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Review

Comprehensive multi-dimensional gas chromatography

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Abstract

In comprehensive two-dimensional gas chromatography, the entire chromatogram eluting from the primary column is submitted to the secondary column for a second independent separation. The resulting two-dimensional chromatogram has peaks scattered about a plane rather than along a line. Peak capacity can be very large allowing much more complete separation of complex mixtures such as petroleum products. Moderately complex samples can be separated much more quickly than is possible with high-resolution one-dimensional gas chromatography. The method is a true hyphenated instrument analogous to gas chromatography–mass spectrometry.

Contents

1. Introduction

Comprehensive two-dimensional gas chromatography (GC–GC) is a multi-dimensional method of analysis. It is one of a great many possible two-dimensional couplings of separation techniques [1]. It is also a member of the class of hyphenated analytical methods in which the coupled techniques are not necessarily separations. In its essential principles, GC–GC closely resembles gas chromatography–mass spectrometry (GC–MS). Both methods combine independent analytical techniques and generating comprehensive two-dimensional data.

Fig. 1 compares GC–MS and GC–GC instrument designs. In both of these instruments, gas chromatography first disperses the sample's components in time presenting them to the secondary analytical instrument either individually or at least in greatly simplified sub-mixtures.

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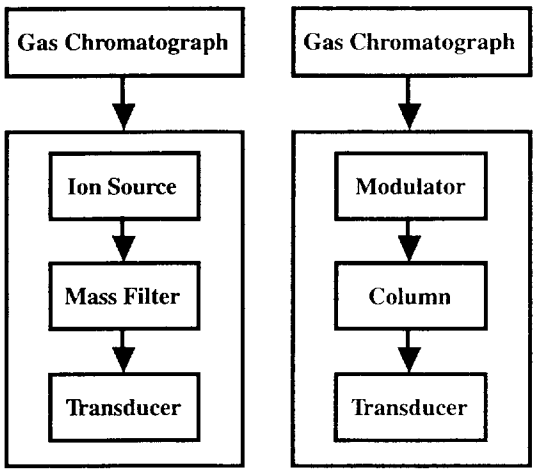

Fig. 1. Schematic diagrams of gas chromatography–mass spectrometry and comprehensive two-dimensional gas chromatography instruments.

This first dispersion creates a primary retention time axis. The secondary analytical instrument operates as a detector for the inlet GC. Unlike the more common GC detectors, however, this detector has resolving power of its own and provides, in some sense, an independent analysis of the dispersed sample eluting from the primary GC. In GC–MS, the secondary instrument, a mass spectrometer, ionizes and fragments sample components dispersing the fragments along a secondary mass-to-charge ratio axis. In GC–GC, the secondary instrument is another gas chromatograph which disperses simplified submixtures along a secondary retention time axis. In both cases, the secondary instrument disperses the sample along an axis which is conveniently placed orthogonal to the inlet GC's retention time axis to form a two-dimensional data space. These two instrument designs really are quite similar. To convert a GC–MS into a GC–GC, one simply replaces the mass spectrometer with a fast gas chromatography changing the orthogonal secondary axis from a mass-to-charge to a retention axis.

GC–MS and GC–GC differ fundamentally in one respect. In GC–MS, one substance can generate numerous signals along the secondary mass-to-charge ratio axis while the GC–GC one substance generates only one signal at one specific location along the secondary retention time axis. This difference affects how the data from the two instrument designs are interpreted. GC–MS data can be interpreted simply as a set of mass spectra with the inlet GC being used to present the sample components to the MS one at a time. The information content of the inlet GC's retention time axis and the two-dimensional nature of the data are ignored reducing the data to one dimension. This is reasonable because the mass spectra alone are usually enough to identify a substance; the information contained in GC retention times is largely redundant. This feature of GC–MS leads to a problem in that either the inlet GC should have very high resolution or the method should be applied only to relatively simple mixtures to guarantee that all substances are separated and the mass spectra are not contaminated by overlapping substances. Interpreting GC–GC data simply as a set of fast gas chromatograms, however, is generally not very useful. The two retention time axes contain complementary information and both are needed to reliably identify substances. Because one substance occupies only a limited and well defined portion of the secondary axis, the inlet, or primary, GC does not have to guarantee separation of all substances and can be of lower resolution and, therefore, faster than that required for GC–MS. GC–GC data are commonly presented in complete two-dimensional form rather than as individual secondary chromatograms to show all the information.

