ADVANCES IN FOOD ANALYSIS

INTRODUCTION TO MULTIDIMENSIONAL (MD) CHROMATOGRAPHY

Marco Beccaria, PhD

University of Liege, Belgium
email: mbeccaria@uliege.be

Monodimensional *vs* Multidimensional Chromatography

Monodimensional *vs* Multidimensional Chromatography

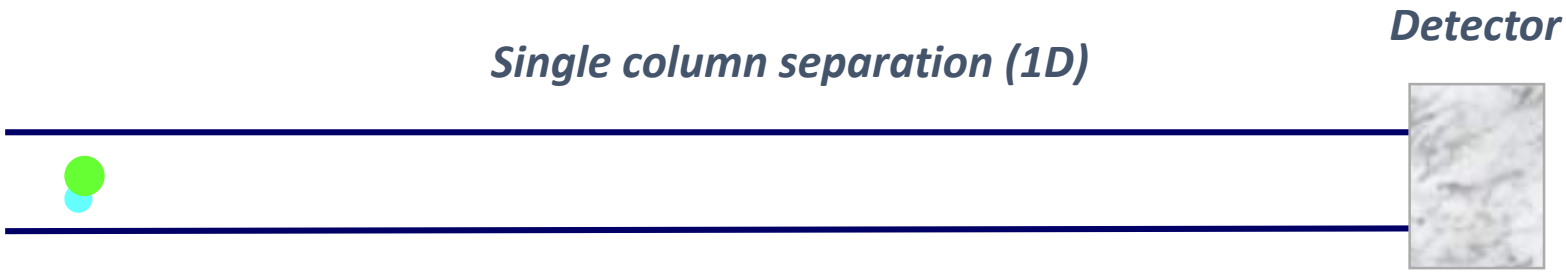
- Monodimensional (1D) chromatographic techniques, both GC and LC, are widely applied to the analysis of real world samples in several fields (e.g. foods, pharmaceuticals, petrochemicals, etc)

- These 1D-methods may not provide a sufficient resolving power for the separation of target and/or untarget components in complex samples.

- A possible solution can be the use of multidimensional systems (MD), where the dimensions are based on different separation mechanisms and selectivity.

MONODIMENSIONAL CHROMATOGRAPHY

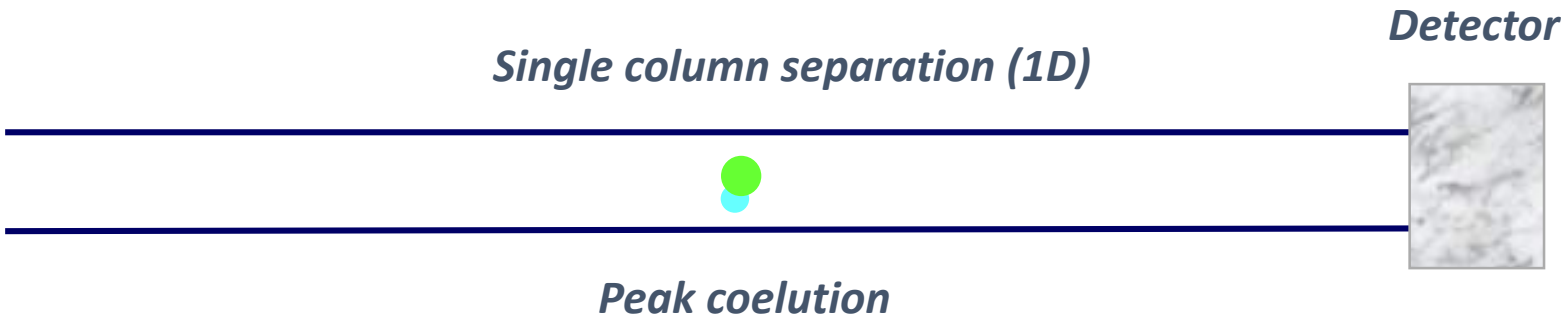
Why 2D Chromatography?



CHROMATOGRAM

MONODIMENSIONAL CHROMATOGRAPHY

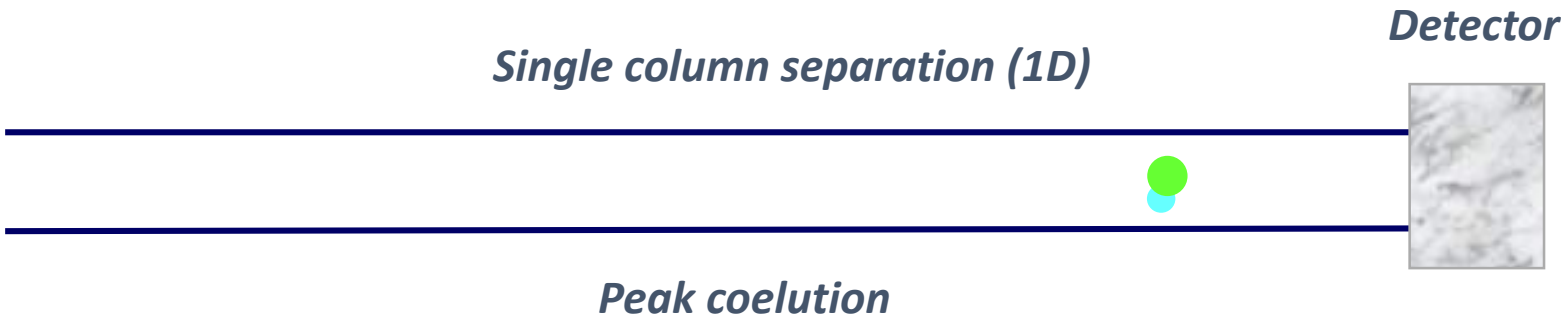
Why 2D Chromatography?



CHROMATOGRAM

MONODIMENSIONAL CHROMATOGRAPHY

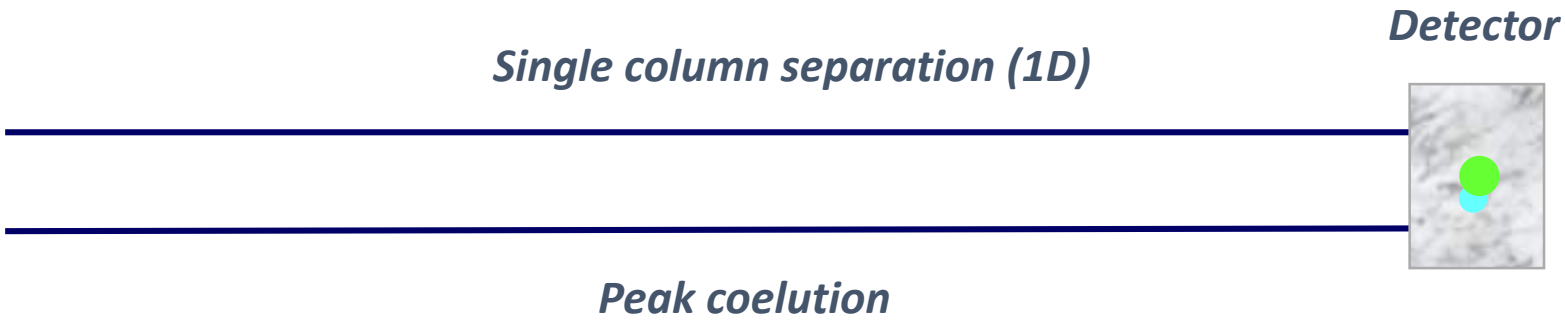
Why 2D Chromatography?

