Chapter 2

Fundamental principles of comprehensive multi-dimensional chromatography

2.1 Multidimensional techniques

The term *multidimensional separation* refers to the combined use of different separation mechanisms to resolve components in complex mixtures. Possible combinations include separation methods such as simple solvent extraction, the different chromatographic mechanisms and electrophoretic techniques. In theory, any physical or chemical selective technique available to the separation scientist can be used to develop a multidimensional system.

2.2 Multidimensional chromatography

In multidimensional chromatography, fractions from a chromatographic system are transferred to one or more additional chromatographic separation systems to improve resolution and sensitivity or to decrease analysis time. This is sometimes referred to as *coupled column chromatography,* but generally the term *heart cutting* is used. With this technique, a selected portion of a chromatogram is transferred to another column that operates according to a different separation mechanism. Simmons and Snyder first demonstrated this in 1958. Since the ingenious invention of valveless pressure switching

by Deans[1], it became possible to collect fractions without fear of reactive sample components absorbing on valve materials. This technique works very well for target analysis. Only a few cuts can generally be made from the first separation. If many cuts or two cuts eluting close together are to be analyzed, re-injection of the same sample is normally required. In cases where the entire matrix needs to be analyzed, as often happens in the petrochemical industry, this system is extremely tedious and time consuming.

2.3 Comprehensive Multidimensional Chromatography

Comprehensive multidimensional chromatography is a special case of multidimensional chromatography. Here, the second column analyzes the entire sample. The initial resolution is conserved by sampling many times across every peak eluting from the first column. The repetitive 2nd dimension analysis represents a two dimensional separation with its injection time corresponding to the elution time of the first chromatogram. Integration of the 2nd dimension gives a data point that can be used to recreate the original one-dimensional chromatogram. This is analogous to the total ion chromatogram obtained from a GC-MS analysis[2]. This digitizing of the first chromatogram is achieved with a modulation device. The modulator samples and often refocuses a small fraction of the first chromatogram, which the second column analyzes in the same time slot in which the modulator collects the next fraction.

2.4 Orthogonality

Multidimensional separations are only effective when unrelated selectivity mechanisms are involved in the different separation stages. When the mechanisms are completely different and independent the components are widely distributed across a plane (See Figure 2-1,A). For each component of a sample, two retention time values are generated, one for each dimension. Two such separations are said to be orthogonal[3]. The word

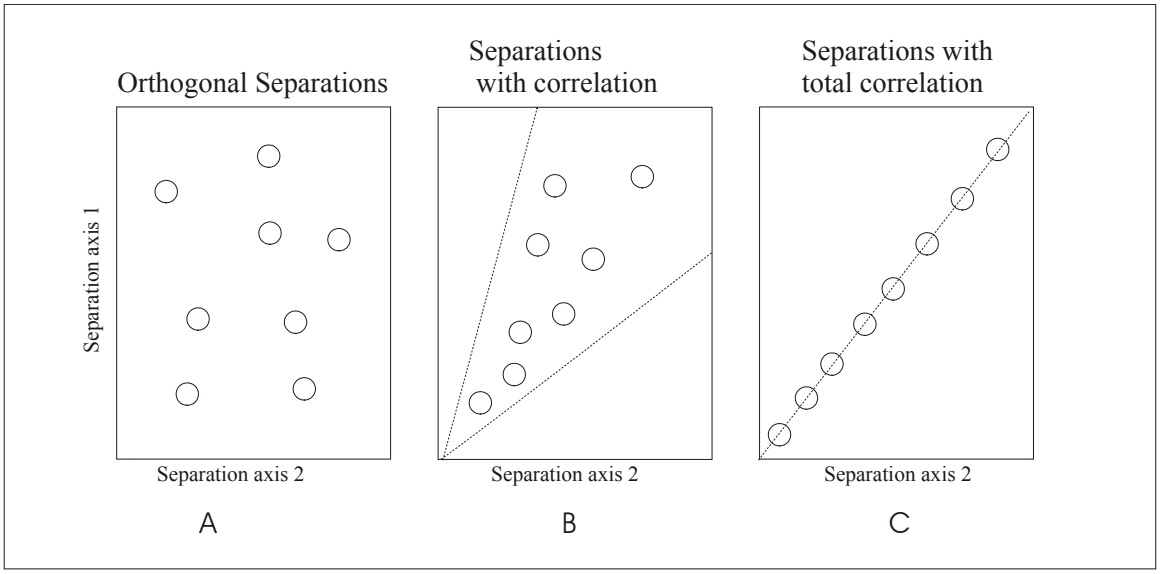
[1] D.R.Deans, Chromatogaphia, 1 (1968) p18

[2] P.J.Schoenmakers, et.al. J.Chromatogr.A. 892 (2000) p29

[3] C.J.Venkatramani, J.Xu, J.B.Phillips, Anal.Chem. 68 (1996) p1486

orthogonal originates from the right angles of the plane that describes the generated separation space. Any correlation between the two dimensions tends to degrade this right angle. At the point where correlation is 100% all the compounds are once again distributed on a single line – the diagonal across the plane (See Figure 2-1, C). Generating inaccessible peak capacity wastes time and reduces the efficiency of the multidimensional system. Minimizing the synentropy or cross-information between the separation dimensions maximizes information production.

