second dimensions, and wrap-around may become significant. Wrap-around is observed when the second dimension retention time for an analyte exceeds the modulation period. Data are transformed into matrix format based on this period, and so the analyte peak will be located at an apparent position on the 2D plane of ${}^{2}t_{R}$ = [actual retention time − n(modulation period)], where n = number of wrap-arounds.

The mechanism(s) which cause elution order changes in single column GC must be operative in GC×GC analysis, and hence peak shifting behaviour and other observations should be taken into consideration in the specific case of the GC×GC experiment.

This paper will demonstrate basic studies to aid comprehension of peak order shifting in GC×GC and interpretation of 2D plots which exhibit this effect, arising from the added separation dimension.

2. Experimental

2.1. Samples

n-Tridecane was purchased from Aldrich (analytical-reagent grade; Milwaukee, WI, USA) and organochlorine pesticide and semi-volatile aromatics mixtures were from Ultra Scientific (Kingston, RI, USA; part numbers M-8080 and SVM-124-1, respectively). The components in these mixtures are identified in Table 1. One of these samples is similar to that chosen by Hinshaw [8] in his discussion of single column GC operating principles, which is not unlike the study investigated here. These standards will be used for system optimisation when studying samples containing these sorts of compound classes, so were deemed suitable to use here rather than use a contrived sample.

2.2. Gas chromatography system

An Agilent 6890GC with Chemstation software (Agilent Technologies, Burwood, Australia) was used with a flame ionisation detector at 250 °C and data acquired at a frequency of 100 Hz. Split injections were made at 240 °C throughout the study, with a split ratio of ca. 20:1–50:1 unless otherwise stated.

2.3. Column sets and GC conditions

Several column sets were used for the studies. A BPX5 (polysilphenylene siloxane phase, 5% phenyl equivalent; non-polar) with column dimensions of 30 m×0.25 mm I.D., 0.25 μm d_f was used as the primary column for most experiments, except for the column length study, when a 25 m length of the same column was used. For the column length study, a 5.5 m×0.1 mm I.D., 0.2 μm d_f BPX50-coated column (polysilphenylene siloxane phase, 50% phenyl equivalent; polar) was directly connected to the primary column. For the other studies, various second columns were directly coupled to the primary column: (i) 1.2 m×0.1 mm I.D., 0.1 μm d_f BPX50 coated column; (ii) 0.8 m×0.1 mm I.D., 0.1 μm d_f HT8 coated column (phenyl carborane phase); (iii) 0.8 m×0.1 mm I.D., 0.1 μm d_f LC-50 coated column (liquid crystalline phase); and (iv) 0.8 m×0.1 mm I.D., 0.1 μm d_f BP20 (polyethylene glycol; polar). Apart from the LC-50 column (from J&K Environmental, Canada), all columns were from SGE International (Ringwood, Australia). The GC oven was operated using different analytical conditions depending on the objective of the particular study. Both isothermal and temperature program conditions were employed as required. The initial temperature

Table 1
Constituents of mixtures M-8080 and SVM-124-1

M-8080	SVM-124-1
(A) α-Hexachlorocyclohexane	(1) Hexachloropentadiene
(B) γ-Hexachlorocyclohexane	(2) 1,2,4,5-Tetrachlorobenzene
(C) β-Hexachlorocyclohexane	(3) 2,4,6-Trichlorophenol
(D) δ-Hexachlorocyclohexane	(4) 2,4,5-Trichlorophenol
(E) Heptachlor	(5) 2-Chloronaphthalene
(F) Aldrin	(6) 1-Chloronaphthalene
(G) Heptachlor epoxide	(7) 2-Nitroaniline
(H) Endosulfan I	(8) Acenaphthylene
(I) Dieldrin	(9) Dimethyl phthalate
(J) *p,p′*-DDE	(10) 2,6-Dinitrotoluene
(K) Endrin	(11) Acenaphthene
(L) *p,p′*-DDD	(12) 3-Nitroaniline
(M) Endosulfan II	(13) Pentachlorobenzene
(N) Endrin aldehyde	(14) 2,4-Dinitrophenol
(O) *p,p′*-DDT	(15) Dibenzofuran
(P) Endosulfan sulfate	(16) 4-Nitrophenol
(Q) Methoxychlor	(17) 2,4-Dinitrotoluene
	(18) 1-Naphthylamine
	(19) 2,3,4,6-Tetrachlorophenol
	(20) 2-Naphthylamine
	(21) Fluorene
	(22) Diethyl phthalate
	(23) 4-Chlorophenyl phenyl ether
	(24) 4-Nitroaniline

was set at 60 °C with a column head pressure of 25 p.s.i. (1 p.s.i. = 6894.76 Pa). Temperature program rates varied from 1.5 to 16 °C/min and flow-rates ranged from 0.8 to 4.0 ml/min, depending on the study. The semi-volatile aromatic mixture was analysed using a temperature program of 40 °C (hold 1 min) heat to 150 °C at 20 °C/min, then to 230 °C at 2 °C/min on column set (i). All analyses were conducted using the constant flow mode.

2.4. Cryogenic modulator

The GC×GC experiment is achieved here by use of a cryogenic modulator, which has been described elsewhere [15]. The present unit, the longitudinally modulated cryogenic system (LMCS) Everest model, was from Chromatography Concepts (Doncaster, Australia). The cryogenic system was retrofitted to the 6890GC. It was operated under various modulation periods from 3 to 5 s cycle time and 0.5 s hold time in the release position. The CO_2 cryogen coolant maintains the temperature of the modulation trap at least 100 °C below the prevailing oven temperature.

The Chemstation event control instructs the modulation system to commence modulation at a precise time, at a fixed modulation frequency. Data are converted and displayed as described elsewhere [15].

2.5. Effects of column length on the separation of analytes

The relationship between the column length and ${}^{2}t_{R}$ was investigated by using the column set listed in Section 2.3, and with a set temperature program and flow-rate.

The ${}^{2}t_{R}$ for *n*-tridecane was determined by employing the targeted mode of the LMCS. The solute (tridecane) is trapped and held in the cryotrap until 7 min, and then the cryotrap is moved to inject solute to D2. The targeted mode allows the cryogenic trap to selectively ‘‘capture’’ solute and pulse the band to the second dimension column at a predefined time. In this way, ${}^{2}t_{R}$ can be accurately determined because the exact time for introduction of the solutes to D2 is

known. By subtracting the introduction time from the total analysis run, we can accurately calculate the time for the solutes to elute from the second dimension column. Details of the targeted mode have been discussed recently, for the analysis of faecal sterols [16].