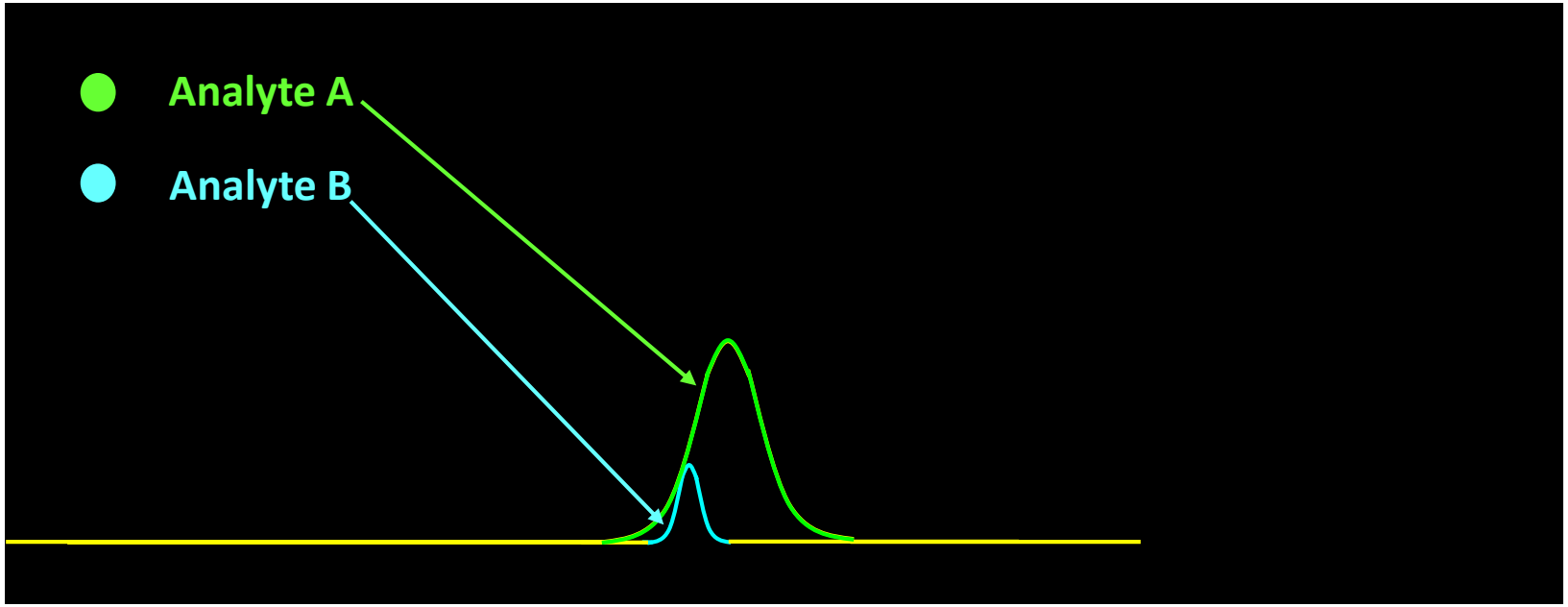


CHROMATOGRAM

MONODIMENSIONAL CHROMATOGRAPHY

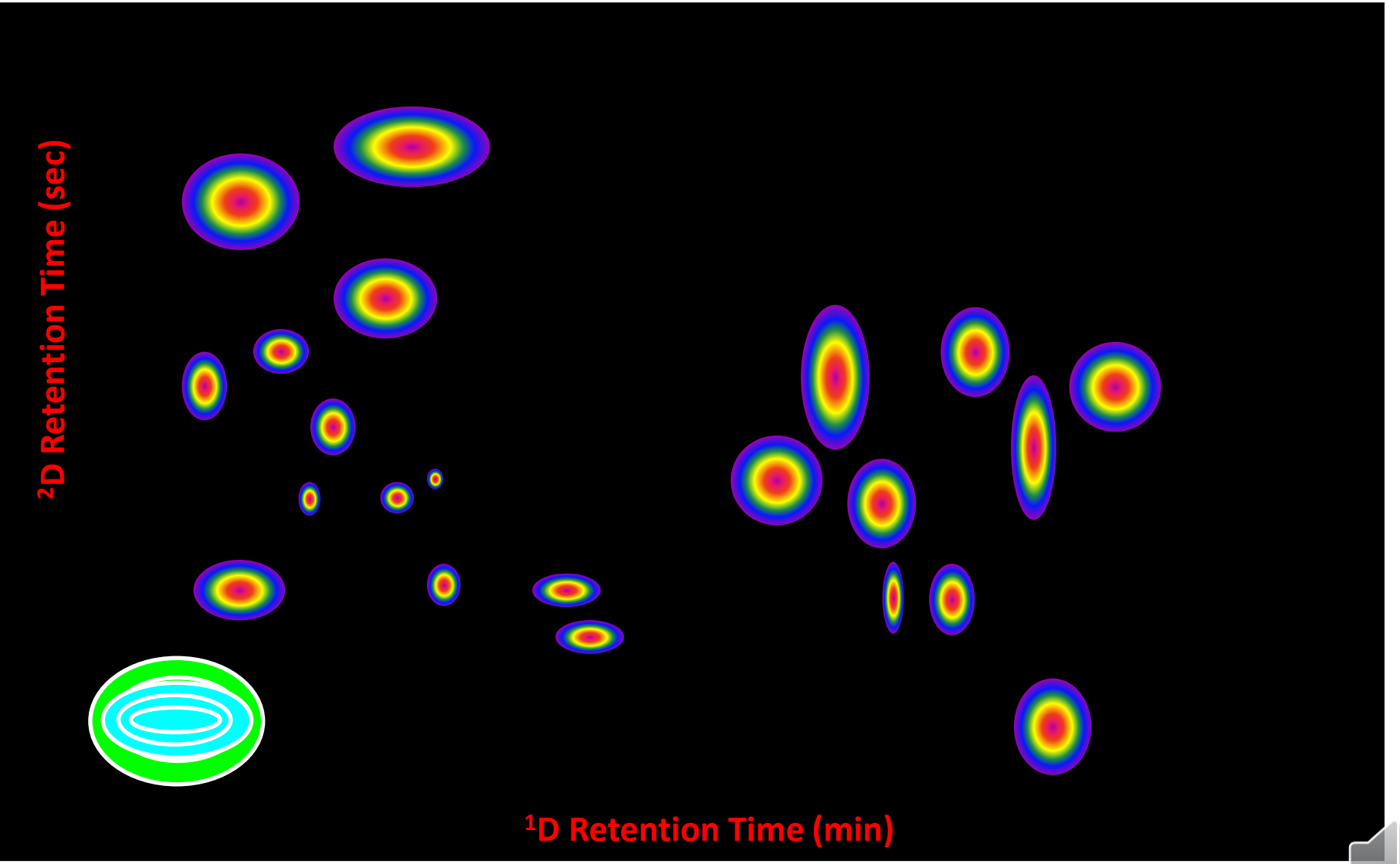
Why 2D Chromatography?





CHROMATOGRAM

Multidimensional Chromatography - 2D plot



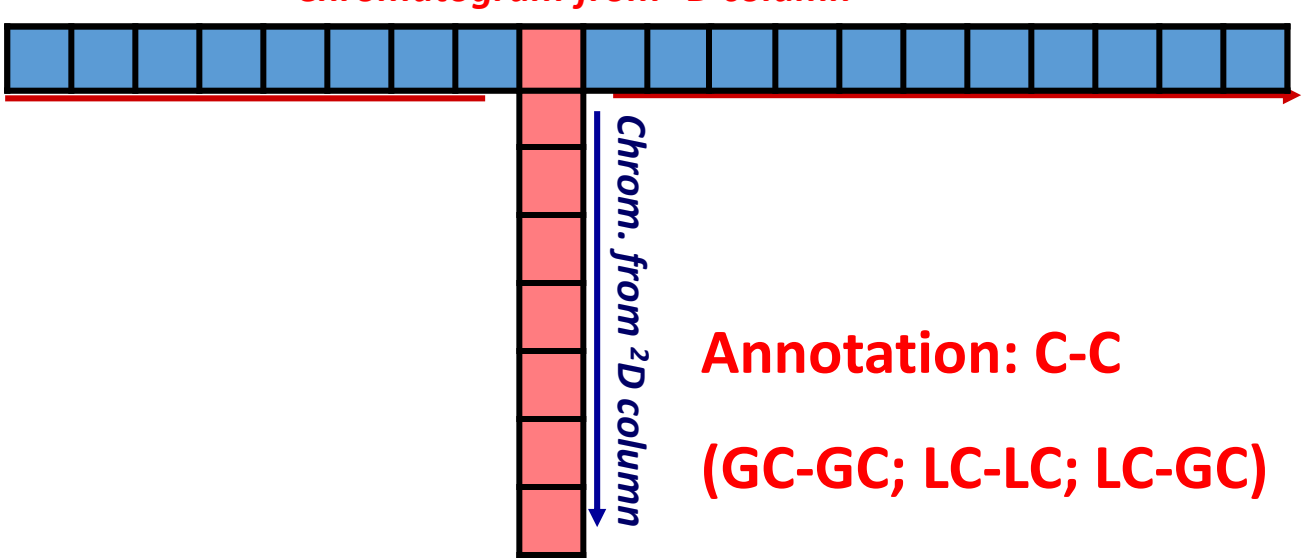
1D-*versus* 2D-chromatography

1D-chromatography

Chromatogram from a single dimension chromatography (1D-C)

Heart-cut 2D-C chromatography

one or few fractions of a sample are subjected to two consecutive separation steps



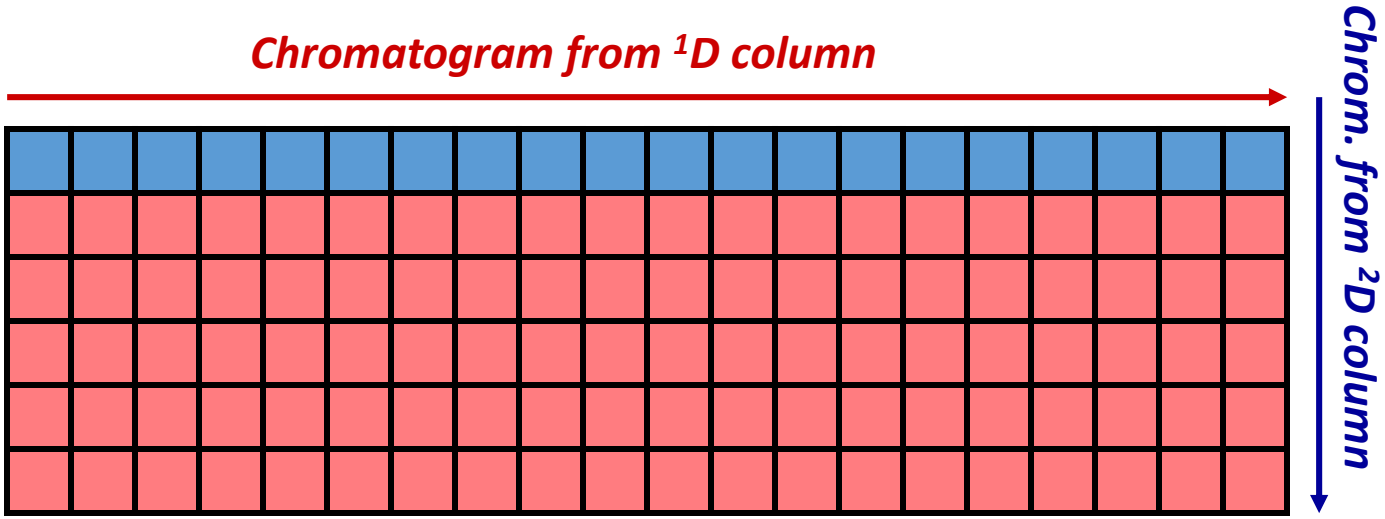
Annotation: C-C

(GC-GC; LC-LC; LC-GC)

Comprehensive 2D chromatography

every component of the sample is subjected to 2 separation steps:

two-dimensional analysis of the entire initial sample through continuous heart-cutting.



