Molecular structure retention relationships in comprehensive two-dimensional gas chromatography

Marriott, Philip; Massill, Tracey; Hugel, Helmut


Published Version: https://doi.org/10.1002/jssc.200401917

Repository homepage: https://researchrepository.rmit.edu.au
Molecular Structure Retention Relationships in Comprehensive Two-Dimensional Gas Chromatography

by

Philip J. Marriott*, Tracy Massil and Helmut Hügel

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

Submitted to

Journal of Separation Science Cs. Horvath Special Issue (REVISED)

* author to whom correspondence is to be addressed

Philip Marriott email: philip.marriott@rmit.edu.au

Keywords: comprehensive two-dimensional gas chromatography, structure-retention; retention correlation; structured retention; dynamic chromatography; decomposition; isovolatility plots

Running Title: Molecular structure and GCxGC
Summary

Comprehensive two-dimensional gas chromatography (GC×GC) offers new opportunities to develop relationships between molecular structure and retentions in the two dimensional (2D) separation space defined by the GC×GC retention in each dimension. Whereas single dimension GC provides only one retention property for a solute, and hence the specific relationship between retention and chemical property is not readily apparent or derivable, the 2D presentation of compounds in GC×GC provides a subtle and exquisite correlation of chemical property and retention unlike any other GC experiment.

The ‘orthogonality’ of the two separation dimensions is intimately related to the manner in which different separation mechanisms, available through use of two dissimilar phases, are accessible to the different chemical compounds or classes in a sample mixture, and indeed the specific chemical classes present in the sample. The GC×GC experiment now permits various processes such as chemical decompositions, molecular interconversions, various non-linear chromatography effects, and processes such as slow reversible interactions that may arise with stationary phases or in the injector or column couplings, to be identified and further investigated.

Here, we briefly review implementation of the GC×GC method, consider the molecular selectivity of GC×GC, and highlight a selection of molecular processes that can be probed by using GC×GC.
**Introduction**

Comprehensive two-dimensional gas chromatography (GC×GC) is now over one decade old. Its uptake in respect of instrument placements has been relatively slow, however recent trends suggest that it is winning an ever-increasing group of converts from amongst the community of GC users. The slow adoption of this innovative technology is probably due to a number of factors, such as lack of readily available or cost-effective instrumentation, perceived complexity of the method over single dimensional GC analysis, and the slow development of user-friendly automated software for interpretation of GC×GC data. Perhaps another factor could be the sheer volume of data (i.e. separated components) that many typical complex sample analyses, accomplished by using GC×GC, has revealed. This in itself may pose a challenge to the comprehension of many chromatographers.

The basic experiment is conducted in an instrument typical of which is shown in **Figure 1**. Central to the successful development of GC×GC is the modulator, located between two different GC columns, which is unique in performance and operation, and is not simply a modification of an interface in methods such as conventional multidimensional gas chromatography. Other method considerations include the use of a very fast elution time on the second dimension column (2D) achieved by the use of a short column which may have a narrow ID and thin film phase, the requirements for fast detection acquisition commensurate with the fast peaks generated by the 2D column, and the option of a different oven to house the 2D column. This essentially defines GC×GC, however the apparent simplicity of this description belies the information content that can be derived from the experiment.

**Modulator function.**

The important functions of the modulator include its ability to sample contiguous segments of a first dimension (1D) peak, and then deliver these to the 2D column as a very narrow injection or introduction band. The two main modulator types are broadly categorised as valve sampling methods, and those based on use of elevated or reduced temperature processes (see the review by Lee *et al.* [1]). The former are represented by diaphragm and sampling loop-type interfaces, which generally do not achieve mass
conservation but rather sub-sample the ⁴D peak (but some loop methods do sample a large portion of the peak). The latter may incorporate a retarding column segment (e.g., relatively thick film column) with a mechanism to provide a higher-than-ambient oven temperature that releases the trapped or retarded solute from the thick phase into the carrier gas stream. Alternatively, they may use a sub ambient temperature process to trap the solute as it travels into the modulator region. In this case, the applied cooling process must be modulated to a higher temperature to permit the trapped solute to be re-mobilised into the carrier phase and thence into the ²D column. Thus the modulator interface might be thought of as slicing the ¹D peak into sampled sub-segments, which may or may not be accompanied by a zone compression effect that effectively concentrates the sampled peak segment. In all cases the delivered distribution to the ²D column faithfully represents the first dimension peak distribution, but only to the extent that the period of modulator sampling allows the ¹D peak to be reconstructed, based on the resultant distribution on the ²D column.