Figure 2-1: Separation space utilization by orthogonal and correlated mechanisms



2.5 Resolution in two-dimensional chromatograms

Some of the definitions for one-dimensional separations need to be extended to cater for the additional complexity of multidimensional separations. Preferably, terms already developed in chromatography should be extended to include all cases of chromatography. One such term is resolution.

There are many ways to express chromatographic resolution, as discussed in detail in Chapter 3.2 . In practice, resolution in a one-dimensional separation is often measured with: $R = \Delta t / 4\sigma$. Δt is the difference in the retention time maxima of two components. σ is the average standard deviation of two Gaussian peaks. When R is larger than 1, the

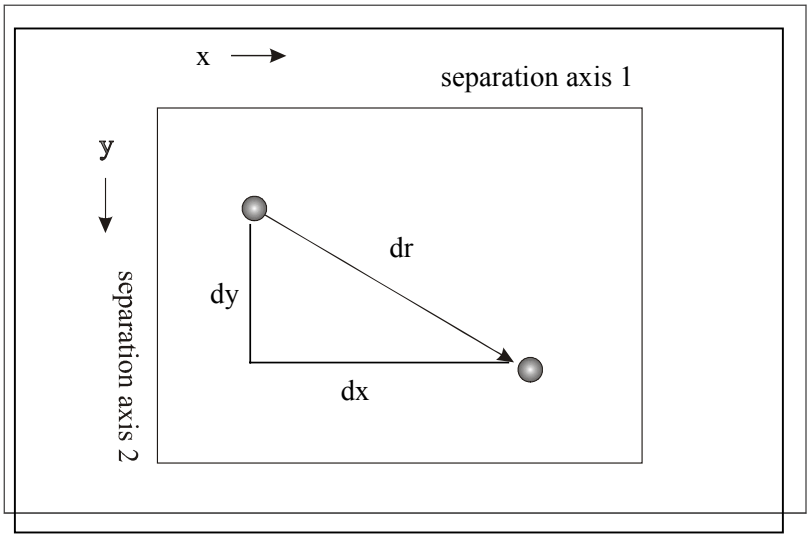
difference in retention time (Δt) is larger than the peak broadening (the standard deviation term) and peak spacing is adequate to observe two distinct component zones. When R is less than 0.5, peaks are completely fused and with R larger than 1.5 the peaks are completely (baseline) resolved. This definition has been extended to the two-dimensional case.

Two treatments of the resolution for two-dimensional bivariate Gausian zones have been proposed [4,5,6]. However, Schure showed both treatments to be equivalent when the zone broadening for two adjacent peaks are equal in both dimensions [7].

The distance between two spots on a plane, δr, is given by the Pythagorean expression (see Figure 2-2)

$$dr = \sqrt{dx^2 + dy^2} \qquad \text{[eq2-1]}$$

Figure 2-2: Cartesian, or Euclidean plane showing the Pythagorean relation



[4] J.C.Giddings,in Multidimesional Chromatography, H.J.Cortes (ed.), Chromatographic Science series, vol 50, Marcel Dekker, New York (1990) p1

[5] J.M.Davis, Anal.Chem. 63 (1991) p2141

[6] W.Shi, J.M.Davis, Anal.Chem. 65 (1993) p482

[7] M.R.Scure,J.Microcol. Sep. 9 (1997) p169

Differential displacement as well as 2D zone broadening determines resolution in two dimensions. If the separation along either axis is large enough to overcome zone broadening and yields good resolution, the separation cannot be undone by any displacement, positive or negative, in the other axis.
The resolution evolving along each one-dimensional axis is:

$R_1 = dx / 4\sigma_1$ and [eq2-2]

$R_2 = dy / 4\sigma_2$ [eq2-3]

Applying the Pythagorean relation the resolution in two dimensions is:

$$R_{2D} = \frac{dr}{4\sigma} = \sqrt{\left(\frac{dx}{4\sigma}\right)^2 + \left(\frac{dy}{4\sigma}\right)^2} \quad \text{[eq 2-4]}$$

σ, the mean standard deviation along the line that connects the two centers, can be approximated by the average of σ_1 and σ_2. This is especially true for spherical zones.