Annotation: C×C

(GC×GC; LC×LC; LC×GC)

ORIGIN OF MULTIDIMENSIONAL CHROMATOGRAPHY

ORIGIN OF MULTIDIMENSIONAL CHROMATOGRAPHY

Comprehensive 2D Planar Chromatography (1944)

Consden *et al*

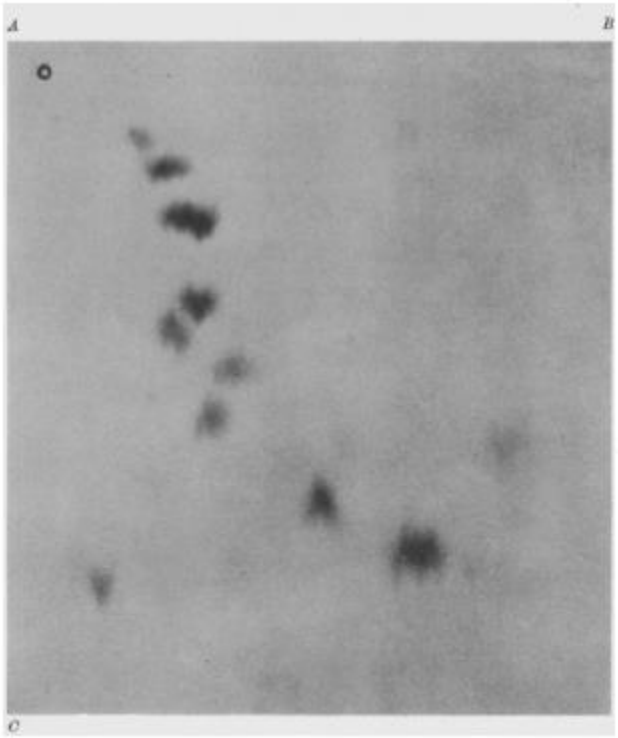
Qualitative Analysis of Proteins: a Partition Chromatographic Method Using Paper

By R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Wool Industries Research Association, Torridon, Headingley, Leeds, 6*

(*Received* 13 *May* 1944)

BIOCHEMICAL JOURNAL, VOL. 38, NO. 3

Any chromatographic method is well adapted for the discovery of further members of a known homologous series, but the two-dimensional chromatogram is especially convenient, in that it shows at a glance information that can be gained otherwise only as the result of numerous experiments. The position of unknown substances on chromatograms developed with suitable pairs of solvents will also provide a clue to the existence of certain groups in the molecules, e.g. hydroxyl, acidic, basic or cyclic.



Two-dimensional chromatogram of a wool hydrolysate (180μg.) on Whatman no. 1 sheet. Hydrolysate applied at circle. Run with collidine for 3 days in direction *AB*, then in direction *AC* with phenol for 27 hr. in an atmosphere of coe gas and NH_3 (produced from a 0·3% NH_3 solution). The filter employed in photographing renders the yellow prolin spot scarcely visible. (Photography by J. Manby, photographer to the University of Leeds.)

ORIGIN OF MULTIDIMENSIONAL CHROMATOGRAPHY

Comprehensive 2D Thin Layer Chromatography (1951)

Kirchner *et al*

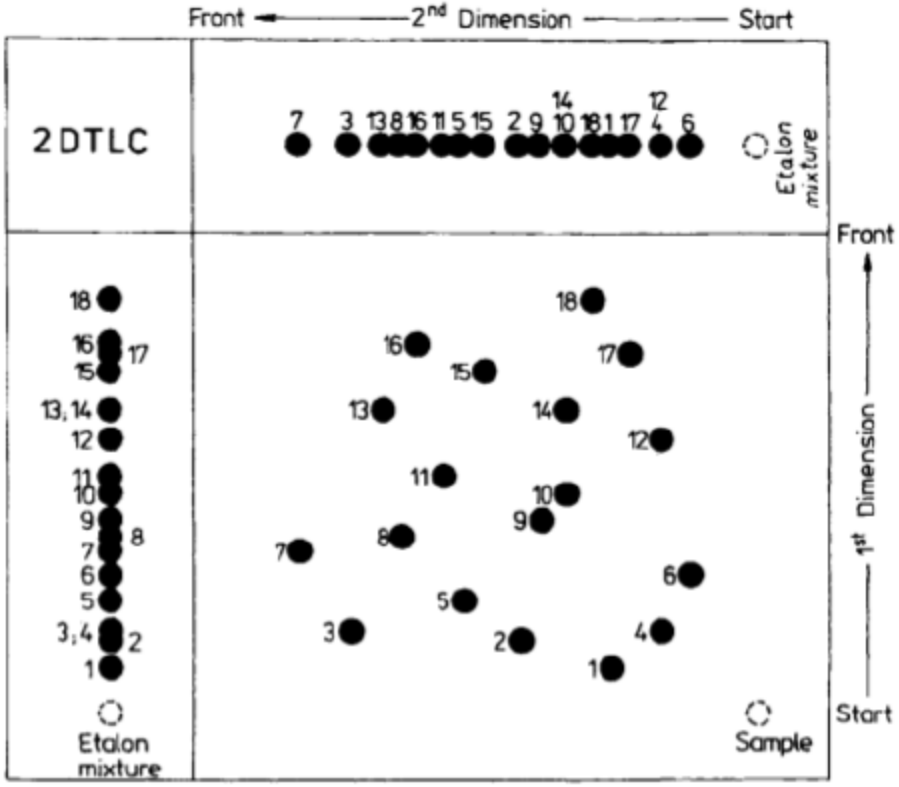


Figure 1. Scheme of spot distribution on a 2D TLC plate.

ORIGIN OF MULTIDIMENSIONAL CHROMATOGRAPHY

Selective 2D Liquid Chromatography (1978) Erni & Frei

Journal of Chromatography, 149 (1978) 561–569

CHROM. 10,733

TWO-DIMENSIONAL COLUMN LIQUID CHROMATOGRAPHIC TECHNIQUE FOR RESOLUTION OF COMPLEX MIXTURES

F. ERNI and R. W. FREI*

Analytical Research and Development, Pharmaceutical Department, Sandoz Ltd., CH-4002 Basle (Switzerland)

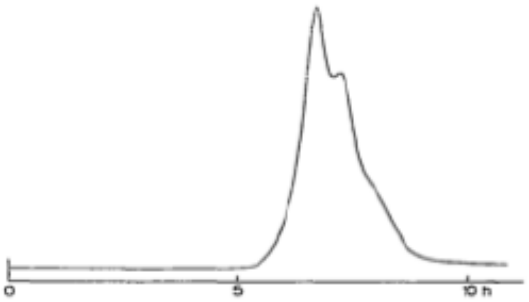


Fig. 5. GPC separation of a senna glycoside extract (trace from recorder 1 in Fig. 4). Mobile phase, buffer, pH 6 (Titrisol; Merck, Darmstadt, G.F.R.); flow-rate, 1.2 ml/h; detection, UV (254 nm). Chromatographic equipment as described in Fig. 4.