**Figure 2A** is a summary of the generation of a 2D GC×GC result from a normal gas chromatogram. Step 1 illustrates that the ¹D peaks are modulated according to a selected modulation period (usually the period of modulation is of the order of magnitude of the primary peak standard deviation \( P_M \sim \sigma \)), then exported as a linear data stream that can then be converted into a matrix format (steps 2 and 3), and then displayed as a contour plot (step 4) which has dimensions of total analysis time on ¹D, and the individual chromatogram duration on ²D, based on the modulation period. Modulated peaks produced from repeated injection, but with different modulation phase (which is the relationship between the peak position and the times of sampling process), are shown in **Figure 2B**. Each preserves the total component area, but show different individual modulated peak maxima times, heights and areas. They all reflect the same ¹D source peak distribution and retention time, and so can be used to derive the equivalent ¹D peak parameters of peak retention time, and peak width, area and height.

Since the modulator provides an essentially instantaneous injection into ²D, the second chromatography column is effectively a discrete, but fast, GC analysis. It also provides a chromatographic result, which is largely independent of the first column separation, and so this is often interpreted as producing separation orthogonality on
the two GC columns. This may be tested, by determining if it is possible to predict the 

$^2$D retention of a randomly selected solute eluted from the first column. Since 

examination of reported GC×GC results demonstrates that there is little overall 
correlation of retention on the two dimensions, then this experiment does provide 

orthogonal analysis. This then implies that it should be possible to differentiate 

individual retention mechanisms on each column, and in turn these should lead to 

retention of solutes based upon their chemical properties that are sensitive towards the 

interaction mechanisms provided by each column stationary phase. Figure 3 

illustrates that the GC×GC result may indeed be interpreted as a chemical resolution 

map, which is defined by the retention mechanisms of each column. This result is a 
two-dimensional separation space, with the dimension magnitudes defined by the total 
retention time on the first column, and the modulation period (time) on the second 
column. It is desirable normally to ensure that compound adjusted retention on $^2$D is 
no greater than the modulation period ($^2t'R < P_M$), because the detector data stream is 
processed into the 2D format based on the time chosen for modulation, somewhat 
similar to generation of a data matrix. If a solute elutes later than this time, then it will 
be located within the effective component elution range in the subsequent matrix data 
column. This may confound the interpretation and may lead to artificial overlap of 
solutes in nominally different modulations, but which fortuitously are processed into 
the same matrix column.

The dissociation of retention on $^1$D from that on $^2$D thus reveals much more detail in a 
gas chromatographic analysis than can be derived from a single dimension time- 
response chromatographic representation of a sample. Simply, the elution time of a 
solute in the 1D experiment does not by itself give evidence of supplementary 
chemical information about the compound; the elution time is determined by solute 
volatility (and in a temperature programmed analysis, volatility as a temperature 
dependent parameter, will vary over the duration of the analysis). Volatility in turn is 
a complex function of dispersion effects and specific molecular interactions such as 
dipole moments, proton donor/acceptor properties, polarizability and similar 
properties [2] between the solute and the stationary phase. By contrast, even though a 
sample still undergoes separation on a first column in GC×GC, and is again subject to 
the same effects as above, the second column is then able to reveal differences 
between coeluting or closely eluting compounds. Simply, the appropriate choice of a
suitable stationary phase specifically capable of targeting molecular properties, which lead to selectivity in the second dimension, achieves this.

If the two column phases are chosen such that there are no selectivity differences in the second column, then correlated retentions will arise – i.e. there is no molecular differentiation (separation) on ²D. This might be considered a poor set column choice, however it is also often a consequence of the separation goals of the analysis. It may be entirely unavoidable that in order to achieve separation of certain components in the GC×GC experiment by using a given column set, other components may be correlated when using this set. Clearly, complex sample analysis will necessarily lead to compromises in choice of column sets.