$$R_{2D} = \sqrt{R_{s1}^2 + R_{s2}^2} \quad \text{[eq2-5]}$$

Using this equation, it is simple to derive a method for determining R_{2D}[7,8] using the easy to measure 'peak to valley ratio', P, defined as

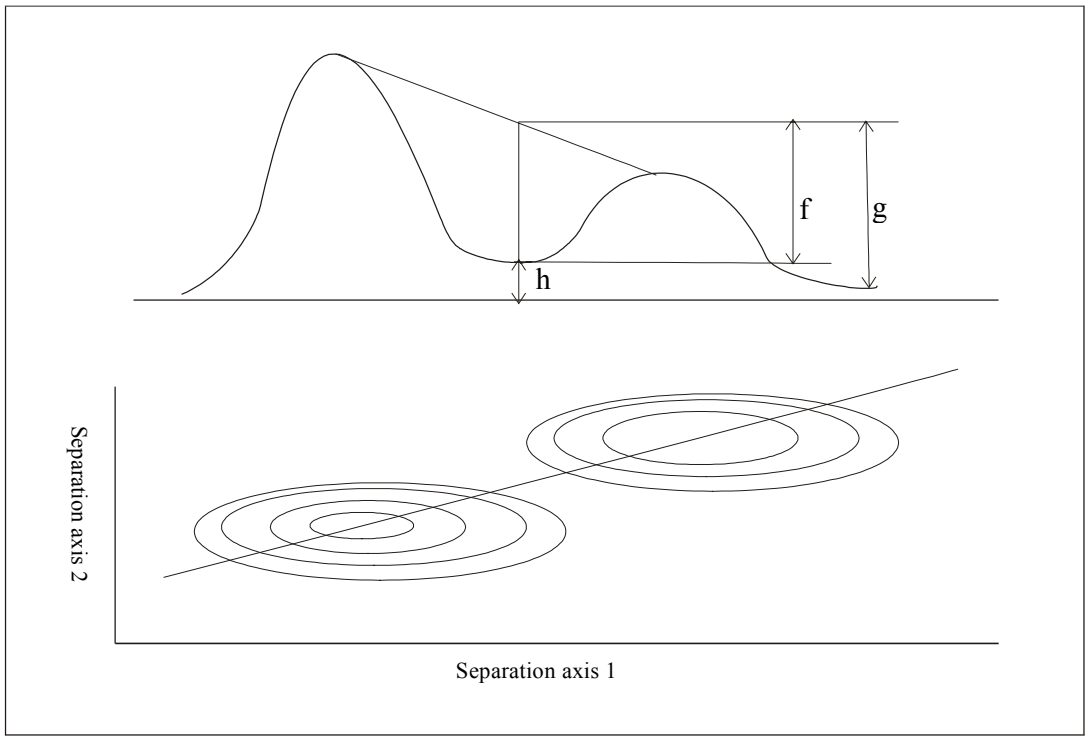
$$P = \frac{f}{g} \quad \text{[eq2-6]}$$

As shown in Figure 2-3, $\boldsymbol{f}$ is the difference between the amplitude at the valley, $\boldsymbol{h}$, and the average peak maximum, $\boldsymbol{g}$. Assuming that peaks are Gaussian, the two-dimensional resolution can also be calculated as [7,8].

[8] R.E.Murthy, M.R.Schure, J.P.Foley Anal.Chem. 70 (1998) p1585

$$R_{2D} = \sqrt{-\tfrac{1}{2}\ln\left(\frac{1-P}{2}\right)} \qquad \text{[eq2-7]}$$

Figure 2-3: Schematic diagram of the two-dimensional resolution measurement using a 2-dimensional contour plot and the corresponding slice for resolution determination.



2.6 Peak capacity of comprehensive multidimensional systems.

The powerful separation capabilities of one-dimensional column chromatography are often inadequate when it comes to the analysis of complex samples[9,10]. As the number of components increase, a drastic increase in plate count is necessary to resolve all the components of a complex sample. Giddings developed a mathematical model to illustrate the limitations of one-dimensional chromatographic systems[4].

[9] G.Guiochon, J.Chromatogr., 185 (1979) p3

[10] G.Guiochon, A.M.Siouffi, J.Chromatogr., 245 (1982) p1

He formulated the overall resolving power of a linear column in terms of the peak capacity $\mathbf{n_c}$. The peak capacity is defined as the maximum number of peaks that can be resolved side by side into the available separation space. For non-programmed runs, peak capacity is related to the number of theoretical plates (**N**) by:

$$n_c = \phi N^{1/2} \qquad \text{[eq2-8]}$$

where ϕ depends on the retention time range or available separation space.
At first glance it would appear as if a column can separate any number of components, **m**, as long as $\mathbf{m \leq n_c}$. This is true as long as all peaks are evenly distributed in the available separation space, filling the entire available space by separated peaks. Unfortunately this is rarely the case. Peaks are randomly distributed over the chromatogram and often overlap. The situation can be improved by ensuring that the number of components in the sample is much smaller than the peak capacity of the column. $\mathbf{n_c >> m}$. In practice, the available plate numbers severely limit the number of randomly eluting peaks, **m**, that can be resolved in a one-dimensional chromatographic run. Using the Statistical Model of Overlap (SMO)[11], Giddings estimated the need for an astounding 400,000,000 plates if 98 out of 100 randomly eluting compounds are to be separated[4] by one-dimensional chromatography.