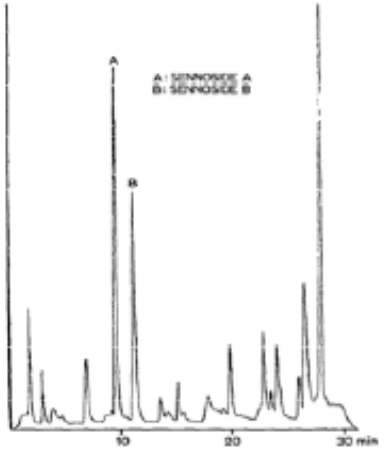


Fig. 6. RPC separation of the same senna glycoside extract as in Fig. 4. Mobile phase, seven steps of acetonitrile–0.01 *N* sodium hydrogen carbonate in water; flow-rate, 2 ml/min; detection, UV (254 nm). The gradient steps were as follows:

ORIGIN OF MULTIDIMENSIONAL CHROMATOGRAPHY

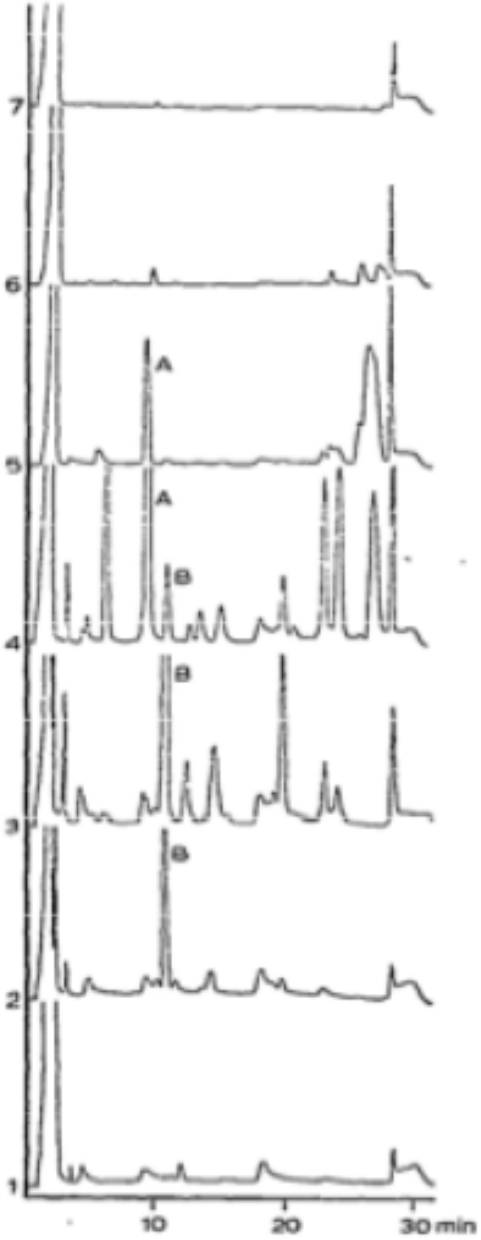


Fig. 7. RPC runs of seven fractions from the GPC run of the same senna glycoside extract as in Fig. 6. Peaks: A, sennoside A; B, sennoside B.

Selective 2D Liquid Chromatography

(1978) Erni & Frei

ORIGIN OF MULTIDIMENSIONAL CHROMATOGRAPHY

First Comprehensive 2D Liquid Chromatography (1990) Bushey & Jorgenson

Automated Instrumentation for Comprehensive Two-Dimensional High-Performance Liquid Chromatography of Proteins

Michelle M. Bushey and James W. Jorgenson*

Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290

ANALYTICAL CHEMISTRY, VOL. 62, NO. 2, JANUARY 15, 1990

Pp 161-167

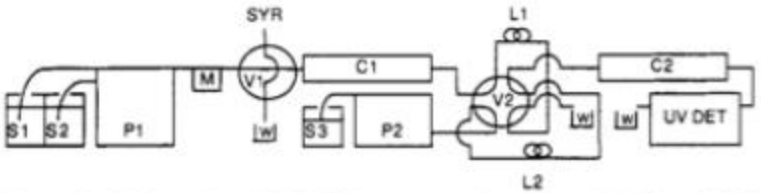


Figure 2. Schematic of 2-D LC instrumental setup. S1, S2, and S3 are buffers A, B, and C; P1, Brownlee microgradient syringe pump; M, 52-μL mixer; V1, Rheodyne 0.5-μL injection valve; SYR, injection syringe; C1, cation exchange column; V2, eight-port computer-controlled valve; L1 and L2, 30-μL loops; P2, Waters Associates Model 6000A piston pump; C2, size exclusion column; UV DET, UV detector operated at 215 nm; W, waste.

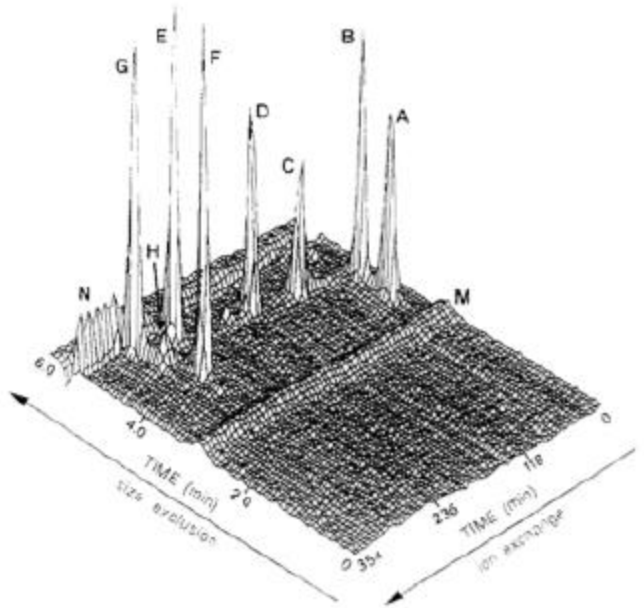


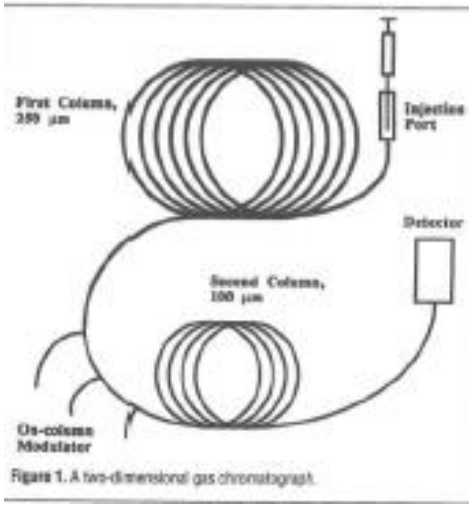
Figure 3. 2-D chromatogram of protein sample: peak A, glucose oxidase; B, ovalbumin; C, β-lactoglobulin A; D, trypsinogen; E, α-chymotrypsinogen A; F, conalbumin; G, ribonuclease A; H, hemoglobin; M, exclusion volume "pressure" ridge; N, inclusion volume "salt" ridge. Ovalbumin and α-chymotrypsinogen A at 0.2%, other proteins at 0.3% (w/v). C1 conditions: 5 μL/min, 0% to 100% buffer B from 20 to 260 min; buffer A, 0.2 M NaH_2PO_4, pH 5; buffer B, 0.2 M NaH_2PO_4/0.25 M Na_2SO_4, pH 5. Valve actuated every 6 min; detection at 215 nm, data collection rate 0.5 point/s; plot shows every other point collected for injection 1 through 60. Each line perpendicular to the IEC time axis represents one injection on the SEC column.

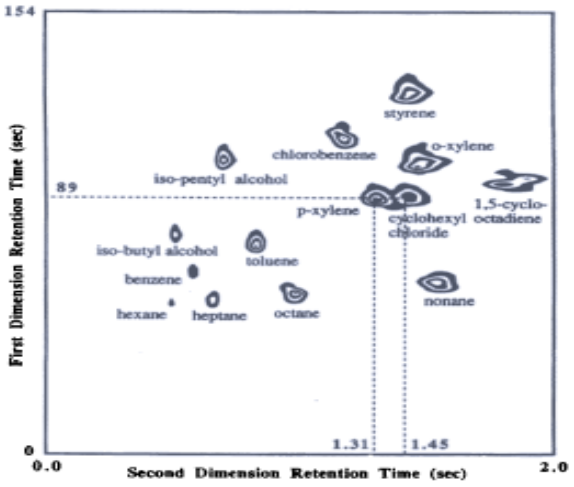
ORIGIN OF MULTIDIMENSIONAL CHROMATOGRAPHY

First Comprehensive 2D Gas Chromatography (1991)
Lui & Phillips

Comprehensive 2D-GC (GC×GC)

GC×GC is an "on-line" multidimensional technique that enables a bidimensional analysis of the entire initial sample, through continuous heart-cutting. The leap from heart-cutting MDGC to comprehensive GC was achieved in 1991 by Liu and Phillips who developed a new transfer system: the thermal modulator.





IT WAS AN EYE-OPENER FOR MANY ANALYTICAL CHEMISTS

Lui ZY and Phillips JB. *J. Chromatogr. Sci.* ***1991,*** *29, 227-231*

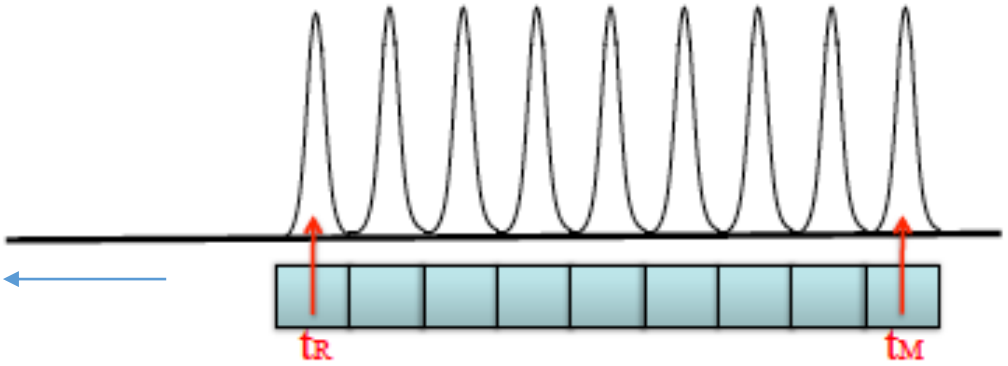
Theoretical consideration in Multidimensional Chromatograpy (MD-C)

- ❑ Peak Capacity
- ❑ Orthogonality
- ❑ Undersampling

Theoretical consideration: Chromatographic "peak capacity"

A measure of the separation power: The peak capacity (n)

Peak Capacity Definition



Peak Capacity

$$n_c = \frac{t_R - t_M}{w_b}$$

t_R = Separation run time

t_M = Void time

w_b = Average peak width

Chromatographic “peak capacity”

The efficiency of a separation system is best demonstrated by its peak capacity, n_c which is the number of solutes that can theoretically be baseline resolved on a given column.