**Structured Retentions in GC×GC**

This is perhaps the most important underlying property of GC×GC that gives rise to a number of novel capabilities of GC×GC compared with 1D GC. Essentially this relates the positions of peaks in the 2D separation space to the trends in chemical properties of the sample set, and most significantly leads to patterns that can be interpreted or recognised as arising from those compounds in the sample. Two samples can be used to illustrate this property. The first is the GC×GC separation of a petrochemical sample, such as diesel. The second is the analysis of PCBs.

**Figure 4** is a diesel sample analysis. Delineation of components is such that there is almost complete separation of alkanes/naphthenes from mono-aromatics, from di-aromatics, and then tri-aromatics. This could be viewed phenomenologically as a boiling point / aromatic separation, without defining what constitutes an ‘aromatic’ separation. It would be useful to be able to isolate the ²D column property that leads to the precise retention differences in that column; often we ascribe this to ‘polarity’ however this is a generalist phrase. The pattern of components within the space is striking. The expanded view of **Figure 5** is an expansion of the di-aromatic region. The rationale for the structural features seen here is that there is a systematic variation in chemical property of molecules that leads to small changes in the relative interaction energies with the stationary phase to give either an increase or decrease in retention position. Whilst it is appreciated that more alkyl substituents on the naphthalene molecule lead to greater retention, there is not always a clear separation
on this basis on the 1D column. This allows in a subtle way to derive possible chemical structural features, which cause these observations. The exact description of this has not been delineated in the GC×GC literature at the moment. It can be supposed that if the structure-retention relationships can be quantified, then greater understanding of both the nature of GC×GC separations, and fine chemical properties arising from molecular geometries could be probed. Note that the particular 2D structure in GC×GC space will be defined by the choice of phases. In this example, the 1D column comprised a 5% phenyl 95% methyl phase, and so will be largely sensitive to dispersion effects (being low polarity). The 2D column was a polyethylene glycol phase, and is thus of high polarity. More commonly, a 50% phenyl phase is used as the 2D column phase for this type of analysis, however it gives a rather similar overall separation. The second column therefore retains the more polar (or higher aromaticity or polarisable) molecules more strongly. If 1D column polarity is altered, for instance if a more polar phase is used, then alkanes will be eluted earlier, and polar compounds (eg. aromatics) elute later. This in turn alters the elution temperature of the components, and will cause their 2D retention to be affected. If the 2D phase is then chosen to be non-polar (i.e. quite different to the 1D phase) then alkanes will be retained more on 2D, and aromatics will be less retained on 2D, than the extent shown in Figure 5. The chromatographic separation can markedly be altered if the non-polar/polar column set is changed to a polar/non-polar set, as seen for coffee volatile analysis [3, 4], and demonstrated in a column orthogonality study [5]. Under conditions that give reasonable retention (i.e. not too long) of the higher aromatics, the alkanes and naphthenes tend to be grouped in a narrow 2D retention range, with low retention.

Two obvious novel conclusions arise from this example. First is the ability to relate chemical structure to retention position, and the second is the more practical outcome that this offers a significant new method to compare petrochemical samples for qualitative and quantitative composition. The ‘pictorial’ format of the GC×GC result provides immediate recognition of chemical speciation within the sample, and may be a powerful basis for chemometric-based interpretations of sample suites, for example.

The case of PCBs and chlorinated dibenzodioxins has analogies to the above example. The combination of low- polarity 1D column, with a carborane phase column (HT8),
produces a series of structured retentions which group congeners with the same number of chlorines into a correlated data set, whilst separating different chlorine-number congeners into different retention horizons on the 2D column [6]. This is illustrated schematically for PCBs in Figure 6. First, if the 1D column retention is effectively according to boiling point, and since for a given 1D elution temperature retentions on the 2D column follow the relation $(\text{Cl})_{n+1} < (\text{Cl})_n$, then it will imply that greater number of chlorine atoms leads to lower ‘polarity’ for structures that have similar BP, even though more chlorines imply higher molar mass. An additional advantage is that whereas the single column analysis of PCBs often requires high resolution MS to be able to confirm chemical composition and to reduce matrix interferences, the clarity of the GC×GC separation space should mean that unit resolution MS is sufficient for adequate identification, providing two GC retention properties and one MS dimension.