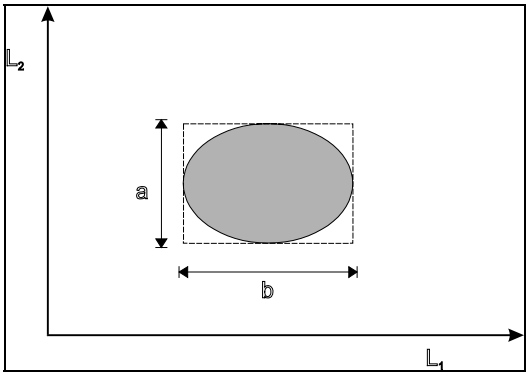
Multidimensional systems provide an alternative method for increasing n_c. Separation by two independent, orthogonal retention mechanisms produces a retention plane. The peak capacity of the retention plane is the product of the peak capacities of the individual columns. If both columns can resolve 100 evenly spaced components then the system has a total peak capacity of $n_c = 10^4$ and the system can separate 98 out of 100 randomly eluting compounds. According to Giddings[4] only 40,000 plates are required in a two-dimensional system to generate a peak capacity of 100. A one-dimensional system would require 4×10^8 plates to achieve the same peak capacity of $n_c = 10^4$.
Due to peak broadening in both dimensions, components are present on the retention plane as two-dimensional ellipses (**Figure 2-4)**. Thus the total peak capacity for an

[11] J.M.Davis, L.C.Giggings, Anal.Chem, 39 (1983), p418

orthogonal two-dimensional separation system comprising two orthogonal separation mechanisms is given by:

$$n_c = \frac{L_1 L_2}{ab} = n_{c1} n_{c2} \qquad \text{[eq 2-9]}$$

Figure 2-4: Peak capacity on a retention plane



Where L_1 and L_2 is the separation space of the first and second dimension respectively (i.e. the total analysis time of each dimension) and *ab* is the area of the rectangle that circumscribes the ellipse on the separation plane. For spherical spots this is the number of spots that can be packed into a body centered cubic structure[12].
This equation holds for a multidimensional coupled column separator only if the number of transfers to a second column equals or exceeds the peak capacity of the first column. This ensures that the first dimension resolution is maintained. This approach is called comprehensive multidimensional chromatography (CMC).

In the case of heart cutting, where only selected cuts are transferred to secondary columns, the total peak capacity is equal to the sum of the peak capacities of the secondary columns. The contribution by the first dimension to the total peak capacity of the system is reduced to the number of cuts transferred to the second dimension.

[12] J.C.Giddings, HRC, 10 (1987) p319

$$n_c = \sum_{i=i}^{k} n_{ci} \qquad \text{[eq2-10]}$$

If, for example, 6 cuts are transferred and analyzed sequentially on one secondary column, then the maximum gain in peak capacity is the sum of every transfer to the second column. $n_c = 6 \times n_{c2}$.

An alternative definition for peak capacity of a 2D chromatogram was put forward by Davis[5]. The peak capacity is defined as the ratio of the total area A of the chromatogram to the area A_0 required for the resolution of any zone.

$$n_{c,alt} = \frac{A}{A_0} \qquad \text{[eq2-11]}$$

Peak capacity defined in this manner is related to the traditional n_c (eq2-9) for zones packed into a body centered cubic structure by a numerical factor: $\pi / 4$

$$n_c = \frac{\pi}{4 n_{c,alt}} \quad \text{or} \quad n_c = 0.79 n_{c,alt} \qquad \text{[eq2-12]}$$

The advantage of defining peak capacity in this way is that a general theory, free from geometric factors that depend on zone distribution and bed shape, could be developed. This theory resembles the theory developed for 1-D separations in that both depend only on the expected number of components (*m*) and the *saturation* (λ) or the component density of the chromatogram ($\lambda = m / n_c$).