Conventional two-dimensional gas chromatography, commonly known as heart-cutting, also serially couples two different chromatographic columns. Heart-cutting is not a comprehensive hyphenated method, however, because the secondary instrument cannot be applied to the entire chromatogram eluting from the inlet GC. The secondary GC, considered as an independent instrument, is much too slow to serve as a detector for the primary GC column.

In any comprehensive hyphenated method, GC–MS, GC–GC, or whatever, the secondary instrument must make measurements fast enough to preserve the information contained in

the primary instrument signal. That is, the primary instrument signal must pass through the secondary instrument with fidelity. In a truly hyphenated or comprehensive GC–GC instrument, the secondary GC must operate fast enough to generate at least one complete chromatogram during the time required for a peak to elute from the primary GC column and it must be possible to reconstruct the primary column chromatogram from the two-dimensional data just as a chromatogram can be reconstructed from GC–MS data.

The determination of one substance in a complex mixture may require a two-dimensional separation to isolate the target substance. A comprehensive separation may not be required if the rest of the mixture is not of interest. For applications like this, heart-cutting methods are more appropriate than comprehensive GC–GC. This is analogous to certain simplified GC–MS methods in which the mass spectral data at only one particular retention time or the signal intensity at only one selected mass-to-charge ratio is examined.

Comprehensive GC–GC has been demonstrated [2]. Key features of this technique are the use of an on-column thermal modulator to collect sample portions from the primary column and transfer them to the secondary column and the variation of retention in the secondary column as a function of progress of the primary column to orthogonalize the separation [3,4]. The method has been applied to the analysis of complex petroleum mixtures [5] and to the determination of pesticides in human serum [6].

2. Experimental

Fig. 2 shows a comprehensive two-dimensional gas chromatograph. A dual oven gas chromatograph equipped with a flame ionization detector based on an IBM model GC/9630 was used. The primary column was placed in one of the two ovens and the secondary column in the other. The two ovens were placed apart and between them a separate length of chromatographic column held at room temperature was installed to

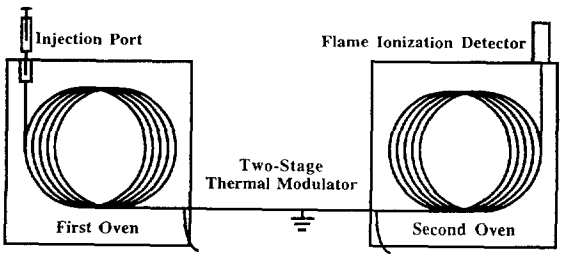

Fig. 2. Comprehensive two-dimensional gas chromatography.

form a two-stage thermal modulator. Sample portions eluting from the primary column were transferred to the secondary as sharp concentration pulses by electrically heating the thermal modulator.

The primary column, obtained from Quadrex Corporation, was 10 m long, 530 μm I.D. with a methyl silicon stationary phase 8 μm thick. The secondary column, obtained from Alltech Associates, was 25 m long, 250 μm I.D. with a Carbograph VOC stationary phase.