Note that for many samples, it is not straightforward as to what structure, if any, might exist in a complex GC×GC chromatogram. Figure 7 is an essential oil analysis, with both 1D and GC×GC results contrasted. The complexity of the sample is displayed favourably by GC×GC, but without further identification, it will not be possible to conclude if there is any structure to this result, although logically it must be possible to relate similar compounds (e.g., homologues) to their positions in the 2D space. This will be an important interpretative tool for general GC×GC sample analysis.

**Reproducibility**

Supporting considerations of structured retentions and especially sample comparison through reliability of component positions in 2D space is the need to ensure reproducible run-to-run analysis. Furthermore, and perhaps more stringent, the ability to compare results for a given column set and conditions from one laboratory to another will give an exceptional level of sample analysis capabilities. Both intralaboratory [7] and interlaboratory reproducibility [8] were studied for a modulated cryotrapping system for a number of different chromatography instruments and modulator units, and column sets of the same geometry. Figure 8 offers data which can be interpreted with respect to repeatability; these data showed rsd values of...
$tR_1 \sim 0.16\%$ and $tR_2 \sim 0.74\%$. In respect of $^2D$, where retention times are usually less than 4-5 s, and peak basewidths are about 100 – 150 ms on a 1 m narrow bore column, this uncertainty translates to retention variability of less than 10-20 ms, and about 0.5 or less peak standard deviation $\sigma$. In order to achieve this, the modulator timing (which corresponds to commencement of each modulation event) must demonstrate at least this level of reliability. Lab-to-lab data reproducibility will be expected to be less reliable, since method uncertainties, especially where different instruments are employed, will give retention variation. Perhaps the most important criterion is that the column sets must be very well characterised, and then appropriate corrections used, to provide matched results. For instance, using the unretained peak to correct for flow variations may be important. For chemometric studies [9,10], it is clear that positions of peaks are critical in assignment of peak responses. Good reproducibility is a function of modulator performance, and then control of GC conditions such as temperature and flow. GC×GC therefore places considerable demand on system performance criteria over and above that of single dimension GC, but also demands excellent timing control of the modulator, regardless of which modulator type [11] is employed.

**Secondary chromatography effects in GC×GC**

**Non-linear chromatography.** From the above discussion, it is evident that GC×GC should demonstrate the differences arising from any process that causes different molecular structural forms to be produced or generated during (or prior to) the analysis of a sample. GC×GC may also yield interpretation of the 2D results that permits information to be derived for the chemical process. As an example, under conditions of linear chromatography, a compound will be located at a precise position as defined by its retention coordinates, and should be symmetrically distributed about the maximum. Whilst the modulated profile of peaks subjected to GC×GC may vary somewhat and can lead to different extents of amplitude enhancement [12], it should have a very well characterised relationship to the peak profile that elutes from the $^1D$ column [13]. Under non-linear conditions, overloading may occur on either of the two columns [14], and will be controlled by phase and solute chemistry (again, often generalised as polarity), temperature, and factors such as column phase ratios. It is now simply a matter of translating the single column interpretation of non-linear
chromatography to the GC×GC experiment. Since non-linearity is very sensitive to injected amount, then the process of zone compression GC×GC may be thought to exacerbate the prevalence of overloading on the typically narrow bore, thin film columns used for the second dimension in GC×GC. Given that solute is only delivered to 2D at relatively high temperature conditions (i.e. after the solute has traversed 1D) it should have relatively sufficient vapour pressure to not badly suffer the mobile phase concentration limitations, which cause peak distortion characteristic of lower temperatures. Figure 9 illustrates an overloaded peak response in GC×GC, where both the first column (Figure 9(A)) and second column (Figure 9(B) inset) exhibit overloading. Note that the modulated peak distribution in Figure 9(B) matches closely the non-modulated (dotted peak profile) in (A). The corresponding 2D plot in Figure 9(C) can be interpreted by considering the contour plot levels as indicating the response slope sensitivity over the distribution. Close lines correspond to steep slope changes, just as the 1D peak also has a gentle slope then a steep slope shape.