Even though the total peak capacity of a two-dimensional chromatogram is theoretically described as $n_{c(total)}=n_{c1} \times n_{c2}$, in practice it is found that the ability to resolve peaks does not increase in direct proportion to the increase in peak capacity. Fewer peaks can be baseline resolved (R=1.5) in a two-dimensional separation than for the corresponding peak capacity in a one-dimensional separation.
Davis offered the following explanation[5]:

In a 1-D separation, in which the baseline width of a single component peak of standard deviation σ is $x_o = 6\sigma$, one must provide x_o units of component free space on both sides of the peak maxima to ensure baseline resolved peaks. Thus, two units of x_o are required for each well-separated peak of width x_o.
For a 2-D separation where the single component zone is $A_o = \pi r^2$ one must provide with and an area $\pi(2r)^2$ of component free space, corresponding to the circle of overlap, to ensure that the zones are baseline resolved.

Thus, for every two component free widths required to achieve baseline resolved peaks in one dimension, four component free areas are required in two dimensions.

This does not contradict the fact that 2-D separations are better than 1-D separations. So much peak capacity is produced by a 2-D separation that one can afford to waste some of it through ineffective utilization of the peak capacity but still achieve better separation. 2-D separations also have the added advantage of producing ordered chromatograms.

2.7 Sample dimensionality and Ordered Chromatograms:

Giddings's Theorem[13]:
Given systematic variation in the molecular structures of a mixture of components, and dimensionality match between that mixture and its separator, the separation will be ordered.

It is evident that an increase in resolution of randomly eluting compounds is possible if the available separation space of a one dimensional line chromatogram is expanded to a plane by two orthogonal separation dimensions.

[13] E.B.Ledford(Jr.), GcXGc: How it works and why, Presented at the ATAS Symposia Uk,Cambridge, May 1, 1997

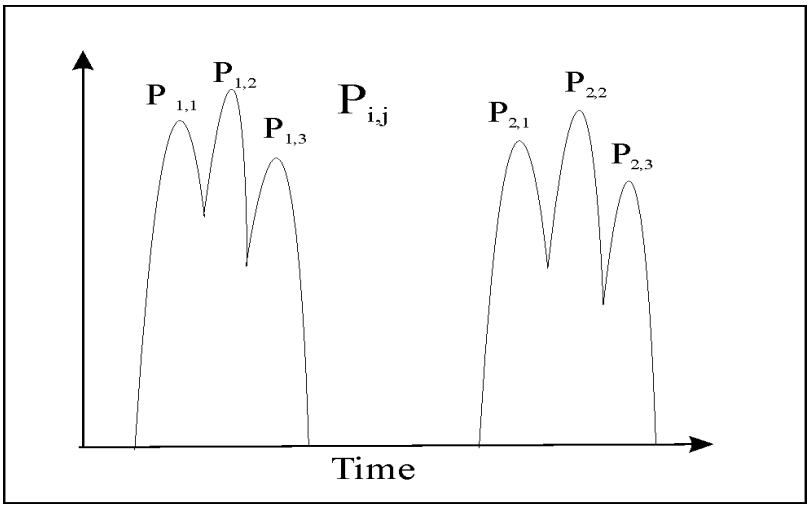
An additional increase in information production can be achieved if the separation system is designed to elute components of a complex sample in a systematically ordered fashion. This calls for a selectivity correlation between the separation dimensions and the properties of the sample. For a truly ordered and unambiguous analysis, there need to be as many separation-dimensions as there are sample properties. Giddings[14] suggested the term *sample dimensionality* (s) for the number of sample properties that defines a molecular entity. This means that the molecular identity of a component is fully established once the displacements along the n-dimensions of the separator are determined. s = n. Generally if two or more retention mechanisms are operative in a single chromatographic dimension, components will not be separated systematically and the separation pattern will appear chaotic. There are exceptions. As illustrated in Figure 2-5, if a sample property p_i is expressed weakly relative to p_j on a single dimension then apparent order might still be observed. Clusters of peaks separated by p_j will be grouped together by the stronger expression of p_i selectivity. This is called apparent dimensionality (s'). Ordered patterns can still be obtained even though s < n. For example, if on a column in one dimension, separation is conducted mainly by molecular weight but also to a lesser extent by shape, then a chromatogram looking like Figure 2-5 will be obtained. Separation need not always be attained by chromatographic separation. When a selective detector, like accurate mass mass-spectrometry is used, similar patterns may be observed in two-dimensions.

When only some of the sample dimensions are of interest, it may be required to analyze a sample along a selected few of the possible sample dimensions only. The required dimensionality (s'') is defined as the number of variables that must be determined for purposes of analysis. If all other sample properties are weakly expressed then the apparent sample dimensionality is equal to the required dimensionality. Thus if s'' = s' a sample can be systematically analyzed in a system of n = s'' dimensionality. However these weaker dimensionalities may produce interesting secondary patterns in the resultant

[14] J.C.Giddings, J.Chromatogr.A, 703 (1995) p3

chromatogram if they are expressed strong enough to produce some resolution but not so strong as to create a chaotic chromatogram.

Figure 2-5: Apparent dimensionality: Ordered patterns despite s < n.