The experiment is briefly explained as follows. The second dimension column (5.5 m long) is connected to the first column by a union, then passes through the cryogenic trap and is fitted to the detector. The effective second column length between trap and detector can be adjusted by pulling the column through the trap resulting in a decrease in the column length from the union to the trap, and an increase in length from the trap to detector. Effective column lengths of 0.5, 1.2, 2.2, 3.2, 4.2 and 5.2 m were used in this study.

3. Results and discussion

3.1. Effect of column temperature on the elution order of analytes

The order of elution of solutes is a complex function of relative interactions between solutes and the stationary phase. These interactions will usually be quite sensitive to temperature variation. Where two or more compounds have similar retention time, but different sensitivities to temperature differences then elution order variation may arise depending on the change in the chromatographic conditions. Thus isomers will usually not show significant variations in relative retentions, but compounds of grossly different chemical class will be more likely to exhibit relative retention variations.

Fig. 1 shows normal gas chromatograms of the mixture using different temperature programming rates and a BPX5–BPX50 column set (without the cryogen applied). Among the 17 components, the following pairs of components, B and C, I and J, L and M and O and P exhibit reversal in elution order over the range of temperature ramp rates used.

The same mixture was analysed using the GC×GC technique (now with cryogen applied) with a modulation period of 3 s under the same four r_T settings, and contour plot presentation of the full GC×GC two dimensional separation space is shown in Fig. 2a, with approximate alignment of elution range from A to Q. Fig. 2b is an expanded section of the contour plot which illustrates clearly the relative shift of solutes in D1 and D2. The changes in first column retention (^1t_R) reflect those seen for GC operation in Fig. 1. A decrease in the temperature program rate will cause the solutes to be discharged from D1 at lower temperatures. The effects of temperature ramp rates on T_e and peak widths are summarised in Table 2, for solutes O and P as typical components. At higher ramp rate, provided that all other analytical conditions remain the same, the solutes will elute earlier but the solutes' T_e will increase. T_e varies by about 80 °C over the r_T range used for the study (Table 2).

The increase in peak widths due to slower temperature programming is observed in both normal gas chromatography and for comprehensive gas chromatography pulsed peaks. The solutes tend to overload when they elute later, at a lower temperature [17] (see the peak asymmetry values in Table 2). Fig. 2c shows the relationship between T_e, ^1t_R and ^2t_R for solutes E and N for different ramp rates, demonstrating that higher T_e means solutes reach D2 column at higher temperatures and so will be expected to have a decreased ^2t_R, and possibly lower resolution (R_s)—but R_s depends more on the effect of temperature on the retention mechanism and magnitude of interaction of each solute on the D2 column. The trend is the same for ^1t_R and ^2t_R. The absolute ^2t_R and hence wrap-arounds are calculated by comparing the modulated results using two different modulation periods. The shift in the relative positions of solutes on the second dimension is better illustrated by Fig. 2d, again for solutes O and P. As r_T increases, solutes O and P have a smaller ^2t_R difference. Since O and P swap ^1t_R positions, Fig. 2e shows their relative elutions by fixing the position of solute O and plotting P according to the corrected ^1t_R time (taking account of different r_T settings) and absolute ^2t_R difference. Thus at lower r_T P will progressively elute earlier than O on D1, and will have a later elution on D2. Similar trends may be plotted for other solute pairs.

The same sample was also analysed using the BPX5–LC-50 column set. The partial contour plots in Fig. 3 shows that solutes O and P again cross-over in the first dimension, which is determined by the