The small peak to higher 1D retention is a sample impurity that has the linear chromatography peak shape of a non-overloaded peak. The broad peak produced by the overloaded component will reduce resolution from nearby peaks, however unlike 1D GC, it may still be possible to resolve on 2D some components that suffer overload peak overlap on the first column.

**Isovolatility curves.** Beens et al. described the observation of what they termed ‘isovolatility curves’ in GC×GC experiments [15], where compounds entered the GC system through use of slow introduction of solute into an injector during the temperature programmed GC analysis. Since the compounds elute continuously through the first column, and their introduction into 2D is modulated, each compound displays a logarithmic decrease in $t_R$. This leads to a series of curves in the 2D space, which should correspond to the temperature dependence of the compounds’ retentions. This observation was used by Western and Marriott to propose methods for determination of retention index values for compounds in GC×GC [16]. However, a new approach was sought for sample introduction. Two methods were tested. One was sequential injection during the temperature programmed analysis, eg at every 5 or 10 min, the other was through using different temperature program rates [17]. This alters elution time and hence elution temperature of each compound in each injection, and produces a series of points which describe the isovolatility curve for each
compound. It should be possible to study the temperature sensitivity of relative retentions of compounds through this approach, and where compounds swap elution order with temperature, their isovolatility lines should cross. There is scope for revising the term isovolatility, since as the elution temperature changes, volatility of components change. If isothermal analysis was employed, then isovolatility curves would be generated, and these would delineate a horizontal line in the 2D space.

Many GC×GC chromatograms exhibit curved ‘streaks’, which can almost invariably be associated with specific peaks in the 2D plot.

**Figure 10** illustrates the presence of the streaks in the 2D space for an essential oil comprising polar solutes, and they can almost all be traced back to an individual starting position terminated at a (major) component. These streaks bear resemblance to the above isovolatility curves, and so they can be interpreted in similar fashion. However their source is not fully established. A compound that is slowly released from the injector subsequent to adsorption on active sites in the injector, or due to unswept voids in the injector, a compound that undergoes on-column slow reversible adsorption, a compound that enters a void located in the join between the 1D and 2D columns (where the column join is before the modulator) will all potentially give rise to this phenomenon. It appears to be most commonly associated with the more polar solutes, and so all of the above effects will be exacerbated by polar compounds. Presently, there is not one explanation that accounts for the observation, and whilst even new capillary columns do show these effects, it is still not possible to rule out column activity. Note that in successive analyses with and then without modulation, almost always there is no indication of the streak in the normal chromatogram, which might be expected to be manifested by a raised baseline. This suggests that it is the increased sensitivity of the GC×GC experiment’s zone compression, which leads to the ability to observe the tailing nature of the peaks. This then poses the possibility that such tailing is a general phenomenon in capillary GC, and may compromise quantitative analysis for susceptible compounds. There is scope for a definitive study to identify the causes of these phenomena.

**Decomposition processes.** The isovolatility and streaking curves referred to above can have a further origin, and that is in the dissociation or decomposition of a
chemical compound, where the product is a chromatographically viable compound.