As an example of sample dimensionality, consider a sample containing only straight-chain n-alkanes. The components of the sample can completely be described with just one variable such as molecular mass or carbon number. This one-variable specification serves to define a one-dimensional sample. As long as separative displacement varies systematically with carbon number, an ordered chromatogram can be obtained with a system of adequate peak capacity. If we now choose to add an additional functionality to our sample, such as a double bond or one substituent group, the displacement along a single separation axis will exhibit a systematic, but unequal, dependency on both variables. Such a sample will require two separation dimensions where the displacements are independent or where the sample properties at least weigh differently in their effect on the two displacements. Extending the sample to include 0,1,2… different functionalities will require three dimensions and so forth[4].

To achieve ordered chromatograms the number of separation dimensions must equal or exceed the sample dimensionality. The sample dimensionality is the number of independent variables needed to uniquely specify members of the mixture. The number of

dimensions required to produce an ordered chromatogram for any defined sample is limited because there are only so many possible independent chemical variables that can be defined. Soon any 'new' variables are likely to be strongly correlated with one or more previous variables[15].

Some examples of possible chemical variables are:

- Dispersion forces responsible for volatility or molecular mass dimensionality,
- Dipole interactions,
- Pi-interactions - aromaticity,
- Hydrogen bonding e.g. oxygenated compounds,
- Size and shape e.g. for level of branching or cis/trans geometrical differences,
- Ion mobility,
- Chiral selectivity.

2.8 Historical overview of multidimensional instrumentation

2.8.1 Thin layer chromatography

To create a 2D TLC chromatogram, the sample is applied on a corner of the plate. The plate is then developed in one direction. After completion the plate is rotated 90° and developed using a different eluent to affect a different separation mechanism. It is extremely easy to implement 2D-TLC compared to other comprehensive 2D methods. However the limited peak capacity of a TLC plate means that n_c x n_c of 2D-TLC does not produce a very high number[16]. The orthogonality of a 2D TLC separation is also limited because the stationary phase is usually the same in both dimensions. Detection limits and quantitation of sample components with TLC is rather poor. Automation and re-use of separation medium is difficult.

[15] J.B.Phillips, J.Beens, J.Chromatogr. A, 856 (1999) p331

[16] G.Guiochon, M.F.Gonnord, A.Souffi, M.Zakaria, J.Chromatogr., 250 (1982) p1

2.8.2 Planar column chromatography

This technique essentially employs the advantages of TLC but the peak capacity of the plate is increased by obtaining control over flow rate of the mobile phase. The bed is enclosed in a leak proof, pressure resistant container. Instead of relying on the capillary forces of the bed and surface tension of the mobile phase a pump is used to force a stream of solvent across the planar column. When the less retained components reach the edge of the bed, a different mobile phase is used in a perpendicular direction to separate the band with a different mechanism and distribute the sample components across the entire planar separation space. The major difference between planar column chromatography (also called multidimensional column chromatography) and TLC is that the compounds are eluted from the column as in HPLC and detected with a UV beam perpendicular to the thin solvent stream and focused on a diode array.
It has proved difficult to design a suitable detector for two-dimensional planar column chromatography. Furthermore the technique suffers from the same fundamental drawback as 2D-TLC: How to obtain orthogonal separation mechanisms while still using the same stationary phase in both dimensions[17].

2.8.3 Electrophoretic techniques

Another example where compounds are separated by spatial dispersion is the multi-dimensional technique of SDS-PAGE combined with isoelectric focusing. This two-dimensional system gives the best resolution for the separation of complex mixtures of proteins. The separation utilizes two independent protein characteristics:

- The isoelectric point (pI) reflects the charge of proteins.
- Slab gel electrophoresis is then used with sodium dodecyl sulfate (SDS) in the second dimension[18,19]. Here, the differences in molecular mass determine the relative mobility of the SDS-protein complexes in a polyacrylamide gel.

[17] F. Geiss, Fundamentals of thin layer chromatography: planar chromatography**,** Huthig, (1987)

[18] P.H.O'Farrel, J.Boil.Chem., 250 (1975) p4007

[19] N.G.Anderson, N.C.Anderson, Anal.Biochem. 85 (1978) p331

Two-dimensional electrophoretic techniques are widely used in the field of biochemistry today.