An estimation of a column’s peak capacity for a retention time window from time t_1 to t_2 is given by:

$$n_C = \frac{t_{R,z} - t_{R,1}}{\overline{w_t}} + 1$$

For isocratic elution *(Giddings J.C. Anal Chem. 1967, 39, 1027-1028)*

For gradient elution *(Neue et al Adv Chromatogr. 2001, 41, 93-136)*

“Peak capacity” in two-dimensional (2D) systems

- Ideally, the total peak capacity , n_{2D}, is equal to the sum in “heart-cutting 2D mode” or the product in “comprehensive 2D mode” of the peak capacities in the two dimensions in fully orthogonal 2D systems with non-correlated selectivity:

I. $n_{2D} = n_1 + n_2$ (Heart-cutting 2D chromatography)
II. $n_{2D} = n_1 \times n_2$ (Comprehensive 2D chromatography)

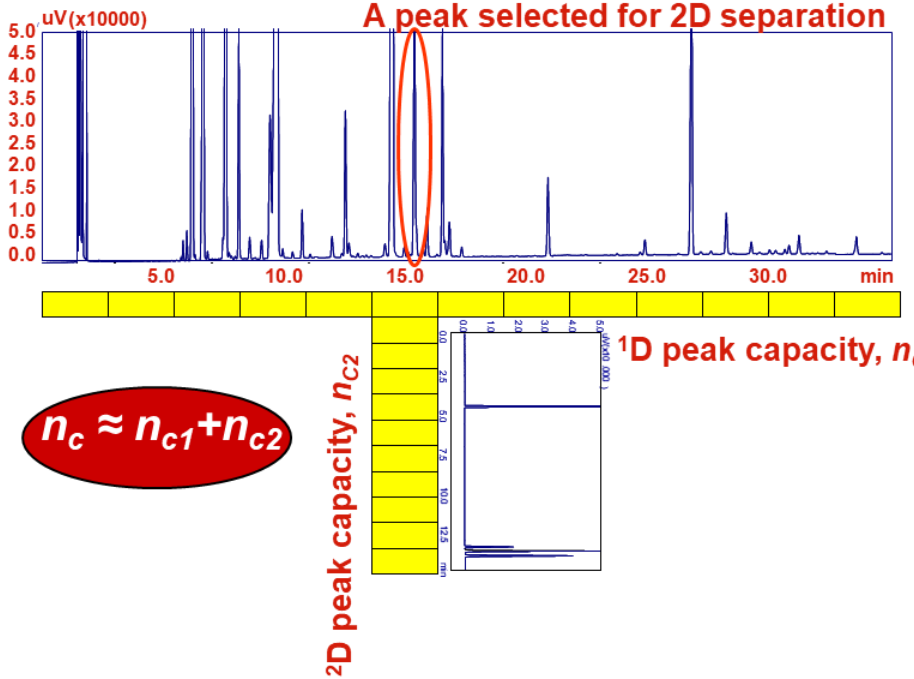
- For complex matrixes, characterised by random peak distribution, a very high value of n_{2D} is required for resolution of all the compounds.

- The product of the peak capacity in the two dimensions, excluding the portion of separation space corresponding to void volume and re-equilibration, gives a value that overstimates the real peak capacity of a real separation, because it includes the region where orthogonality in not achieved.

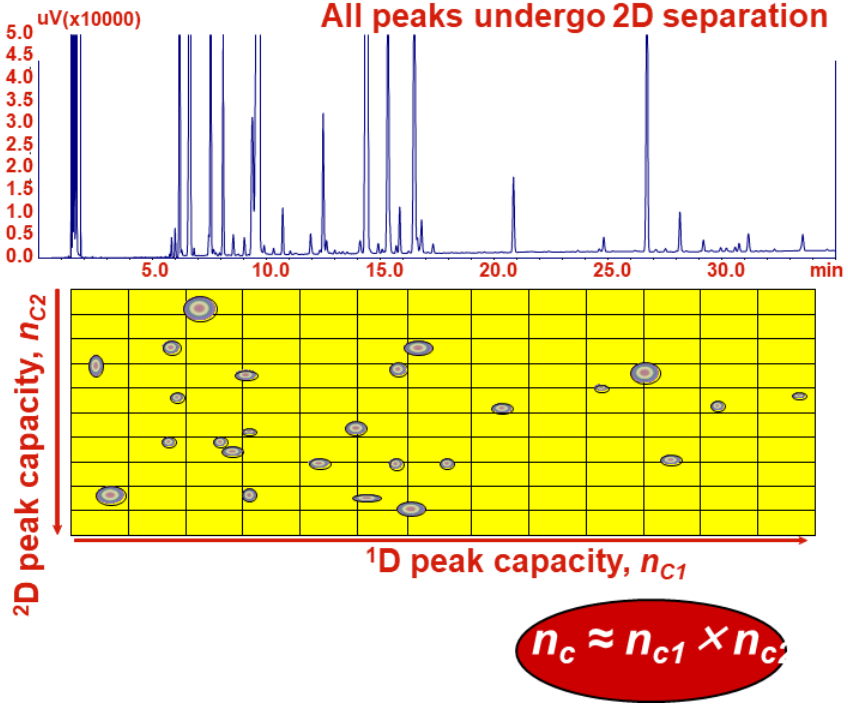
PS: Accurate calculation of n_{2D} is difficult to obtain.

“Peak capacity” in two-dimensional (2D) systems

Heart-cutting mode

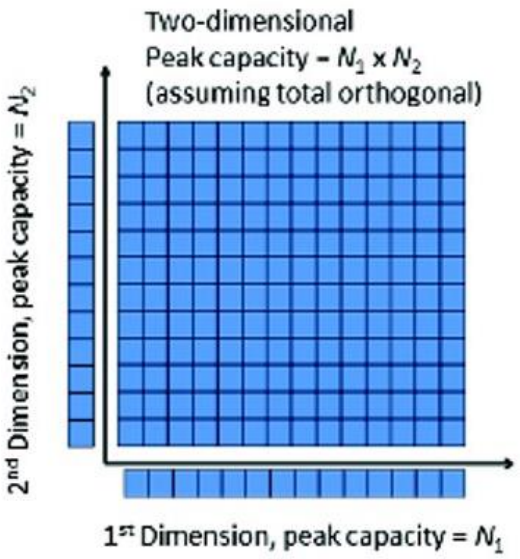


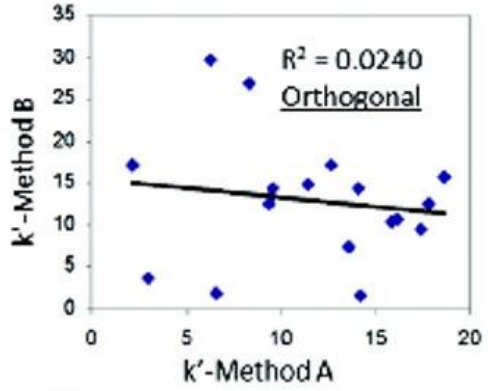
Comprehensive mode

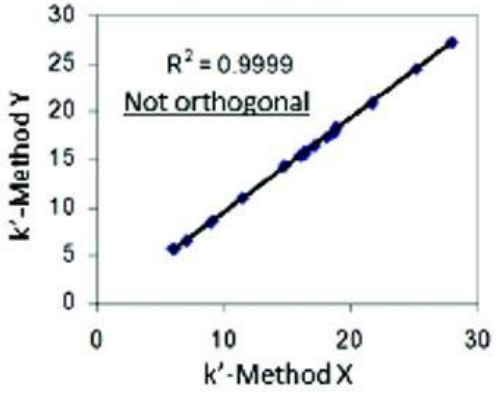


PS: even if less used, it is possible the hyphenation of more than 2 chromatographic dimensions (in term of different analytical column selectivity)

Theoretical considerations: Orthogonality







- Orthogonality metrics (OMs) have been used to measure space occupancy and the uniformity of the spreading of components within these spaces