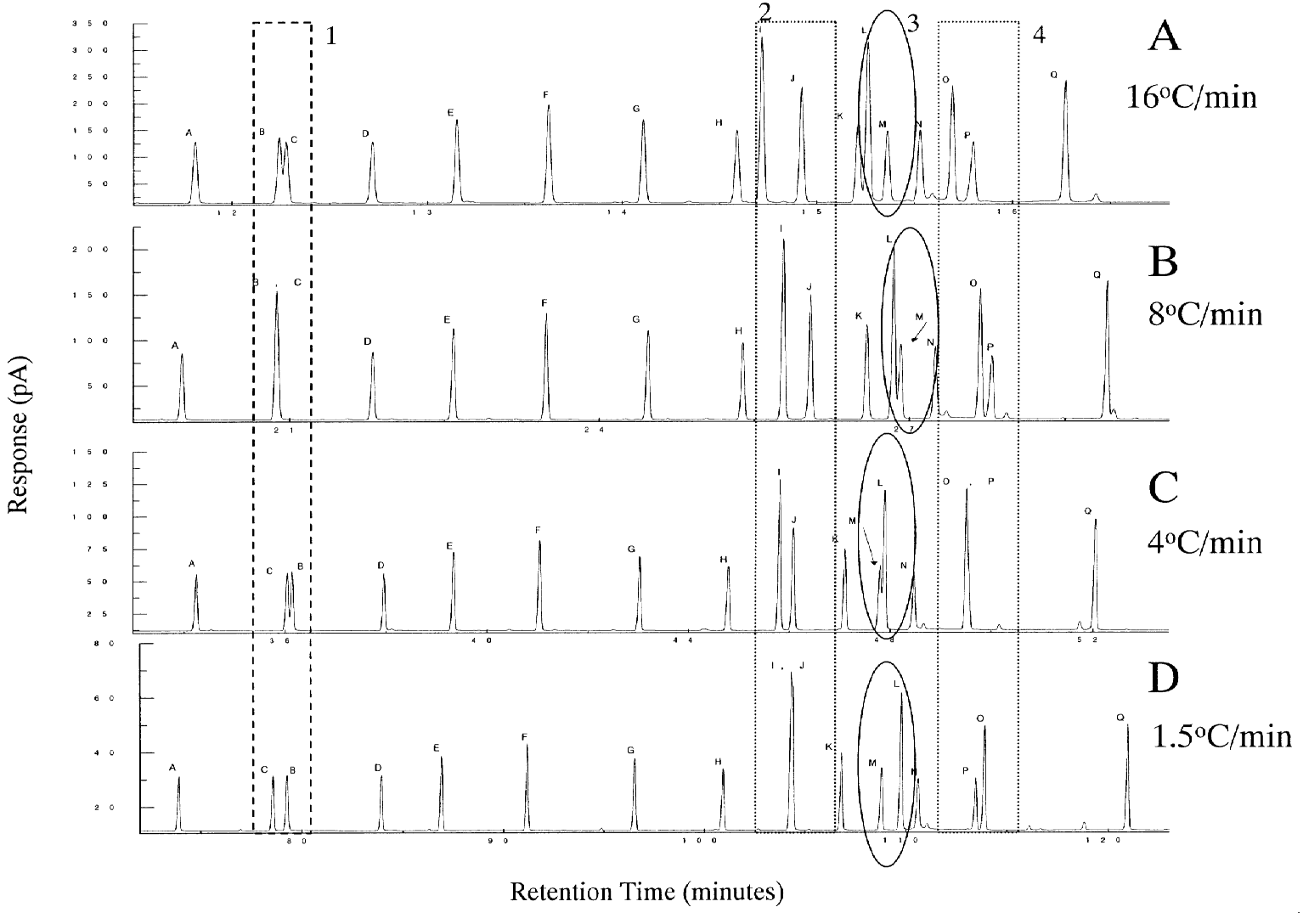

Fig. 1. Effect of temperature programming rate on the elution order of organochlorine pesticides (refer to Table 1) in normal GC using the BPX5–BPX50 column set. Temperature programming rates (°C/min): A=16, B=8, C=4, D=1.5. Solute pairs B and C, I and J, L and M and O and P showed elution reversal on D1.

BPX5 column common to both column sets. There is no cross-over in the second dimension. Due to the low temperature limit of the liquid crystal column, the highest temperature program rate was restricted to 8 °C/min otherwise the solutes would elute beyond the temperature limit of the column. The effect of different stationary phase as the second column is discussed further below. However, in no instances were solutes found to swap relative positions on D2 over the r_T range investigated.

3.2. Effect of flow-rate on the elution order of analytes

Here, two effects are in play. Temperature programming is used, and as $\bar{u}$ increases, T_e decreases, and so contradictory effects arise. The higher $\bar{u}$ causes ${}^2t_R$ to decrease, but resultant lower T_e will counteract the effect on ${}^2t_R$, tending to increase ${}^2t_R$.

Similar peak reversals as those observed for r_T variation occur when the same mixture was analysed using different flow-rates (at a set r_T), although the effect of gas velocity on the elution order is less pronounced compared to the effect of temperature programming rate change. Fig. 4a shows the region where an example of the reversal in elution order between components O and P is observed in the first dimension. Again these solutes did not reverse their elution order in the second dimension. There are also more subtle differences in peak widths and peak broadening, as tabulated in Table 3. The efficiency realised by the second column is between 2500 and 6000 plates/m.

The relationship between the elution temperature and ${}^1t_R$ as well as ${}^2t_R$ is presented in Fig. 4b for

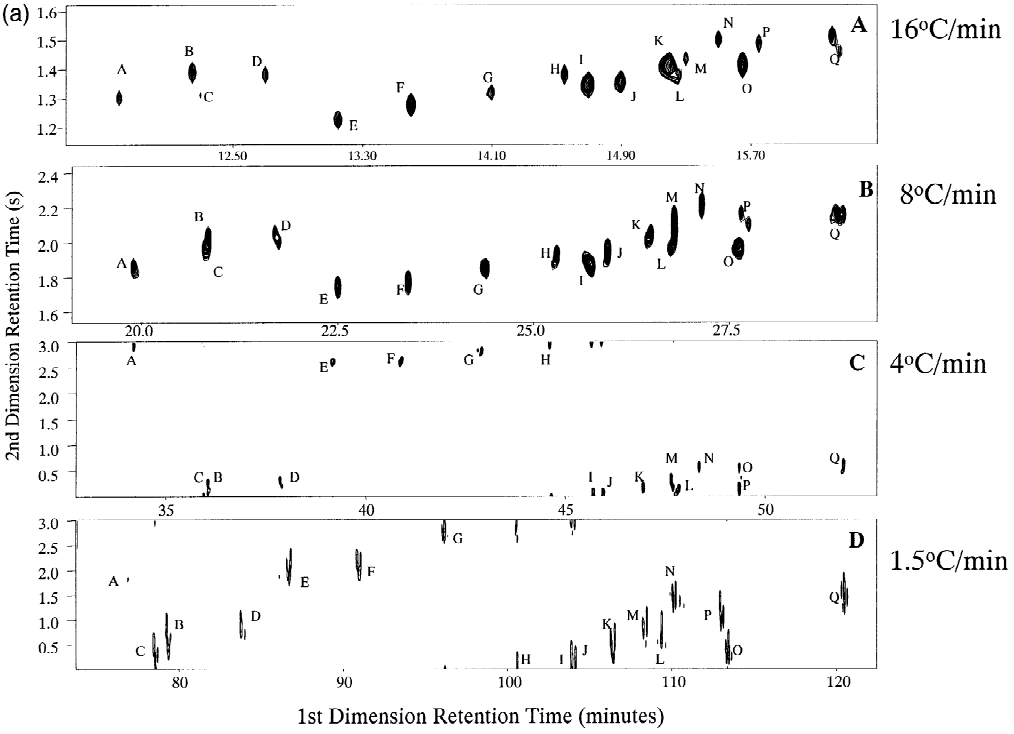

Fig. 2. (a) Contour plot of a GC×GC experiment analogous to the GC experiment described in Fig. 1. Modulation period 3 s. The plots are approximately vertically aligned. (b) Expanded section of (a) from solute I to solute P, illustrating the relative elution orders for solutes L and M and solutes O and P in both dimensions. Data are reported in Table 2 for solutes O and P. (c) Plot of temperature ramp rates (r_T) against first dimension (${}^1t_R$) and second dimension (${}^2t_R$) retention time for GC×GC and GC operation as indicated. Elution temperature (T_e) values for solutes E and N are also shown. (d) Plot of r_T against the absolute ${}^2t_R$ for solutes O and P. (e) Graph of the relative elution of solute P with respect to solute O for different r_T settings. The ${}^1t_R$ values have been corrected by multiplying the actual time difference by the program rate. $\Delta{}^2t_R$ is the difference between ${}^2t_R$ values for P and O.

