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TRENDS

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# Comprehensive two-dimensional gas chromatography

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The perceptive ideas of Giddings in 1984 [1], which identified the "extraordinary promise" of two-dimensional technology must be acknowledged when considering the origins of comprehensive two-dimensional gas chromatography (GC×GC). Giddings suggested that only in the suitable coupling or interfacing of two appropriate dimensions of separation could the full power of two-dimensional systems be demonstrated. Some years later, the visions of Giddings were realised by Liu and Phillips [2] who reported this new mode of high-resolution gas chromatography in 1991. In this work, two serially coupled columns with complementary separation mechanisms, ensuring a high degree of orthogonality, were interfaced using a two-stage thermal modulator, which ensured the comprehensive analysis of the entire solute composition. This proved significantly more powerful than previously developed solute-selective heart-cutting techniques. Liu and Phillips acknowledged the limitations of such heart-cutting methods, and commented that "true" comprehensive two-dimensional gas chromatography had not been previously demonstrated. Today, in the decade or so since this first report, GC×GC has attracted considerable interest, and there now exists a considerable body of literature, which validates the fundamental concepts of this technology, and clearly places it as premier among the high-resolution technologies for volatile and semi-volatile sample analysis. To date, GC×GC has been applied to the analysis of petroleum and oil, pesticides, atmospheric contaminants and environmental pollutants, essential oils, foods, drugs and polymers, and will expand to other areas as work gathers pace.

Figure 1 illustrates the generic instrumental set up of the GC×GC instrument, whereby two serially coupled columns, contained in the GC oven (or ovens), are interfaced by using a modulator. Thermal modulators are usually positioned at the head of the second column, whilst a valve modulator will be at the column confluence. The choice of columns or column-set should ensure orthogonal separation and separate solutes according to different chemical properties. A typical column-set is composed of a standard low-polarity column ($^{1}$D), with typical dimensions 25 m×0.25-mm ID×0.25-µm film thickness ($d_f$), coupled to a much shorter, and more polar (or a column providing a separation mechanism capable of further differentiating target sample components) second column, $^{2}$D. The reduced length of $^{2}$D, combined with its comparatively reduced ID and $d_f$ (for example 1 m×0.1-mm ID× 0.1-µm $d_f$) ensures very fast analysis can be performed on the peaks eluting from $^{1}$D after they have been trapped and focussed by the modulator.

The role of the modulator is critical in that it is responsible for the quantitative transfer and compression of all solutes, or a representative fraction thereof, from $^{1}$D to $^{2}$D. Essentially, two types of modulators have been developed, based either on valves, or on thermal differences for peak focussing. The former however, only samples part of the effluent from $^{1}$D and up until recently has not been considered to satisfy the definition of comprehensive. Dimandja et al. [3] stated that *comprehensive* multidimensional chromatography is achieved by using a "valve-less on-column interface" or modulator. The present definition of comprehensive, as determined by consensus at the recent First International Symposium on Comprehensive Multidimensional Gas Chromatography, extends to include valve-based modulators provided that the peaks eluting from the first column are representatively and faithfully sampled by the second column, since orthogonal separation is still achieved using these modulators. Research groups including those at the University of Washington [4] and Seeley (Oakland) [5] have introduced such modulators.

Thermal modulators, which provide mass conservation, utilise temperature gradients for the focussing and rapid pulsing of peaks between $^{1}$D and $^{2}$D and include such modulators as the thermal desorption modulator (thermal

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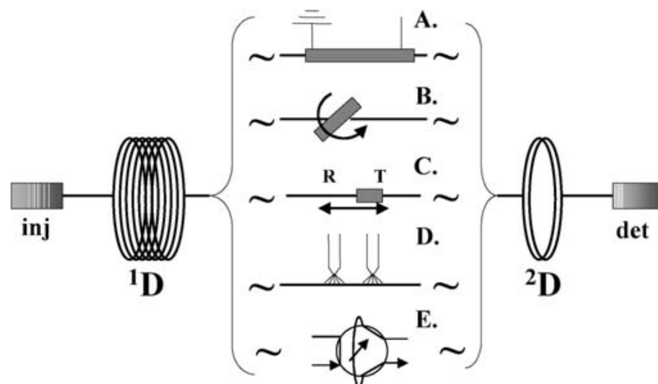


**Fig. 1** Generic schematic diagram of the GC×GC instrument. The modulator is positioned at or near the interface of columns $^1D$ and $^2D$, with the latter employing fast GC conditions. Note that it is possible to contain both columns in the one oven, or use a separate oven for each column. Different modulator types used in GC×GC are shown between the two dimensions. Modulators may be classed as thermal modulation (mass conservative) (*A–D*), or valve (*E*) types. Adapted from ref. [10] with permission