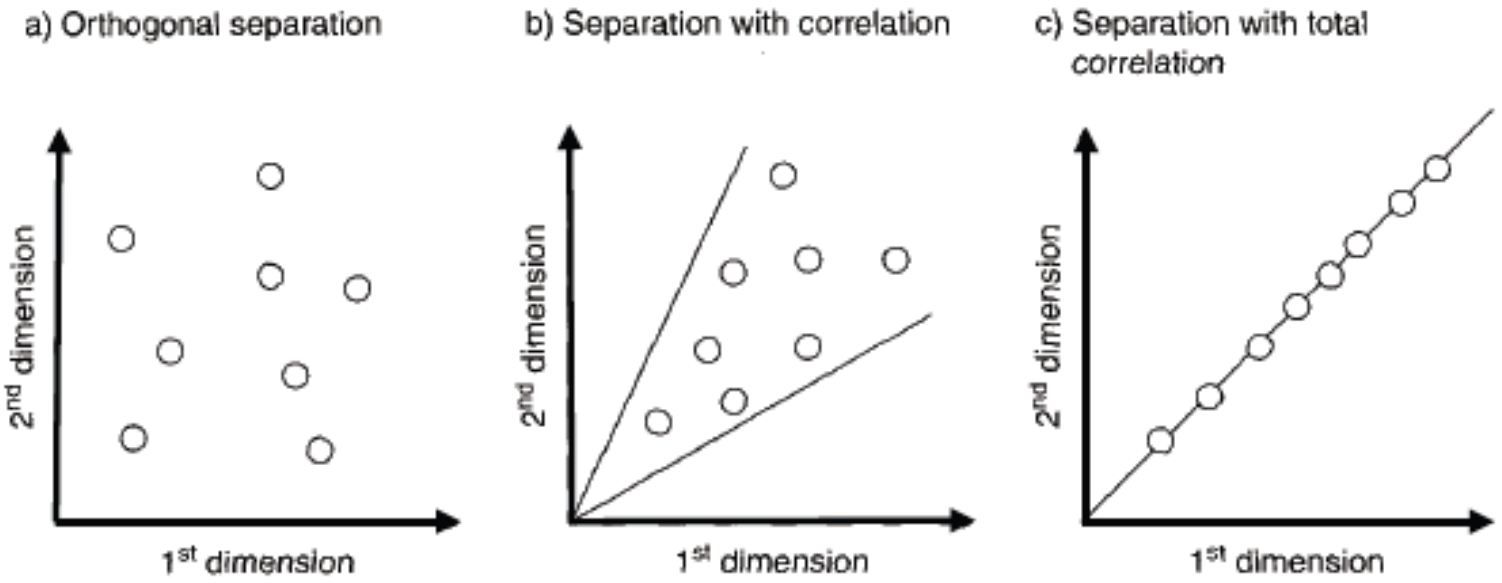
- In 2D chromatography, OMs measure the utilization of the separation space with occupancy metrics, peak spacing metrics and uniformity metrics, among others.

Orthogonality in multi-dimensional chromatography provides information on the spread of the peaks within the separation space without the need for complex computations or division of the separation space into bins.

Theoretical considerations for “Orthogonality”

Separation Orthogonality

Level of correlation between the retention behavior in the 2 separation dimensions in the 2D system



Orthogonality tuning: optimization of the resolution for a 2D column set.

Theoretical considerations for “Orthogonality”

Orthogonality

- (Liu et al. *Anal. Chem.* 1995, *67,* 3840)

A **Geometric Approach** to Factor Analysis for the Estimation of orthogonality and Practical Peak Capacity in Comprehensive Two Dimensional Separations was introduced.

- (Camenzuli & Schoenmakers, *Anal Chim Acta*, 2014, *838*, 93)

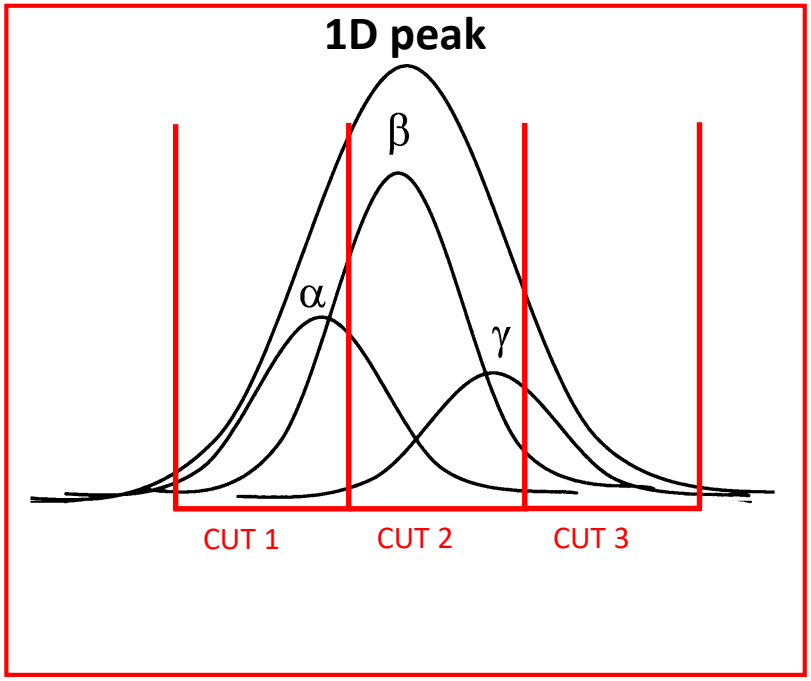
A new measure of orthogonality for multi-dimensional chromatography was introduced by using a **number of equations** which provides information on the spread of the peaks within the separation space without the need for complex computations or division of the separation space into bins.

Theoretical considerations for “Sampling”

Undersampling (Effect of sampling rate on effective ^{1}D bandwidth)

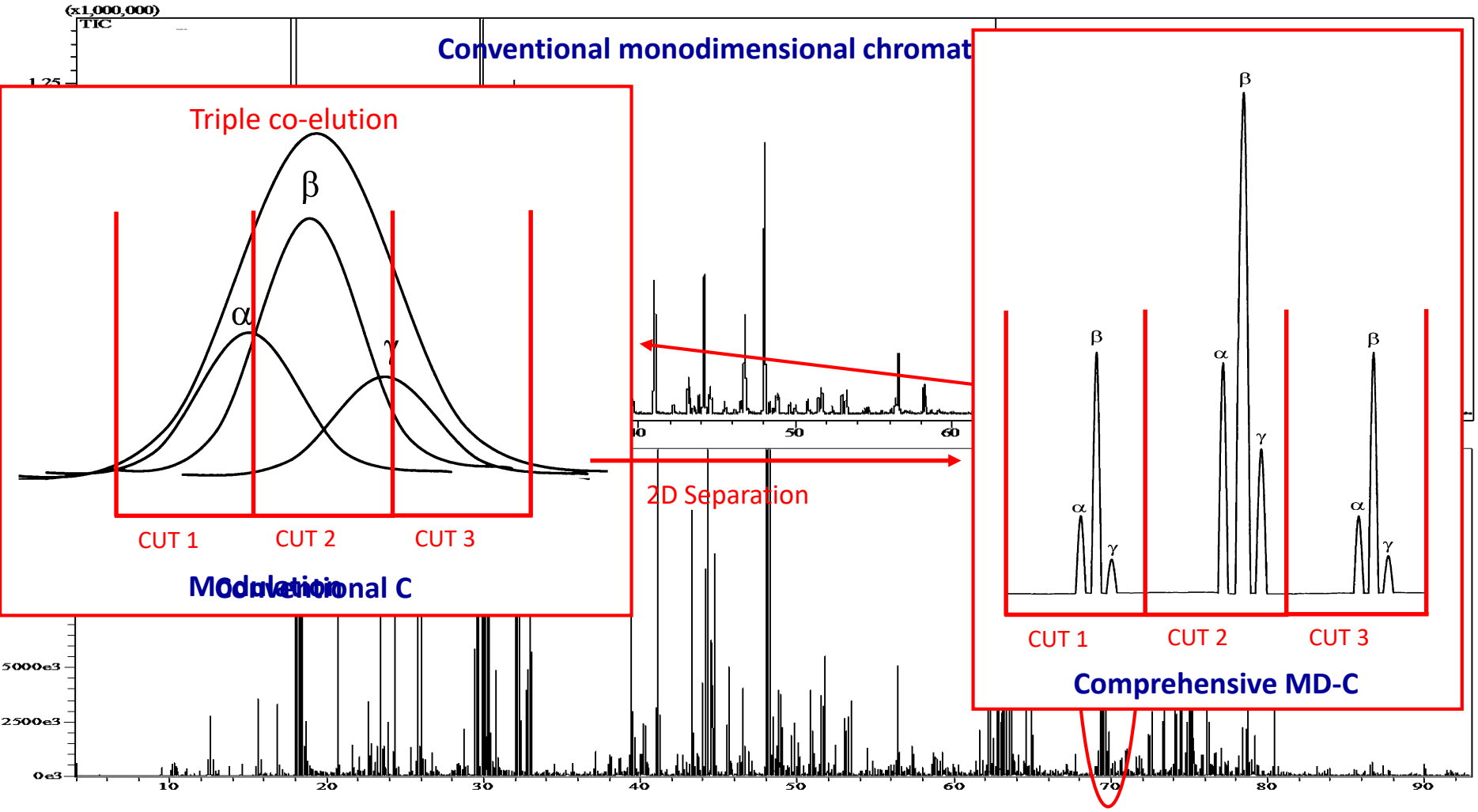
- *(Murphy et al, Anal. Chem. 1998, 70, 1585)*

For high-fidelity separations each **^{1}D peak should be sampled at least three times** into the second dimension when the **sampling is in-phase**. If the sampling is maximally out of phase, there should be at least four samples per peak