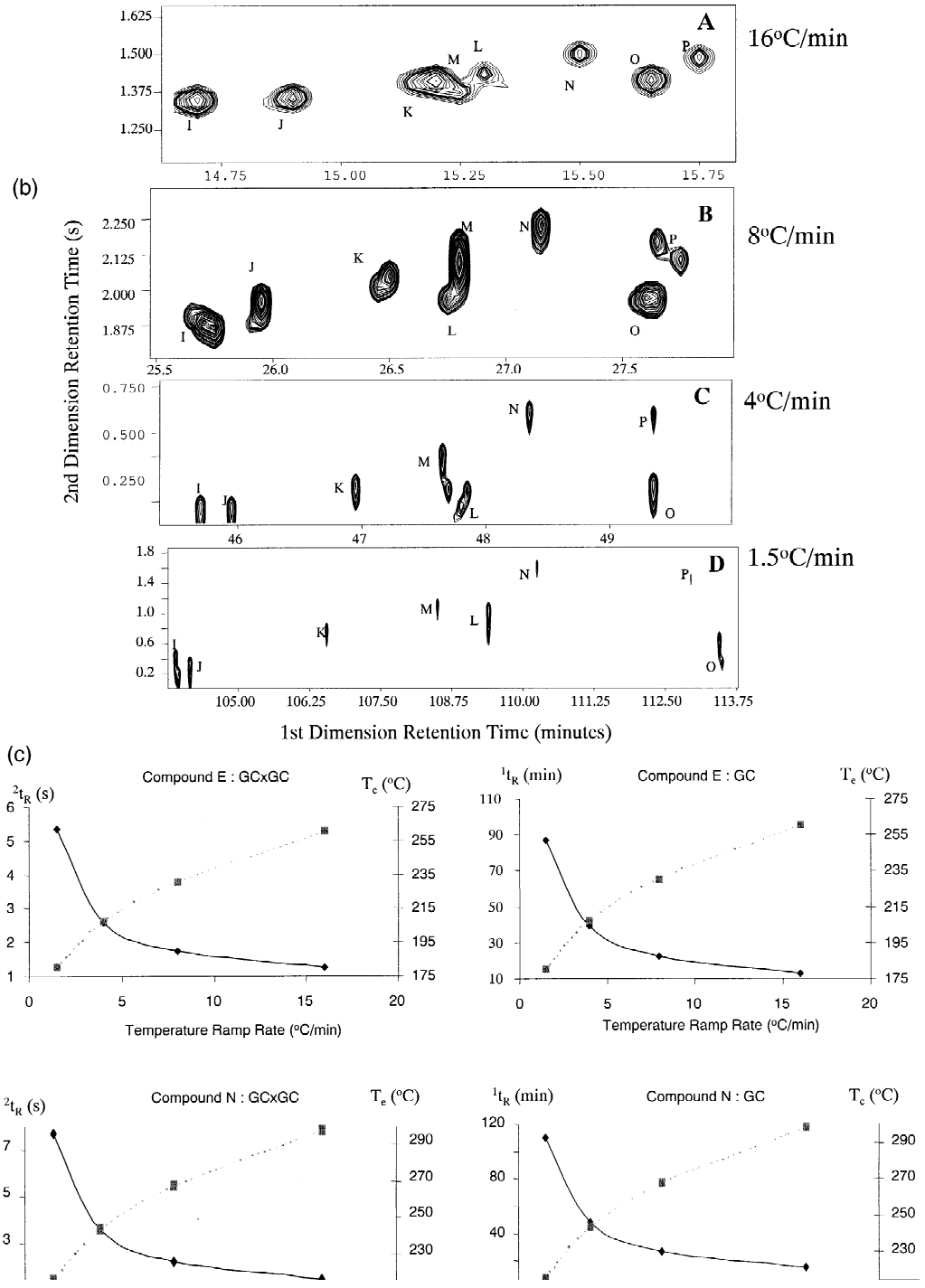

Fig. 2. (*continued*)

Table 2
Peak parameters and elution temperature for solutes O and P using different temperature program rates (Fig. 2b)

r_T (°C)	t_R (min)[a]	^2t_R (s)	T_e (°C)	$w_{0.5}$ (ms)[a]	w_b (ms)[a]	A_s[b]
Peak P: GC×GC						
1.5	113.01	7.43	219.5	264	441	0.40
4.0	49.36	3.53	247.4	177	327	0.23
8.0	27.78	2.11	272.2	87	153	1.08
16.0	15.77	1.49	302.4	73	142	0.81
Peak O: GC×GC						
1.5	113.4	6.66	220.1	229	406	0.21
4.0	49.35	3.14	247.4	171	287	0.29
8.0	27.63	1.99	271.0	70	141	1.00
16.0	15.67	1.41	300.7	71	129	0.94
Peak P: GC			(s)	(s)		
1.5	113.47		220.2	8.36	15.63	0.36
4.0	49.57		248.2	–	–	–
8.0	27.79		272.3	2.38	4.17	0.50
16.0	15.80		302.8	1.63	3.06	0.55
Peak O: GC						
1.5	113.90		220.8	7.89	14.37	0.39
4.0	49.52		248.0	3.76	7.02	0.55
8.0	27.68		271.5	2.11	3.72	0.72
16.0	15.69		301.1	1.36	2.49	0.79

[a] t_R for GC×GC is for the major pulsed peak, $w_{0.5}$ is the peak width at half height, w_b is the peak width at base.
[b] Asymmetry (A_s) values for GC×GC data given for the major pulsed peak.

understood that a 15 °C change in temperature results in change of K_D of about a factor of 2. This can be applied to these results as follows.

For solute F (Fig. 4b), T_e changes from 230 to 195 °C as flow-rate increases by 5. The T_e decrease will result in about a 4.5-fold increase in retention in D2. The fivefold flow-rate increase should reduce ^2t_R by five times. The net effect is that ^2t_R should be reduced by about 1.1–1.2 times. It is observed that ^2t_R goes from 3.0 to 2.2 s, a reduction of about 1.4. It is possible that the flow-rate change is not an exact measure of the carrier flow velocity change, nor the rule-of-thumb of retention sensitivity to T, accounting for the inexact match in the ^2t_R trend. It can be seen that a similar trend is found for solute P (Table 4), with a T_e increase by 33 °C as the flow is altered from 4.0 to 0.8 ml/min, and ^2t_R changes from 3.8 to 2.9 s.

Solutes will travel through the second dimension faster due to $\bar{u}$ increase, however the lower temperature which they arrive at the second dimension at higher flow-rate means the band velocity will not be as high as computed simply based on flow-rate, i.e., K_D increases at lower T_e. Higher flow should make peak widths narrower, but at the same time, lower column efficiency can be expected due to a less optimal flow-rate. Hence peak widths do not vary greatly.

The changes in flow-rate did not cause a profound change in the relative retention of the solutes on D2. At lower flow, O and P were better resolved in D2. However in complex mixtures, changes in the gas velocity might be expected to affect the resolution and elution order of solutes, and so should be considered in any studies dealing with optimisation of GC×GC separations.