The thermal modulator interface was prepared from a 40-cm length of 250 μm I.D. column containing a 0.25-μm film of DB 5 stationary phase. The external polyimide film was removed from the modulator and replaced by a thin film of gold metal. The gold metal film was prepared by painting the modulator section with Bright Gold N (Englehard Industries, East Newark, NJ, USA) and heat curing at approximately 500°C using a heat gun. The modulator had two states. The first was 25 cm long with an electrical resistance of 180 Ω and the second stage was 15 cm long with a resistance of 100 Ω. The computer generated current pulses of 800 ms and 400 ms durations to the modulator first and second stages, respectively, at a fixed interval of 60 s with a delay of 1 s between pulses applied to the two stages. A variable power supply operated at 60 V D.C. provided current to the modulator stages through ODC5P optically coupled relays (OPTO22, Huntington Beach, CA, USA). More details of modulator preparation and operation are given elsewhere [3,4,7].

Both columns were held isothermal for 60 min, the primary at 40°C and the secondary at

85°C. After 60 min both ovens were increased in temperature at rate of 0.1°C/min. Hydrogen at 2.8 atm pressure and 12.8 ml/min flow was used as the carrier gas. The entire flow emerging from the primary column passed through the thermal modulator and into the secondary column. The flow through both columns was the same.

A Macintosh IIci computer (Apple Computer, Cupertino, CA, USA) equipped with an NB-MIO-16X interface card and LabVIEW 2 software (National Instruments Corporation, Austin, TX, USA) was used for instrument control and data acquisition. A QED single board computer (Mosaic, Inc., Newark, CA, USA) was used to generate modulation timing pulses. Spyglass Transform and Format software (Spyglass, Inc., Savoy, IL, USA) was used to prepare two-dimensional chromatographic plots.

3. Results and discussion

3.1. Comprehensive GC–GC of kerosene

Fig. 3 shows a portion of a two-dimensional gas chromatogram. The primary column retention time axis is calibrated in minutes and the secondary is calibrated in seconds. Signal intensity is indicated by color code as shown by the color bar. To reduce dynamic range, the square root of the signal intensity is plotted. The largest few peaks, such as the *n*-alkanes, are clipped to bring smaller peaks into the range of the color code. The clipped peaks have uniformly gray flat tops. Some peaks have been tentatively identified. These are listed in Table 1. A few substances (naphthalene and its derivatives) have second dimension retention times which exceed the modulation period. Each secondary chromatogram in which one of these substances appears, extends beyond the time allocated for a secondary chromatogram and overlaps the beginning the following secondary chromatogram. These substances wrap around in the two-dimensional chromatogram overlapping low retention peaks.

Approximately 600 secondary chromatograms are plotted to generate this two-dimensional chromatogram. Every secondary chromatogram in this range contains at least ten peaks. Kerosene is an extremely complex mixture with many thousands of individual substances present at detectable concentrations. At no time during the range of the chromatogram shown here does any substance emerge from the primary column separated from all others. The primary column only has sufficient peak capacity to partially separate the mixture and present a series of simplified sub-mixtures to the secondary column.

Each substance eluting from the primary column is present in at least three secondary chromatograms. Thus, the second dimension is comprehensive and the primary chromatogram can be reconstructed from the two-dimensional data by summing out the second dimension. The reconstructed chromatogram is equivalent to that which could have been obtained using the primary column alone. The reconstructed primary chromatogram is not very interesting because the primary column's peak capacity is much too small for this extremely complex mixture.

3.2. Independence of separation axes in comprehensive GC–GC

The chromatogram in Fig. 3 clearly demonstrates that the separations in the two dimensions are independent of each other. The retention of a substance along one axis is not related to its retention along the other. For example, the *n*-alkanes distribute along the primary column retention axis according to their volatilities but all have very nearly the same retention on the secondary column axis. The first dimension separation within this homologous series is due to the variation in substance volatility. Members of the series do not separate significantly in the second dimension because they do not differ significantly in any chemical property other than volatility. Other substances which happen to have the same volatility as one of the *n*-alkanes are not separated from them in the first dimension but are separated in the second. Whatever chemical mechanism results in distribution of substances along the first dimension axis is unavailable in the second dimension. The second

Fig. 3. Two-dimensional gas chromatogram.