2.8.4 High performance liquid chromatography. HPLCxHPLC

Three online multidimensional HPLCxHPLC systems were demonstrated in 1978[20], but only the system by Erni and Frei[21] resembles comprehensive multidimensional chromatography. An 8-port valve with two sampling loops of the same size was used to perform repetitive sampling of the first column chromatogram. Because only a small number of cuts were transferred to the second column their system was not strictly comprehensive. Using a similar eight-port valve configuration, Bushey and Jorgenson demonstrated comprehensive two-dimensional HPLCxHPLC in 1990[22]. They coupled cation exchange to size exclusion columns for proteins analysis.
HPLCxHPLC is a complementary method to 2D gel electrophoresis for the analysis of proteins. This was demonstrated with a cation exchange column coupled to two reverse phase columns in parallel.[23,24]. While proteins with molecular weight <20 000 are difficult to separate with 2D-gel electrophoresis, the HPLCx HPLC method offered high-resolution protein separations. The total analysis time was less than 20 minutes. HPLC x HPLC separation schemes were also applied to the analysis of nitro, chloro and aminophenols[25], the analysis of organophosphorous and organochlorine pestcides[26] etc. Numerous articles have also been published to demonstrate comprehensive multidimensional LCxCZE for peptide analysis [27], for urine analysis[28] and other applications.

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[20] E.L.Johnson, R.Gloor, J.Chromatogr. 149 (1978) p571

[21] F.Erni, R.W.Frei, J.Chromatogr., 149 (1978) p561

[22] M.M.Bushey, J.W.Jorgenson, Anal.Chem. 62 (1990) p161

[23] K.K.Unger et.al, HRC 23 (2000) p259

[24] K.Wagner et.al. ,J.Chromatogr.A, 893(2) (2000) p293

[25] A.P.Koehne, T.Welsch, J.Chromatogr.A, 845 (1999) p463

[26] R.W.Martindale, Analyst (London), 113 (1998) p1229

[27] M.M.Bushey, J.W.Jorgenson, Anal.Chem., 62 (1990) p978

[28] T.F.Hooker, J.W.Jorgenson, Anal.Chem., 69 (1997) p4134

2.8.5 Gas chromatography GCxGC

The late Prof. J.B.Phillips pioneered comprehensive two-dimensional GCxGC in the early 1990's[29,30]. His initial work on multiplex chromatography[31]- a form of chromatography where a sample is repeatedly injected - required the design of a modulator. The difference between multiplex chromatography and comprehensive multidimensional chromatography is that in the latter case the sample stream is continuously changing as a first chromatographic run develops. Instrumentation for comprehensive multidimensional gas chromatography in its simplest form consists of two columns coupled together with a modulator. A detector is connected to the exit of the second column and a series of chromatograms is produced. These are arranged into a two-dimensional matrix and presented graphically as a contour plot.

2.8.5.1 Modulators

The function of the modulator is to continuously sample the eluent from a first chromatographic column. The cuts are refocused and injected into a second column. It is the presence of the modulator that differentiates this technique from other multidimensional systems. By continuous sampling, the modulator effectively digitizes the first chromatogram. Each point on the chromatogram is expanded into a second chromatogram that provides information about a different sample dimensionality (e.g. polarity distribution) at that specific vapor pressure point or other sample dimensionality. A number of different modulation devices have been demonstrated using valves or stationary phase focusing. For stationary phase focusing both thermal[32] modulators and cryogenic [33,34,35,36,37] modulators have been used. These are essentially the same: With the

[29] J.B.Phillips, C.J.Venkatrami, J.Microcol.Sep. 5 (1993) p511

[30] J.B.Phillips, C.J.Venkatrami, US patents 5,135,549 and 5,196,039

[31] M.Zhang, J.B.Phillips, J.Chromatogr. A 689 (1995) p275

[32] E.Ledford et al, HRC 22 (1999) p3

[33] P.J.Marriott, R.M.Kinghorn, Anal.Chem. 69 (1997) p2582

[34] R.M.Kinghorn,P.J.Marriott, HRC 22 (1999) p235

thermal modulators a relatively thick film is often used for retention and the compounds are launched into the second column by supplying heat. With cryogenic modulators, designed by P.J.Marriot and his group, the compounds are retained in the interface by reducing the temperature when the coolant flow is switched off the interface quickly heats up to the temperature of the GC oven. The various modulator designs are discussed in detail in Chapter 7.

2.8.5.2 Columns

While any combination of columns with different mechanisms of selectivity can be used, the only practical arrangement for GCxGC is to use a non-polar column for volatility separation in the first dimension. A polar column is generally used in the second. This combination is ideally suitable for complex samples that often span a wide volatility range while the number of chemical classes that compounds can belong to is generally limited. The discrete nature of chemical functionality also implies that compounds are only distributed into a handful of groups or chemical classes when the molecular mass or volatility dimension is adequately suppressed. GCxGC is most useful when the second column can produce chromatograms faster than the first chromatogram generates peaks. Therefore the second dimension is an ideal high-speed GC application where each cut consists of only a few compounds of different chemical classes or polarities.

Volatility always plays the biggest part in GC retention mechanisms with secondary interactions such as polar interactions superimposed on this. Polar and chiral interactions are usually a strong function of temperature. The strength of these interactions decreases with an increase in temperature, which leads to a reduced selectivity at the higher analysis temperatures [38].