3.3. Effects of stationary phase on separation space and analyte elution order

The elution order of the analytes may be affected when a different stationary phase is used, depending on the nature of the solute and solute–stationary phase interactions. In the absence of basic chromato-

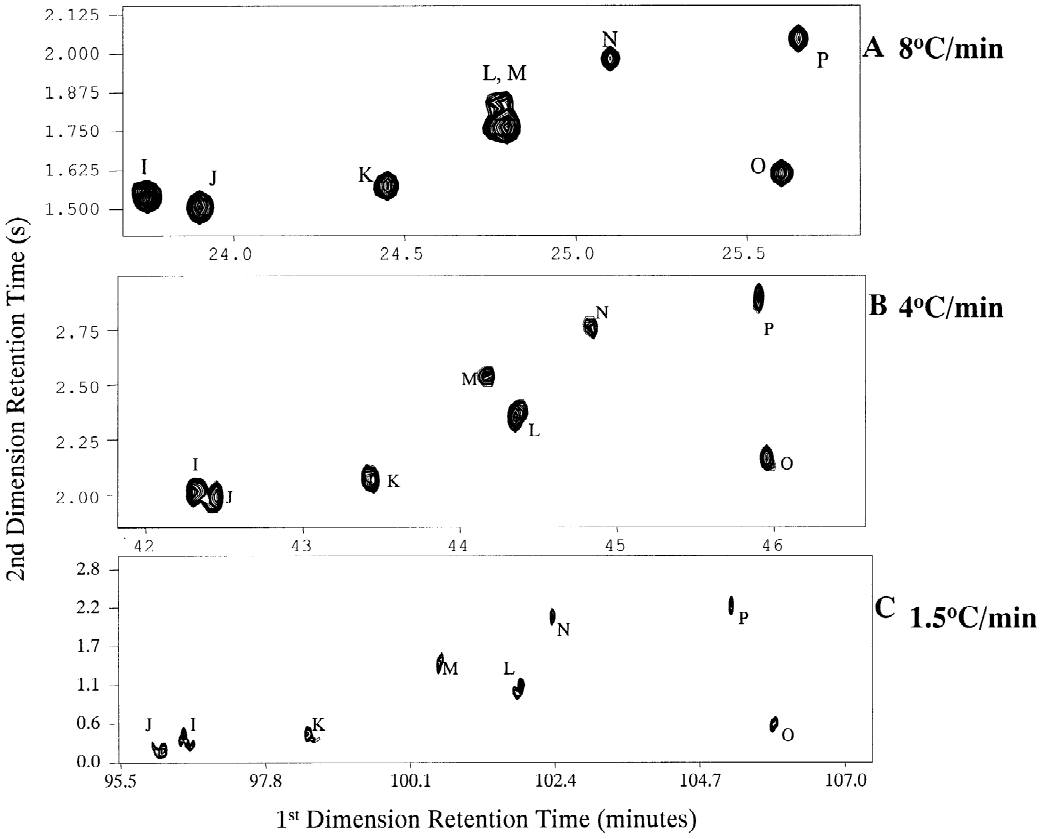

Fig. 3. GC×GC contour plot for the analysis of organochlorine pesticides using the BPX5–LC-50 column set. Expansion from solute I to P is given, for r_T settings of: A=8 °C/min, B=4 °C/min, C=1.5 °C/min.

graphic retention data on each individual stationary phase, this will be difficult to predict. The essence of GC×GC is to choose a column set which maximises the use of the separation space for the sample components. Thus the D2 column should comprise a stationary phase that allows a range of selectivities (i.e., range of ${}^2t_R$ values) for sample components. It may not be always obvious which column set is preferred for a particular analysis. For instance, it is not immediately obvious which column will be preferred for the organochlorine pesticide sample.

Four columns coated with different types of stationary phases were tested as D2. A HT8 column was included since it comprises a carborane backbone, which is supposed to retain analytes with easily delocalised electrons. Columns coated with a medium polarity phenyl polysilphenylene siloxane (BPX50; 50% phenyl equivalent) or a polar polyethylene glycol (BP20) phase were chosen since they retain polar analytes. Finally, a shape selective liquid crystal column was tested.

The comparison of stationary phases is demonstrated in Fig. 5. The HT8 column leads to very little difference in ${}^2t_R$ times for all mixture components—they appear to be located at about ${}^2t_R$=2.5–3 s in the contour plot. Thus the ''orthogonality difference'' required in GC×GC is not achieved for the BPX5–HT8 set and little separation advantage is realised in the GC×GC experiment. The BPX5–BP20 set appears to employ more of the separation space. The different selectivity can be seen for the pairs of solutes B and C, and O and P. The elution order for these pairs of solutes in the second dimension varies considerably. With BP20 and LC-50 as D2, solute C is well resolved from solute B in the second dimension, with B more strongly retained. Both the HT8 and BPX50 column did not resolve solutes B and C as efficiently as the other columns.