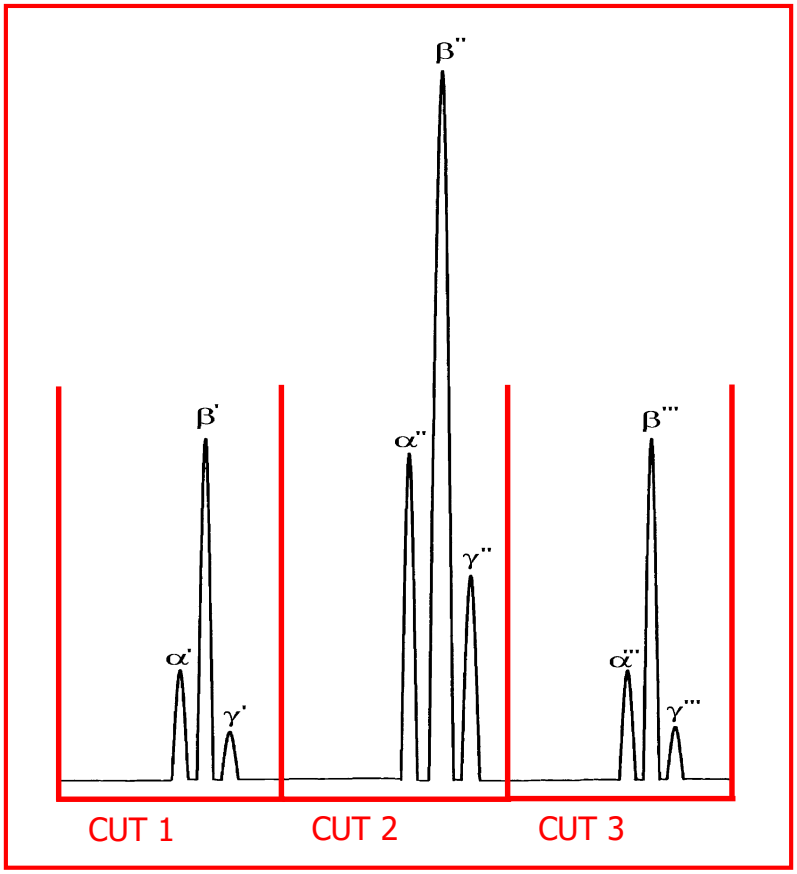


The Effects of Modulation on a Multi-Component Peak

The Effects of Modulation on a Multi-Component Peak



Peak Quantitation

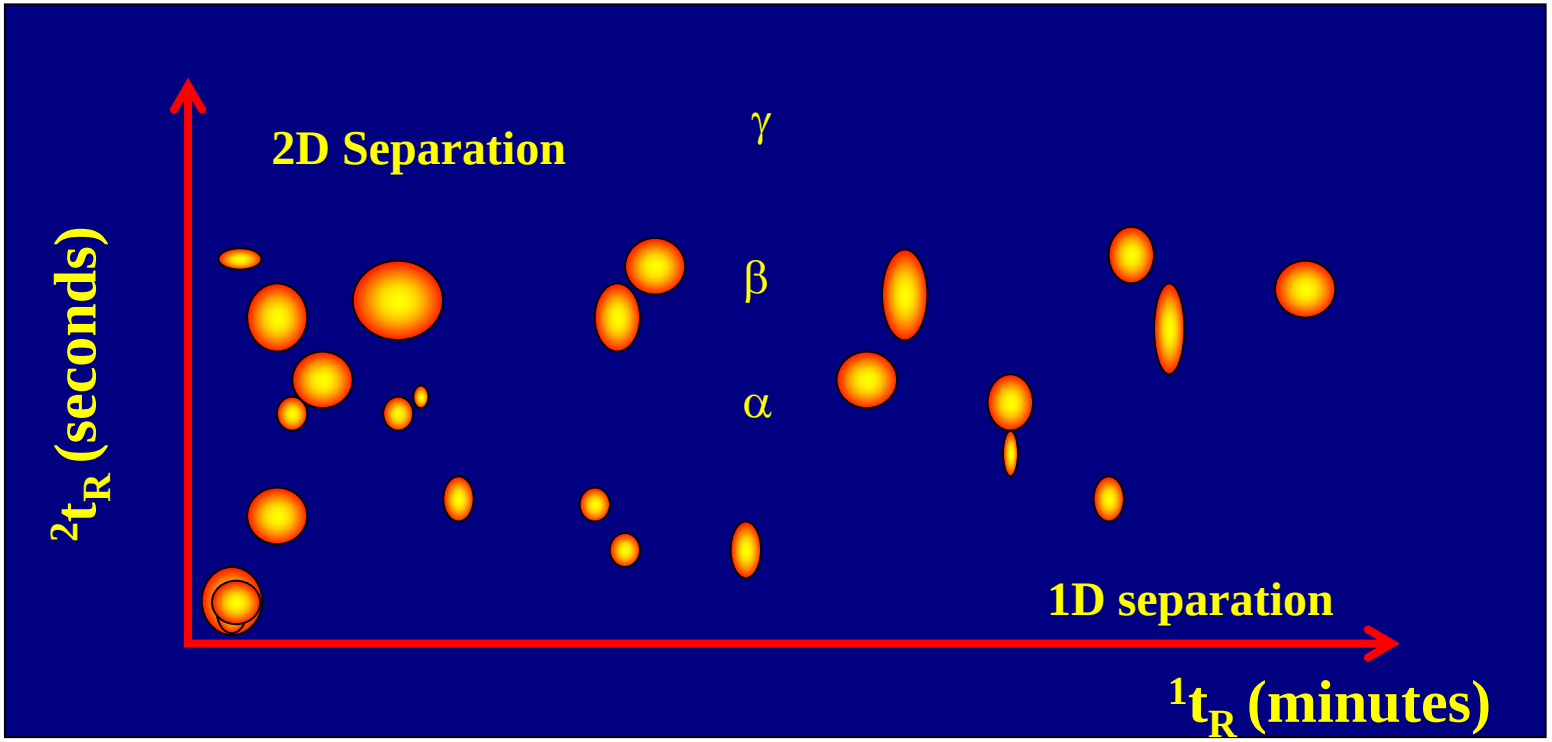


Area $\alpha = \alpha' + \alpha'' + \alpha''' = \Sigma$ **Area** α

Area $\beta = \beta' + \beta'' + \beta''' = \Sigma$ **Area** β

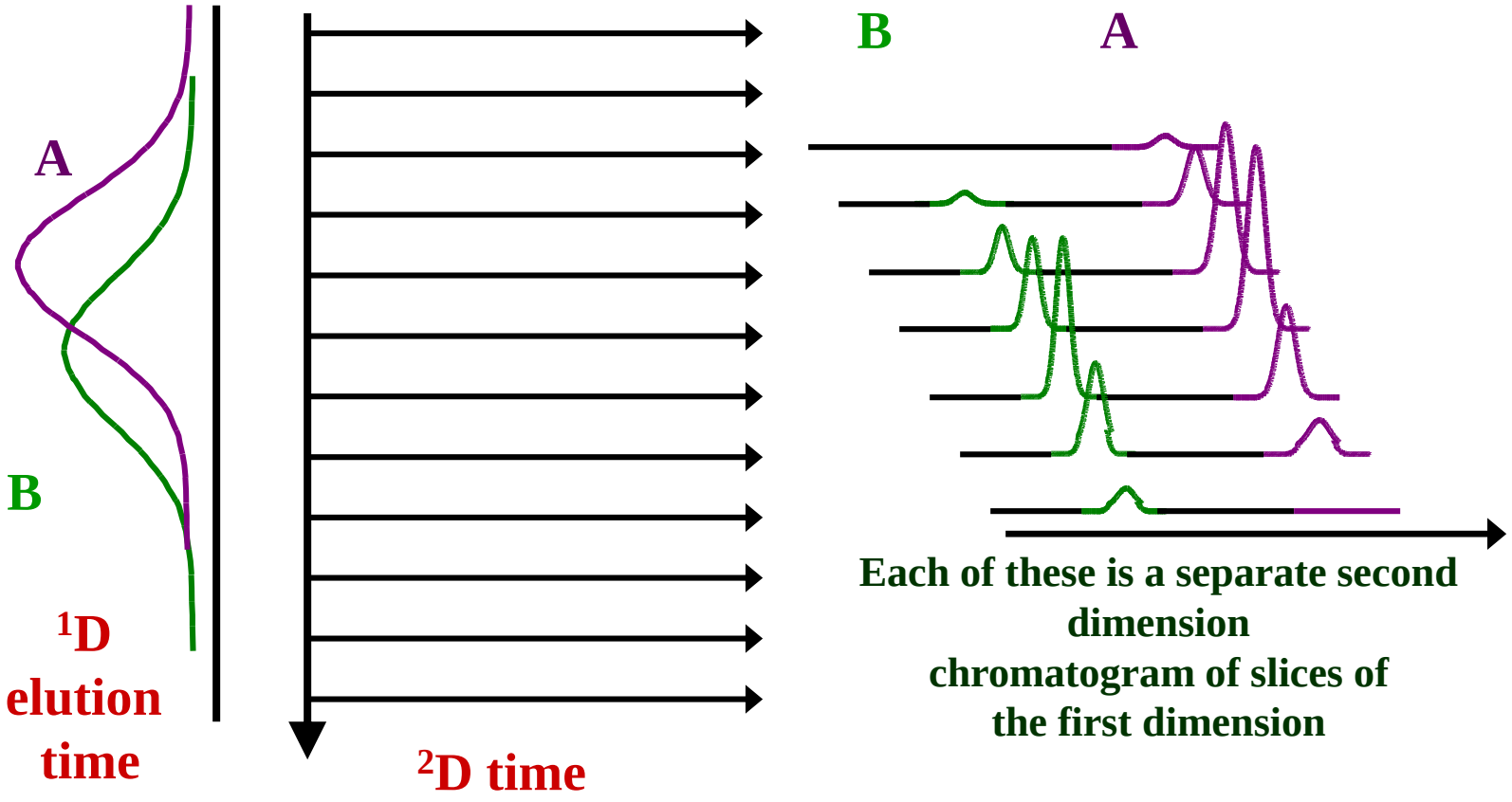
Area $\gamma = \gamma' + \gamma'' + \gamma''' = \Sigma$ **Area** γ