[35] R.M.Kinghorn,P.J.Marriott, HRC 21 (1998) p620

[36] R.M.Kinghorn, P.J.Marriott, HRC 21(1998) p32

[37] R.M.Kinghorn, P.J.Marriott,P.S.Dawes HRC 23 (2000) p245

[38] H.Rotzsche, Stationary phases in gas chromatography, J.Chromatogr. Library Vol.48, Elsevier (1991)p80

Despite the tremendous technical difficulty in coupling three columns together, Ledford successfully demonstrated 3D GC[39]. However, it proved difficult to find GC columns significantly different in selectivity to beneficially apply comprehensive three-dimensional GCxGCxGC. Another way to address increased sample dimensionality was attempted by Sharpe et al. They split the effluent from the first column into two different columns in the second dimension. In the process, compound identification and separation efficiency were facilitated[40]

2.8.5.3 Detection

The 2nd dimension column needs to be very fast to ensure that a transfer is completely analyzed before the next injection. This results in very narrow peaks requiring very fast electrometers. Until recently, standard electrometers on GC instrumentation were not fast enough to cope with peak widths in the 100-200 ms range. Most electrometers that are built into GC's contain low pass filters to remove high frequency noise and improve signal to noise ratios. Very fast changes in the signal can thus not be observed with these boards. Alternative electrometers had to be used or modifications to existing boards had to be made. Some modern instruments like the Agilent HP6890 series have been designed to cope with such fast peaks.

So far the flame ionization detector (FID) has primarily been used for GCxGC. There seems to be some disagreement[41] in the literature about whether the many other detectors that are routinely used for GC can potentially be used for GCxGC[14].

The use of mass spectrometry (MS) has provisionally been demonstrated[42] using an ordinary quadrupole instrument. However it is only the time-of-flight (TOF) instruments that can produce the necessary minimum of six scans per peak to reconstruct total ion chromatograms for the fast peaks produced by the second dimension. State of the art

[39] E.B.Ledford, C.A.Billesbach, Q.Zhu, HRC, 23 (2000) p205

[40] J.V.Seeley, F.J.Kramp,K.S.Sharpe, J.Sep.Sci., 24 (2001) p444

[41] W.Bertch, HRC, 23 (2000) p167

[42] G.S.Frysinger, R.B.Gaines, HRC 22 (1999) p251

TOF's can produce up to 500 scans per second. It is hoped that computers will soon be able to handle the vast amount of data that will be produced by GCxGC-MS.

2.8.6 SFCxGC

Although early expectations were not all met, good applications have been developed with supercritical fluid chromatography (SFC) for chemical group separation using normal phase chromatography[43] and for enantiomer analysis[44].

Lee et al used the same technology developed for GCxGC namely the single stage thermal modulator as described in Chapter 7 to demonstrate comprehensive multidimensional SFCxGC[45]. Two columns were placed in the same oven. Supercritical CO_2 was used for the first dimension and the expanded CO_2 after the restrictor was used as carrier gas in the second dimension. Due to slow diffusion coefficients, CO_2 is not a very good mobile phase to use when speed is important. A 50% cyanopropyl polysiloxane stationary phase was used as the first dimension to achieve group type separation based on the number of aromatic rings. In the second dimension a liquid crystal stationary phase was used to separate the groups further according to shape. Each of the second-dimension runs was essentially isothermal for its duration as in GCxGC. Hence the second dimension does not require conditioning for each next injection delivered by the thermal modulator. While strong on simplicity, the instrument limits the range of compounds that can be analyzed and leaves much desired for separation efficiency in the second column.

The instrument was applied to mixtures of poly-aromatic hydrocarbons and to a coal tar sample. Group type sample bands from the first column were separated into individual components on the second column. The entire sample passed through both columns and generated two sets of retention data, which could be used for more accurate compound identification.

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[43] Annual Book of ASTM standards, vol.05.03, American society for testing and materials, Philadelphia (1991), Method D5186.

[44] K.E. Markides, M.L.Lee, SFC applications, Workshop on Supercritical Fluid Chromatography held in Park City, Utah, on January 12-14, 1988. Provo, Utah : Brigham Young University Press (1988)

[45] Z.Lui, I.Osrtovsky, P.B.Farnsworth, M.L.Lee, Chromatographia 35 (1993) p567

2.9 Chapter Conclusion

The theory behind comprehensive multidimensional chromatography is already quite advanced and the technique has been in use for many years in various embodiments. Yet the practical implementation of the technique is still hampered by the lack of reliable instrumentation, particularly as concerns the interface between the two dimensions. While many combinations of comprehensive chromatography have been demonstrated, the possibilities are far from exhausted. The combination of SFC and GC has not yet received the attention it deserves.