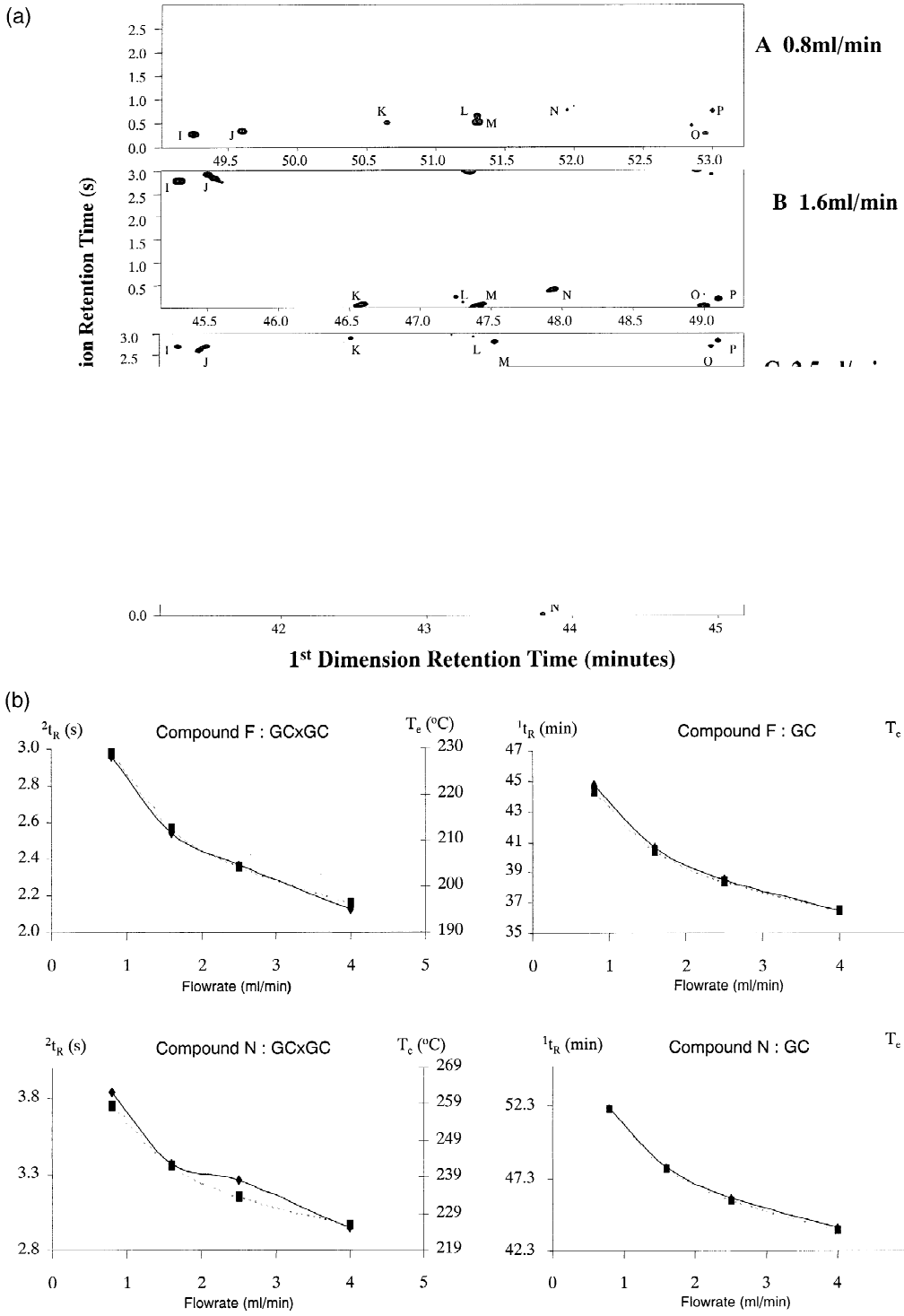

Fig. 4. (a) Effect of carrier gas flow on GC×GC plots, using the same column set as for Fig. 2. Flow rates (ml/min): A=0.8, B=1.6, C=2.5, D=4.0. r_T=4 °C/min. (b) Graphs of effect of flow-rates on absolute ^2t_R or ^1t_R and T_e for solutes F and N, using GC×GC and GC operation as indicated. Time data are shown by (♦) and temperature data by (■).

Table 3
Peak parameters and elution temperature for solutes O and P using different flow-rates (Fig. 4a)

Flow-rate (ml/min)	t_R (min)[a]	^2t_R (s)	T_e (°C)	$w_{0.5}$ (ms)[a]	w_b (ms)[a]	A_s[b]
Peak P: GC×GC						
4.0	44.94	2.89	229.7	145	254	0.94
2.5	46.94	2.92	237.7	121	208	1.04
1.6	49.10	3.23	246.4	116	198	1.01
0.8	53.01	3.75	262.0	121	212	1.02
Peak O: GC×GC						
4.0	44.99	2.68	229.9	126	220	1.00
2.5	49.94	2.72	249.7	112	191	1.27
1.6	49.00	3.01	246.0	100	177	1.12
0.8	52.95	3.31	261.8	102	170	1.00
Peak P: GC			(s)	(s)		
4.0	45.02		230.0	3.24	5.90	0.98
2.5	47.07		238.3	–	–	–
1.6	49.17		246.6	–	–	–
0.8	53.19		262.7	5.05	8.88	0.98
Peak O: GC						
4.0	45.12		230.5	3.35	6.16	1.05
2.5	47.07		238.3	4.77	8.82	0.55
1.6	49.17		246.6	3.79	6.49	1.08
0.8	53.06		262.2	4.62	8.10	1.04

[a] t_R for GC×GC is for the major pulsed peak, $w_{0.5}$ is the peak width at half height, w_b is the peak width at base.
[b] Asymmetry (A_s) values for GC×GC data given for the major pulsed peak.

The slight differences in the retention time for all four chromatographic runs was due to the minor differences in column lengths for the secondary columns as well as the different solute–stationary phase interactions. In contrast to B and C, solutes O and P are not resolved with BP20 phase. The LC-50 phase is best for both pairs of solutes, however it still does not use much of the separation space for most solutes in the sample.

3.4. Effects of column length on the separation of analytes

Effective D2 column lengths were altered by drawing different lengths of the 5.5 m BPX50 column through the cryotrap, keeping all other parameters constant. This maintains the average linear velocity of the column set, which might have otherwise changed if the BPX5 column length was physically altered. Thus the total column length (primary + secondary column) is maintained at 30.5 m, whilst allowing selection of different effective second column lengths. This will not affect the first dimension separation in any major way. Likewise total retention time is unaffected. This study should also allow investigation of the effect of carrier flow-rate variation at or near the outlet of the column. If $\bar{u}$ is constant over this length, then retention per unit length of column should be constant: $^2t_R \propto L$. This will imply that the local pressure at each section of the column is constant. This is indeed found, for second column lengths from 5.5 to 0.5 m lengths. This is perhaps surprising since it is believed that the pressure should rapidly decrease at the column outlet, with an increase in carrier flow towards the column outlet. Fig. 6 shows a plot of ^2t_R versus L for *n*-tridecane under isothermal conditions (115 °C). As the column length increases for D2, ^2t_R increases. The study did not include column length shorter than 0.5 m.

Analysis of the semi-volatiles mixture with different D2 lengths is shown in Fig. 7. A similar study was conducted using the targeted mode by the present authors [18]. Table 4 shows the total re-