Table 1
Some tentatively identified substances in a comprehensive two-dimensional chromatogram of kerosene

C11	*n*-Undecane
C12	*n*-Dodecane
C13	*n*-Tridecane
C14	*n*-Tetradecane
C15	*n*-Pentadecane
A22	2,3,4,7-Tetrahydro-1H-indene
A23	1-Methyl-3-*n*-propylbenzene
A24	1-Methyl-4-*n*-propylbenzene
A25	*n*-Butylbenzene
A26	1-Ethyl-3,5-dimethylbenzene
A27	1,2-Diethylbenzene
A28	1-Methyl-2-*n*-propylbenzene
A29	1,4-Dimethyl-2-ethylbenzene
A30	1,3-Dimethyl-4-ethylbenzene
A31	1,2-Dimethyl-4-ethylbenzene
A32	1,3-Dimethyl-2-ethylbenzene
A33	1,2-Dimethyl-3-ethylbenzene
A34	1,2,4,5-Tetramethylbenzene
A35	2-Methylbutylbenzene
A36	*trans*-1-Butyl-2-methylbenzene
A37	*n*-Pentylbenzene
A38	1-*tert*.-Butyl-3,5-dimethylbenzene
A39	1,3,5-Triethylbenzene
A40	1,2,4-Triethylbenzene
A41	*n*-Hexylbenzene
NA1	Naphthalene
NA2	2-Methylnaphthalene
NA3	1-Mentylnaphthalene

dimension separation must, therefore, be based on a different and independent mechanism. In Fig. 3, the first dimension separation is based largely upon substance volatility because the primary column contains a non-polar stationary phase. Separation in the second dimension is independent of volatility and is based on substance polarity.

The independence of separation mechanism in the two dimensions of a comprehensive two-dimensional gas chromatograph is at first surprising. Both dimensions are gas chromatography and, therefore, interact with sample components in chemically similar ways. Substances which are strongly retained in the primary column might be expected to be strongly retained in the secondary creating a two-dimensional chromatogram with highly correlated retention times. The temperature program applied to both columns, however, reduces the strength of retention in the secondary column as a function of progress of the primary column separation to eliminate retention correlation. In creating a hyphenated method, it is not necessary to use two instruments based on different principles (as in GC–MS). Instead, two very similar instruments can be coupled and tuned to generate orthogonal data.

3.3. Comparison to other methods

Methods using multiple heart-cuts have some of the characteristics of comprehensive GC–GC operation. Gordon et al. [8] described a method in which heart-cuts are taken at several locations in a complex gas chromatogram. Each additional heart-cut determination requires an additional injection into the primary column. Wilkins and co-workers [9] described an instrument with multiple cold traps to save multiple heart-cuts from one chromatogram on the first column for sequential analysis on a secondary column. In both of these techniques, the number of secondary chromatograms is quite limited—in the first case by the time and sample required for repeated chromatograms and in the second case by time and the number of cold traps that can be connected. The rate of sample transfer to the secondary column is quite insufficient for preservation of the first dimension chromatogram through the secondary column analysis.

In liquids, planar methods, either chromatographic or electrophoretic, can be sued to generate comprehensive two-dimensional separations of large peak capacity [10]. Jorgenson and co-workers have described comprehensive two-dimensional liquid chromatography and liquid chromatography / capillary electrophoresis [11–13]. These methods, especially those using fast capillary electrophoresis as the second dimension, have the potential to generate peak capacities comparable to those obtained with comprehensive GC–GC. LC–CE methods differ from this GC–GC method in one fundamental way; the two coupled techniques are not based on the same chemistry and tuned to be independent but instead depend on the inherent differ-

ence in retention mechanism to scatter peaks over the retention plane.