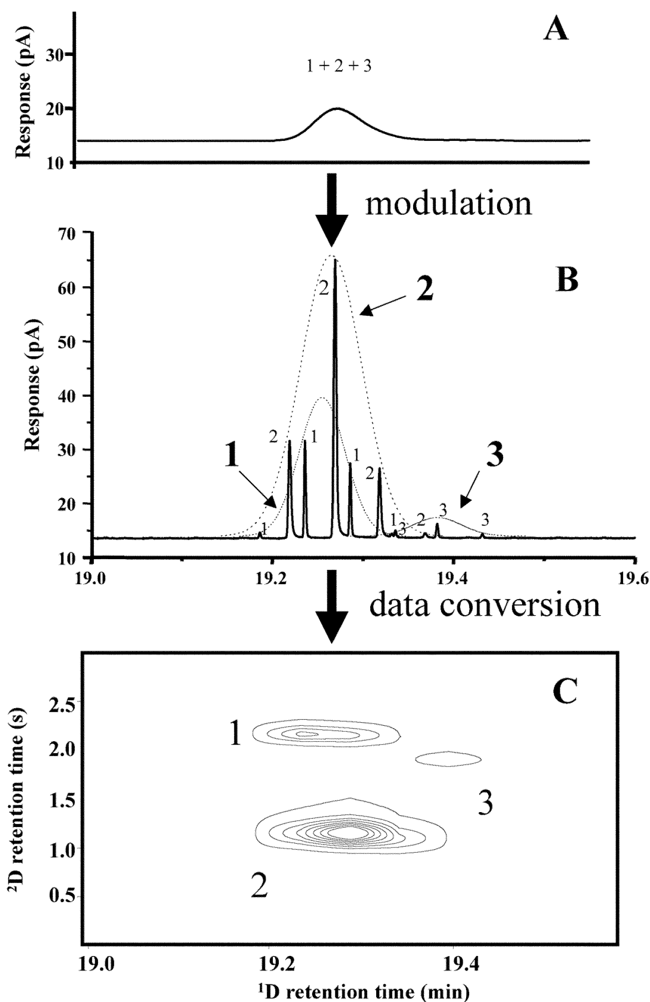


**Fig. 2A–C** Description of the GC×GC process. **A** Normal capillary GC was unable to resolve the three drugs heptaminol *1*, prolintane *2* and pseudo-ephedrine *3*. **B** By using the GC×GC modulation process, three overlapping peak sets, each of which is one compound, are seen and denoted *1*, *2* and *3*. These are shown as the *dotted peak envelopes*, with *individual pulses* labelled similarly giving 4 pulses for compound 1, 4 for compound 2, and compound 3 has only 3 pulses. **C** Converting data to matrix form, the 2D contour plot of each peak is obtained, with each compound fully resolved. The contour levels are in 5-pA steps

sweeper), the longitudinally modulated cryogenic system (LMCS), and cryogenic jet modulators. Figure 1 contrasts the operational modes of these modulators (consult [6] for further modulator details). New modulators continue to be proposed [7, 8]. The performance of modulators should be rigorously tested, although presently there is no specific protocol for this. Excellent retention time reproducibility for the thermal sweeper [3] and LMCS [9] has been reported. The phase of modulation was investigated by Ong and Marriott [10] using the LMCS, where phase effects alter the pulsed peak presentation of data, with a larger modulation period potentially increasing the retention time variation of the largest pulsed peak, with respect to the expected retention time. Such studies provide fundamental information for users, and serve to validate GC×GC technology. Figure 2 illustrates how the modulation process alters the single-dimension chromatographic profile (Fig. 2A), generating pulsed peaks and permitting greater resolution and peak response (Fig. 2B). The modulation process then allows data conversion to a 2D format (Fig. 2C). Here three drugs are now fully resolved, compared with their unresolved composite peak in the one-dimensional analysis.

Detectors used for GC×GC analyses must be adequately fast in order to reliably detect the multiple peaks rapidly emerging from $^2$D which typically have a base width of 150 ms or smaller. Detection acquisition frequency of 50–200 Hz is required. The universal flame ionisation detector and a micro electron capture detector capable of 50-Hz operation give such performance. Time-of-flight mass spectrometry (TOFMS) is rapidly emerging as an important spectroscopic detector for fast GC, including GC×GC. This detector can present data at 500 Hz (it acquires thousands of spectra/s). Conversely, quadrupole MS detectors are normally operated at lower frequencies (e.g. 4 Hz) and cannot cope with the influx of fast GC peaks. Quadrupole detection has therefore been largely overlooked in GC×GC analyses; however, reduced mass range acquisition (e.g. $m/z$ 41–228) allowing faster scanning (up to 20 Hz) has allowed MS characterisation of GC×GC peaks [11].