There are many instances in GC where such processes arise, such as the dissociation of dicycolopentadiene into its monomer, as demonstrated by Langer and coworkers [18]. These have not been addressed in the GC×GC context, but Dallüge et al. at the Free University of Amsterdam reported the chromatography of trace sulfur interferences in various environmental samples [19], which were identified by use of TOFMS (note that FID will not reveal these compounds). An illustration of this is given in Figure 11. Here, 64 u shows up all the S species. Use of selected ion 192 u indicated the S₆ and S₈ species, and so confirmed their identities. This chromatogram can be interpreted based on the previous understanding of chemical processes in the GC×GC experiment. The curved line for S₆ shows the effect typical of this compound eluting into ²D during the progression of the analysis as its elution temperature into ²D increases. Similar ²D retentions for S₆, S₇ and S₈ also confirms this analysis is under temperature programming. If the cause is simply due to S₈ adsorption with slow desorption (perhaps accompanied by decomposition) in the injector, then the S₈ peak will likewise show a ‘tail’, however it does not. If it was fast decomposition in the injector, then only a discrete single peak will be seen for S₆ and S₇. If it is on-column thermal dissociation, then the kinetics of the process will increase at higher temperature, and will also be greater for larger amounts of starting material. So the amount of product formed will be a function of oven temperature and instantaneous reactant amount. It is probable that the individual spots (peaks) for S₆ and S₇ arise from either their presence in the original sample, or are indeed formed at the high temperature of the injector during the injection step. This would be greater for splitless than split injection. It is likely that a systematic study of the GC×GC results will permit various thermodynamic parameters to be derived for this and like processes, which thus far has attracted little concerted effort. Isothermal analysis by using GC×GC would also be informative in this regard.

**Dynamic comprehensive two-dimensional chromatography, DGC×DGC.** The observation of molecular interconversion in chromatography has had an interesting genesis and development. In respect of the broader classification of chemical change during chromatography elution, this is a specific subset, which also leads to a number of novel chromatographic profiles. Decomposition (irreversible) processes also lead to
chromatographic peaks, which are indicative of the chemical changes that take place within the chromatography column. Whilst a review of this area is beyond the scope of the present discussion, it is noteworthy that these types of processes are observed in HPLC, capillary electrophoresis, as well as GC. Horvath and co-workers investigated secondary equilibria and molecular change in liquid chromatography [20,21], in particular dynamic molecular motions associated with proline peptides [22] and proposed methods for interpretation of the chromatographic result to obtain physical-chemical data. Figure 12 illustrates the typical 1D chromatography result of a dynamic (reversible) interconversion process. The two terminal peaks A and B correspond to the species injected into the column, however the ‘reaction’ \( A \rightleftharpoons B \) causes a certain portion of each species to convert into the other compound (often these will be isomers that may be resolved on the stationary phase). This will be a continuous on-column process, and so a smooth plateau forms between each of the terminal peaks. The extent of reaction is a function of temperature and time.

Normally, the plateau is not differentiated into its A and B constituents. However, in GC×GC it is possible to modulate the elution into a fast, high resolution capillary column to separate A and B (with only negligible interconversion on 2D) over this region [23]. Figure 13 presents the original 1D GC result Figure 13(A), with its modulated counterpart (B). Finally the GC×GC presentation of (B) is given in Figure 13(C). Since A and B can be separated on 2D, then they form two distinct bands in the 2D space. The contours of Figure 13(C) correspond to different response levels. The tail of A that extends to the 1D elution time of B arises from species B converting to species A, during the process of B traversing the column. Whilst A has a shorter \( t_R \) than B, any A formed from B at some time after injection cannot elute with original injected A molecules. Operation under isothermal conditions produces the ‘horizontal’ tails seen in Figure 13(C). Temperature programmed conditions will lead to the logarithmic decay in \( t_R \) for each of the A and B tails [24].

Enantiomeric interconversion processes have been studied by Schurig’s group [25]. These provide certain challenges in DGC×DGC; not surprisingly these have not yet been reported. The 2D column must be fast, and capable of producing enantiomeric resolution if the destiny of each enantiomer’s interconversion is to be tracked. Thus whilst 1D enantiomeric analysis has been demonstrated in a number of different studies in GC×GC [26, 27], and 2D chiral columns have been employed with vacuum
outlet conditions for fast chiral analysis in GC×GC applications [28], enantiomeric DGC×DGC has not been studied. Notwithstanding this, it is possible to predict the types of results this might lead to. The model chosen here is the chalcogran example, which has two pairs of diastereomers; its dynamic GC property has been reported and simulated. Figure 14(A) illustrates the non-modulated enantioselective GC result for chalcogran, with adequate separation of the 4 isomers, which was achieved on a dual-enantioselective column ensemble. Isomers A1 and A2 are the enantiomers of one diastereomer, B1 and B2 the other. Figure 14(B) is the proposed GC×GC result that should be obtained if both diasteromeric resolution and enantiomeric resolution is achieved on both columns. Whilst the diastereomers can be resolved on achiral phases, and the enantiomers can be resolved on selected enantiomeric phases in the first dimension on regular long columns, the diastereomers exhibit only incomplete resolution on a short, fast 2D column. Enantiomeric separation is not achieved in similar short chiral columns. Thus an achiral 2D column would only partly allow the A1 ↔ A2 and B1 ↔ B2 processes to be observed in a ‘correlated’ manner – without 2D resolution, but without the confounding overlapping of the two interconversions. In the Figure 14 case, the two B isomers elute within the A isomer boundaries on the chiral 1D column, but elute earlier on the achiral 2D column. It has been reported that other chiral columns lead to a retention order A1 < B1 < A2 < B2, and so this would lead to a different interconversion plateau shape.