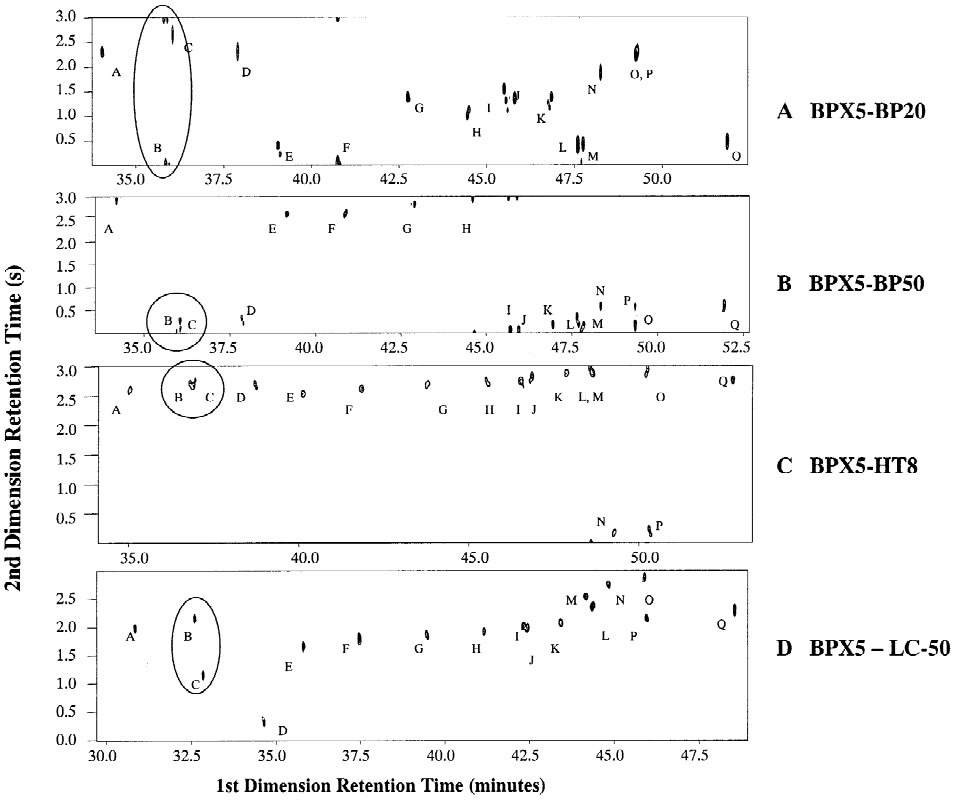

Fig. 5. Effect of stationary phase choice for D2 column. Columns used were: A=BP20, B=BPX50, C=HT8, D=LC-50 for the organochlorine pesticide mixture, and r_T of 4 °C/min.

tention time and peak widths of components 1 and 6. The total retention time remains constant throughout the experiment as the same column set was used throughout the experiment. As column length between the trap and the detector increases, the peak widths increase although the elution temperature for the same solute is approximately the same. The peak widths of the modulated peaks will increase because they are dependent on the length of the second column. In this case, even though the total length of the second column remains unchanged, the length between the trap and the detector is varied. The column efficiency for the effective D2 column is about 5000–6000 plates per meter for peak 6 for all cases. The above observations are similar to what would be found for a single column study. In the present case, it assists us in determining absolute retention times in the GC×GC study below.

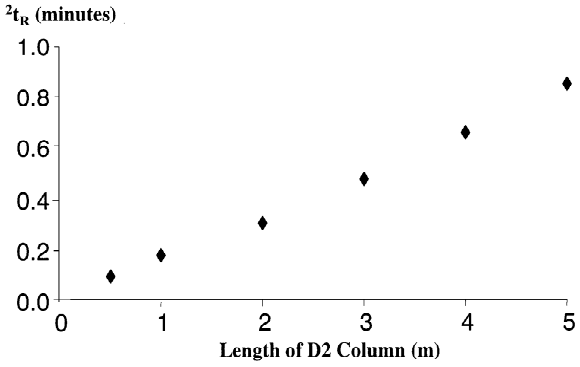

Fig. 6. Effect of D2 column length on *n*-tridecane ^2t_R retention, using targeted operation mode for the cryomodulator.

The probability of wrap-around for the semi-volatiles is higher with the increase in D2 column length. Due to the longer effective second column, there is a significant difference in absolute ^2t_R for all components (refer to Table 4). The more strongly

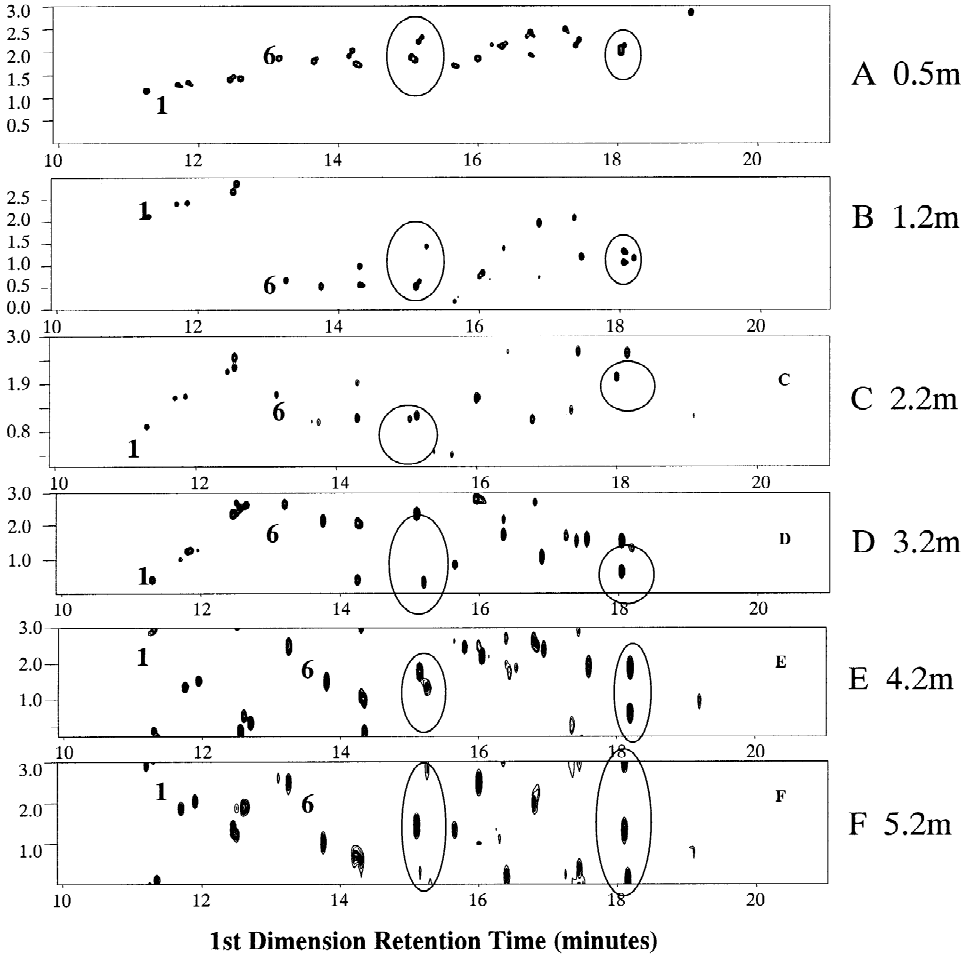

Fig. 7. Contour plots of GC×GC results for semi-volatile sample analysis for a BPX5–BPX50 column set with different effective D2 column lengths: A=0.5 m, B=1.2 m, C=2.2 m, D=3.2 m, E=4.2 m, F=5.2 m, with r_T of 4 °C/min.

retained components will experience more wrap-arounds when plotted in a 2D presentation compared to the less retained components on the second column. Again the number of wrap-arounds is found by conducting the same experiment using a different modulation timing period. Although the components should be better resolved as the column length increases, wrap-around may cause the components to apparently co-elute with longer column length. Refer to the circled components at ~15 min in Fig. 7. The components have similar ${}^{1}t_R$ times. As D2 length increases, they exhibit similar apparent ${}^{2}t_R$ at D2=2.2 m, are well separated at D2=3.2 m and are again close to overlapping at D2=4.2 m. It is interesting to note that the overall component separation is indeed very good in some instances for the 5.2 m D2 column length. The above observations suggest that the use of a short D2 column commonly proposed for GC×GC may not always be optimum. As D2 length increases, however, the peak response decreases due to greater peak broadening.