Data generated from GC×GC analyses are presented in a two-dimensional array, whereby $^2$D retention(s) is(are) plotted against $^1$D retention (min) to produce a contour or surface plot. This 2D chromatogram may permit generation of an "ordered chromatogram" [12] where members of the same compound class having an identifiable $^2$D position in the 2D plane, leading to an ordered structure. This phenomenon results from coupling of columns with "orthogonal separation mechanisms" (i.e. $^1$D might separate compounds based upon boiling point, whereas $^2$D separates compounds based upon a solute-specific retention mechanism). Figure 3 illustrates this phenomenon. The attraction of ordered chromatograms is that unknown ana-

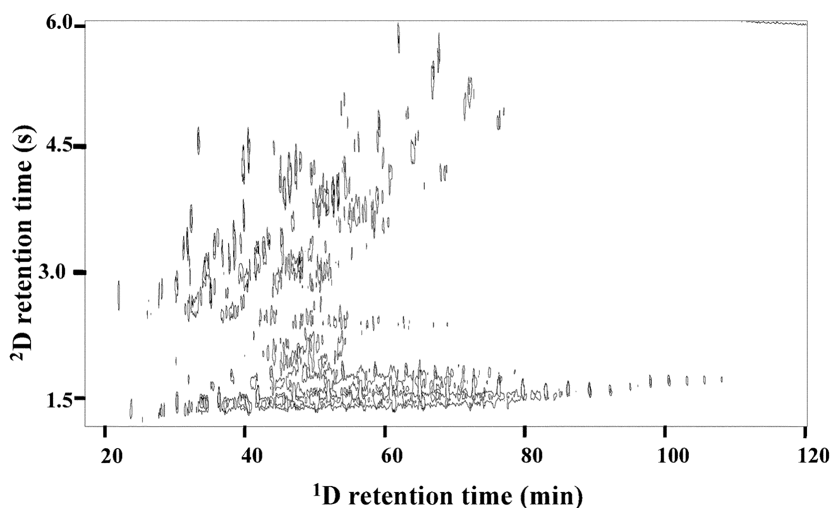


**Fig. 3** GC×GC of a diesel sample. The saturated compounds are located at low ²D retention values (approximately 1.5 s) and will include *n*- and branched alkanes, with cyclic alkanes eluting just after these. Unsaturated hydrocarbons (mono-, di- and poly-aromatics) elute later again, from about a ²D retention time of 2.5 s. Generally, it is possible to state that at any given elution temperature, the more highly unsaturated compounds will elute later on the ²D column, and this leads to a structured 2D separation space. Conditions: ¹D; 3 m× 0.1-mm ID×3.0-μm $d_f$ BP1 phase column: ²D; 0.7-m×0.1-mm ID×0.1-μm $d_f$ BPX50 phase column. 6-s modulation, 40 °C (10 min) to 300 °C at 2.5 °C min$^{-1}$

lytes can be easily ascribed to a specific compound class based upon their relative position in the 2D plane; this has been exploited for class/structure assignment [13] in the analysis of atmospheric samples, with aliphatic, carbonyl, aromatic and bi-aromatic bands easily designated in the 2D chromatogram. Similarly the work of Shell Amsterdam researchers, and Frysinger et al. (US Coast Guard Academy) have established the role of GC×GC for petrochemical and biomarker analysis, and structural chemical features of such samples permit facile recognition of compounds [14, 15]. In the area of environmental analysis, Free University of Amsterdam researchers have made a significant contribution to our appreciation of the breadth of GC×GC applicability including samples as complex as cigarette smoke [16]; GC×GC applications have been reviewed recently [17, 18].

Dedicated data presentation and software packages for the interpretation and handling of GC×GC data are still in a developmental stage; most GC×GC users develop inhouse data conversion both to handle large amounts of data and more importantly to ensure that all "pulses" of a compound are correctly assigned or grouped and reported. For various reasons, peak height is less useful quantitatively than areas. It is likely that real-time 2D data presentation packages, which can generate a comprehensive data report, showing, at the very least, peak height and area data for each individual component, and their averaged ¹D and ²D retentions will be eventually available. Clearly, data analysis is much less advanced than that for 1D GC. Chemometric interpretation of GC×GC data, primarily for peak deconvolution for quantitation purposes, has largely been undertaken by Synovec and co-workers [19, 20]. It is expected that lack of user-friendly software will impede the uptake of GC×GC for routine analysis.

Future trends for GC×GC should see much wider use of TOFMS detection; GC×GC separation will permit generation of significantly cleaner mass spectra with peaks better resolved from interfering species and so more reliable library matching. It is expected that the number of GC×GC applications will dramatically increase, and that special case applications such as enantiomer-GC×GC and chemometric principle component analysis and fingerprinting will advance. Opportunities for compound identification and complete sample characterisation and multiresidue screening have never been more promising.

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