**Conclusion**

Comprehensive two-dimensional gas chromatography offers a unique manner by which to study molecular processes in gas chromatography. Its ability to present comprehensive molecular-specific separations in two dimensions permits simplified interpretations of qualitative sample chemical information. Structural features within the 2D space allow relationships between homologous compounds to be identified, and trends in molecular properties delineated. It is to be anticipated that as further systems are studied by use of GC×GC, even greater scope will be realised for probing molecular interactions. It will therefore be only a matter of time before a sufficiently large portfolio of unique chromatographic molecular features will be assembled in GC×GC, that confirms yet again the position of GC×GC as revealing greater understanding of gas chromatography.
Acknowledgements
The authors acknowledge the RMIT University Australian Centre for Research on Separation Science group members, whose research results form the basis for this paper. Also, Dr Jens Dallüge and colleagues at the Free University of Amsterdam are acknowledged for kind provision of the result shown in Figure 11.
Figure Legends

Figure 1
Schematic diagram of a typical comprehensive two-dimensional gas chromatography instrument set-up. The modulator M here is the moving cryogenic modulator used in the authors’ laboratory. A secondary oven may be used to assist in system optimisation and tuning. For mass spectrometry detection, either a time-of-flight or quadrupole device may be used.

Figure 2A
Data processing in GC×GC follows the following steps. (A) illustrates two overlapping peaks, which are modulated about 6 times. The data stream is processed such that the data are formed into their separate modulated 2D chromatogram data sets. This forms a data matrix, which can be plotted as a contour or surface plot. Higher-level data systems then can present data in various formats as chromatographic reports.

Figure 2B
The modulation process generates a distribution of peak pulses which matches that which elutes from the first column, and here a Gaussian peak shape is overlaid on three different modulation phase results for repeat analyses of a compound.

Figure 3
The GC×GC separation space defines a chemical property retention map. Here, a non-polar / polar column set (1D / 2D) determines the distribution of solutes within this space. Note that although 1D is nominally non-polar, since the elution of components on this column is according to both dispersion and specific interactions between solutes and column, compounds of different polarity will be spread over the total elution space. For the generic case, the retention of compounds on each column may be classified according to the mechanism of retention on each column.
Figure 4
GC×GC separation of diesel on a BPX5 – BP20 column set. On the polar second column, alkanes are poorly retained, whereas aromatics are increasingly retained as the number of rings increase.

Figure 5
Within the di-aromatic region of Figure 4, retention trends can be identified suggesting there are subtle molecular-specific chemical properties that influence component relative retentions. naph = naphthalene, Cx-N = naphthalene with x carbon substituents.

Figure 6
On a properly tuned GC×GC column set, PCBs also are characterised by individual series of peaks, which are related by the same number of chlorines, but are resolved on the second column through differences in their polarity.

Figure 7
A typical essential oil modulated GC×GC result presented as a linear data stream (A), compared with its two-dimensional presentation format (B). Peaks were identified in (B) and so are numbered for this chromatogram (individual identifications not included herein) but are not given their corresponding peak number in (A) since the format of (A) does not permit the respective unique peaks to be unambiguously labelled.