Changing the actual D2 column length will give different observations to those above. When a longer D2 column is used, with constant pressure inlet conditions, the flow-rate will decrease. This leads, in temperature program operation, to solutes reaching the second dimension column later (at higher temperature) and this has been discussed in the above

Table 4
Peak parameters for semi-volatile aromatic components 1 and 6 using different D2 column lengths (refer to Fig. 7)

D2 length (m)	t_R (min)[a]	^2t_R (s)	$w_{0.5}$ (ms)[a]	w_b (ms)[a]	N/m[b]	A_s[c]	N
For peak 1 (GC×GC)							
0.5	11.72	1.15	70	124		1.06	1500
1.2	11.74	2.11	89	158		1.13	3130
2.2	11.72	3.95	119	219		1.03	6100
3.2	11.71	6.41	144	254		1.13	11 000
4.2	11.77	9.00	173	314		1.05	15 000
5.2	11.73	12.14	206	360		1.00	19 110
For peak 6 (GC×GC)							
0.5	13.18	1.84	85	151	5250	0.89	2624
1.2	13.26	3.69	113	202	4920	0.96	5900
2.2	13.17	7.60	157	277	5900	0.84	12 982
3.2	13.24	11.80	208	367	4570	0.97	17 830
4.2	13.29	17.46	321	559	3910	0.69	16 409
5.2	13.29	23.56	340	622	5120	0.65	26 606

[a] t_R for GC×GC is for the major pulsed peak, $w_{0.5}$ is the peak width at half height, w_b is the peak width at base.
[b] N/m~5000 theoretical plates per metre.
[c] Asymmetry (A_s) values for GC×GC data given for the major pulsed peak.

section on flow-rate. However, the longer second dimension column itself will result in a greater ^2t_R and better resolution, but this depends again on the effect of the higher temperature on the overall ^2t_R and resolution achieved.

4. Conclusion

The studies in this paper demonstrate the influence of chromatographic conditions, in particular the elution temperature of analytes and the average gas velocity, on the effect of elution order of analytes both in the first and second dimensions. Careful consideration of the operating conditions as well as column dimensions can lead to a better understanding of comprehensive gas chromatographic separation. Different solute elution temperatures will result in significant shifts within the two dimensions, although inversion of elution order is likely to be more considerable in the first dimension. Larger peak widths are obtained with lower T_e. Flow-rate plays a less important role in inversion of elution order of the analytes and in affecting the peak dimensions and ^2t_R. Clearly, the shifts in elution order is most significant when the stationary phase of the second column is altered, as it changes the solute–stationary phase interactions. It is demonstrated that D2 column length is particularly important to control the separation as well as peak parameters such as peak widths and peak heights of the analytes. Naturally ^2t_R will increase with incrementing D2 column length. Although a longer column will lead to better separation, wrap-around may cause otherwise resolved components to co-elute due to the pulsing period and ^2t_R values of neighbouring peaks. The above observations suggest that optimisation of a GC×GC analysis will be a tedious exercise, and more complex than single column GC.

5. Nomenclature

2D	Two-dimensional
T	Temperature
r_T	Temperature program rate
D1	First dimension
D2	Second dimension
^1t_R	Retention time in the first dimension
^2t_R	Retention time in the second dimension
T_e	Elution temperature
K_D	Distribution constant
$\bar{u}$	Average carrier gas velocity
L	Column length
β	Phase ratio
d_f	Film thickness

r	Column radius
k	Retention factor
T_i	Initial temperature
T_f	Final temperature
R_s	Resolution

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References

[1] Z.Y. Liu, J. Phillips, J. Chromatogr. Sci. 29 (1991) 227.
[2] K. Yabumoto, W.G. Jennings, M. Yamaguchi, Anal. Biochem. 78 (1977) 244.
[3] W. Jennings, in: Gas Chromatography with Glass Capillary Columns, 2nd ed., Academic Press, London, 1980, p. 140.
[4] W. Jennings, in: Analytical Gas Chromatography, 1st ed., Academic Press, London, 1987, p. 99.
[5] M. Mehran, W.J. Cooper, N. Golkar, M.G. Nickelsen, E.R. Mittlefehldt, E. Guthrie, W. Jennings, J. High Resolut. Chromatogr. 14 (1991) 745.
[6] R.J. Pell, H.L. Gearhart, J. High Resolut. Chromatogr., Chromatogr. Commun. 10 (1987) 388.
[7] J.V. Hinshaw, LC·GC Int. 9 (2) (1991) 94.
[8] J.V. Hinshaw, LC·GC Int. 13 (12) (1995) 944.
[9] K. Grob, G. Grob, Chromatographia 17 (9) (1983) 481.
[10] W. Bertsch, J. High Resolut. Chromatogr. 22 (1999) 647.
[11] W. Bertsch, J. High Resolut. Chromatogr. 23 (2000) 167.
[12] V. Pretorius, T.W. Smuts, J. Moncrieff, J. High Resolut. Chromatogr., Chromatogr. Commun. 1 (1978) 200.
[13] C.J. Venkatramani, J. Xu, J.B. Phillips, Anal. Chem. 68 (1996) 1486.
[14] J.B. Phillips, J. Beens, J. Chromatogr. A 856 (1999) 331.
[15] R.M. Kinghorn, P.J. Marriott, P.A. Dawes, J. High Resolut. Chromatogr. 23 (2000) 245.
[16] T.T. Truong, P.J. Marriott, N.A. Porter, J. AOAC Int. 84 (2) (2001) 323.
[17] R.C.Y. Ong, R. Shellie, P.J. Marriott, J. Sep. Sci. 24 (2001) 367.
[18] P.J. Marriott, R.C.Y. Ong, R.M. Kinghorn, P.D. Morrison, J. Chromatogr. A 892 (2000) 15.