3.4. Advantages of the comprehensive GC–GC method

The chromatogram in Fig. 3 illustrates some of the advantages of the comprehensive GC–GC method. First, peak capacity is very large, much larger than that of any practical one-dimensional chromatographic separation. Fig. 3 has a peak capacity of approximately 30 000 and contains about 4000 peaks. Second, retention in the second dimension is independent of retention in the first dimension and so is a measure of a secondary molecular property. Since the secondary column used here is more polar than the primary column, second dimension retention measures substance polarity. Third, each substance is identified by two independent retention measures and so can be much more reliably distinguished from other substances. The secondary GC axis provides confirming identification much like the mass-to-charge ratio axis of GC–MS. For extremely complex mixtures, identification by GC–GC may actually be more reliable than by GC–MS because interfering substances are more cleanly separated. And fourth, the two-dimensional chromatogram provides a much more complete picture of the mixture as a whole. The distribution of substances with both volatility and polarity is readily apparent and structure within the chromatogram illustrates chemical relationships among mixture components. The details of substance distribution over the retention plane depends on the stationary phases chosen. Previously published chromatograms obtained using a different set of stationary phases show a somewhat different distribution (5).

3.5. Speed of comprehensive GC–GC separations

Because comprehensive GC–GC generates much more information than either one-dimensional or heart-cutting GC methods, it is reasonable to expect it to require more time. More information requires a more complex signal which requires more time to generate and transmit through the detector. The temperature programming rate used for the chromatogram in Fig. 3 is quite slow to allow time for the large number of secondary chromatograms. Operating the primary column at this very slow rate does not affect detection and quantitation, however, because peak duration at the detector is determined by the much faster secondary column. The sensitivity of comprehensive GC–GC is generally substantially better than that of high-resolution one-dimension chromatography because peaks at the detector are sharper and, therefore, taller.

Substantially faster operation is possible. The speed of the secondary column could be increased without loss of resolution simply by decreasing its inner diameter and length. Using a 50-μm secondary column in place of the 250 μm used here should decrease analysis time by at least a factor of five which would make the time required by this method comparable to that required by high-resolution one-dimensional chromatographic methods with an order of magnitude less resolving power. Such a reduction in column size is practical because the thermal modulator works equally well with any diameter column. For mixtures not quite as complex as this, both columns could be shortened reducing analysis time dramatically while still providing more peak capacity than any practical one-dimensional method. The detector time constant ultimately limits analysis speed.

Comprehensive multi-dimensional gas chromatography is not limited to only two dimensions. A GC–GC–GC method could be created by coupling three independent chromatographic separations in the same way that two have been coupled here. Each successive dimension must be substantially faster than the previous dimension. Others, for example van Es et al. [14], have demonstrated extremely fast gas chromatography. Total retention times of well under one second are possible. Even a relatively low peak capacity third dimension, if it is made orthogonal to the first two dimensions, could be extremely valuable because peak capacity of a multi-dimen-

sional comprehensive chromatography is equal to the product of the peak capacities of the constituent dimensions. GC–GC–MS using a fast mass spectrometer would also be a very powerful method.

4. Conclusions

Comprehensive GC–GC is still a new and relatively undeveloped method. It has the potential to dramatically increase the resolving power that can be applied to extremely complex mixtures such as petroleum products and to increase the speed of analysis for mixtures of more moderate complexity. Ultimately, any mixture containing more than about thirty components could probably be separated faster by this technique than by any one-dimensional method and any mixture in which more than a few hundred components are to be separated will require this technique.

High resolution gas chromatograms with components distributed over two or more dimensions differ in fundamental ways from the familiar one-dimensional gas chromatograms. Unambiguous information about molecular properties such as polarity and shape can now be provided. Interpretation and utility of this information is as yet undeveloped, however. Patterns formed by distributing a mixture over a retention plane are potentially valuable in understanding the chemistry of the mixture as a whole, but as yet little has been done to understand or interpret these patterns.

The technical demands of comprehensive two-dimensional gas chromatography are greater than those of conventional one-dimensional GC but significantly less than those of other well accepted hypenated instrument methods such as GC–MS. At present, the technique is relatively undeveloped and, thus, more technically challenging than it need be. As the required instrument components such as thermal modulator devices and computer software are developed and become commercially available, the technique should become as routine as GC–MS presently is.

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