Figure 8
The reproducibility of peak position in GC×GC now appears to be well established. Here, repeat analyses of tea tree essential oil demonstrate that molecular identification should be possible from run-to-run based on reliable peak position in the 2D space.

Figure 9
Overloading causes non-linear effects in GC, and is especially common in thin-film stationary phase columns. (A) The first column shows characteristic overloading peak shape. (B) The modulated chromatogram distribution follows that of the original
peak, however the larger pulses may also exhibit overloading. The smaller pulses will still be linear and so have symmetrical peak shapes. (C) The peak contour in 2D space has a shape that is indicative of overloading in both dimensions; the small tail section (circled) arises from a small peak, which is not overloaded, and so is located at the proper 2D retention time.

**Figure 10**
Streaks often appear in the 2D plot and are clearly related to discrete ‘starting’ point compounds. The tail delineates a logarithmic decay curve shape due to the increased elution temperature at which the compound causing the tail enters the 2D column. Refer to the text for comment on the potential source(s) of the streaks.

**Figure 11**
Chromatogram of sulfur compounds in a GC×GC-TOFMS experiment. This result is the selected ion 64 u data, which shows all S compounds. The individual compound identities were deduced by use of selected ion plots of 192 u, 208 u, and 224 u, for S_6, S_7 and S_8 respectively.

**Figure 12**
Typical 1D chromatogram of the interconverting isomers of a compound (here, acetaldoxime on a polyethylene phase column), each of which has different retention time. The plateau corresponds to the ‘product’ of the ‘reaction’ of each of the isomers into the other isomer during the chromatography process, and this occurs continuously during the chromatographic elution.

**Figure 13**
Comparison of 1D chromatogram (A) with the modulated result (B), whilst (C) is the 2D contour plot of data in (B). Since this is isothermal analysis, each compound ‘tail’ occurs at a specific 2D retention time.

**Figure 14**
Proposed GC×GC contour plot for the example of chalcogran, which has two pairs of enantiomers (denoted 1 and 2), for two diasteromeric isomers (denoted A and B). Thus 4 isomers are obtained (only one structure shown), but only the enantiomers can
be interconverted during their chromatography elution. Provided they can all be satisfactorily separated in the 1D and 2D columns, the GC×GC result will display all 4 compounds. If the kinetics allows observation of the interconversion, then each will have a ‘tailing’ shape linking its elution with that of its enantiomer. (A) The analysis of chalcogran on a dual-enantioselective first dimension column indicates that four isomers can be separated. (B) The proposed GC×GC 2D plot, which would arise if the separation and kinetic factors were suitable for this system.

References


FIGURE 1
FIGURE 2A

step 1

step 2/3

step 4
FIGURE 2B
FIGURE 3 The GCxGC chemical property retention map. Non-polar / polar column set

<table>
<thead>
<tr>
<th>2D retention time</th>
<th>1D high volatility</th>
<th>1D low volatility</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D low polarity</td>
<td>2D high polarity</td>
<td>2D high polarity</td>
</tr>
<tr>
<td>1D high volatility</td>
<td>1D low volatility</td>
<td>1D low volatility</td>
</tr>
</tbody>
</table>

1D retention time
Figure 4

![Graph showing molecular structure and GC×GC retention times for 1, 2, and 3 ring compounds and alkanes.](image-url)
Figure 5
Figure 6

2D; polar

1D; non-polar

4Cl-PCB
5Cl-PCB
6Cl-PCB
7Cl-PCB
Figure 7

(A)

Retention time (min)

(B)

2D retention time (s)

Retention time (min)
Figure 7

(A) Retention time (min)

(B) 2D retention time (s) response

Retention time (min)
FIGURE 9

(A) 1D peak shape

(B) GCxGC modulated result

(C) 2D peak

Response, pA

1D retention time, (min)

2D retention time, (s)
Figure 10
Figure 11

64 u indicates all S compounds

192 u gives this peak

S6

S7

S8
FIGURE 12

Internal standard

Species A

Species B

Zone of interconversion

Relative Response

Retention Time (min)
FIGURE 13

(A)  

(B)  

(C)
Example chalcogran structure (1 of 4 isomers)