Bidimensional Visualization



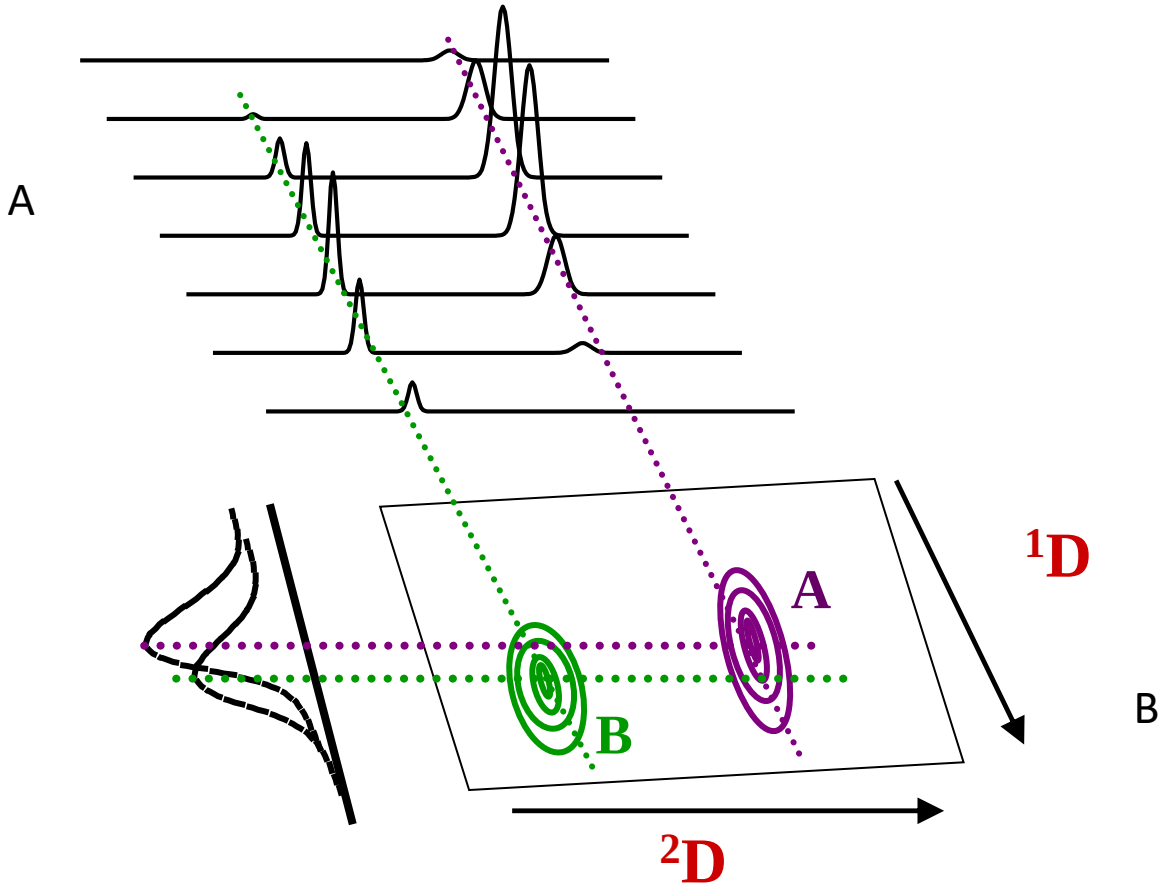
The Effects of Modulation on a Multi-Component Peak

Generation of peak slices in ^{2}D



The Effects of Modulation on a Multi-Component Peak

Peaks distributed in 2D space as ‘contour’ or ‘colour’ spots



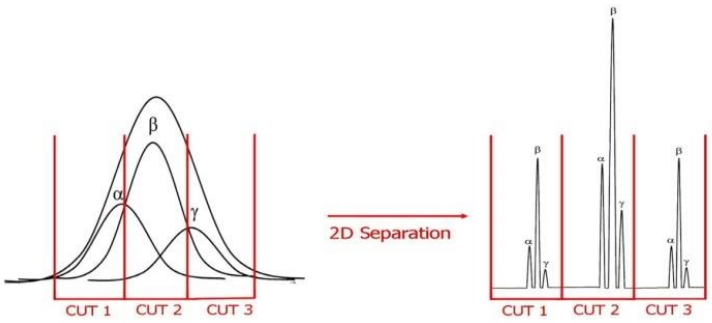
Comprehensive chromatographic separation

- ❑ Requirements
- ❑ Data processing

Comprehensive chromatographic separation

Requirements

- Bands injected onto the secondary column must undergo elution before the following re-injection.
- Any two components separated in the first dimension must remain separated also in the second dimension.
- Elution profiles in both dimensions must be retained.
- To obtain high comprehensive resolution, each peak in the first dimension should be sampled at least three-four times into the second dimension*.



** Murphy R. et al., Anal. Chem. 1998, 70, 1585-1594*

Comprehensive 2D Chromatography

Requirements

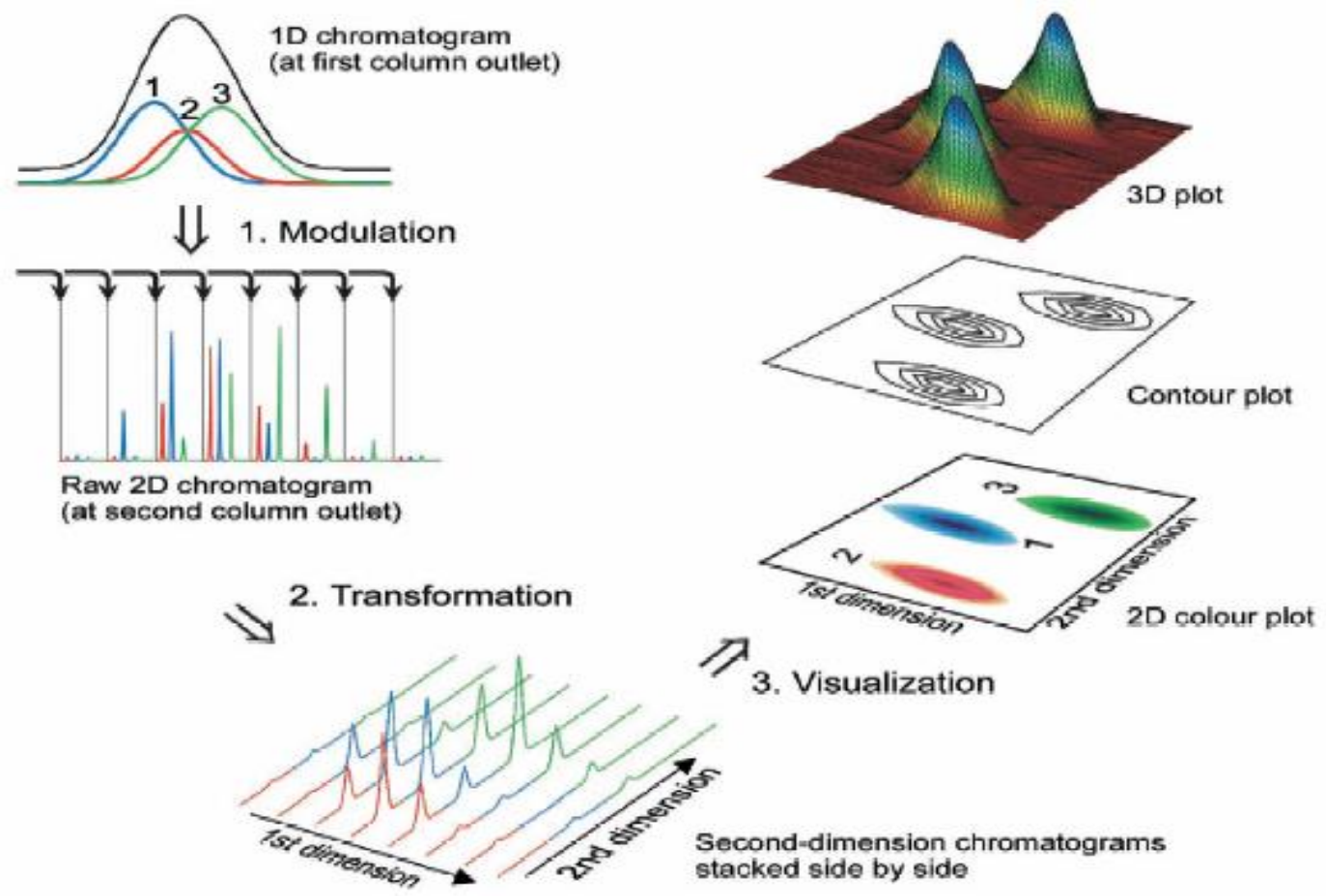
- a typical comprehensive separation is achieved, generally, on two distinct columns connected in series with a special transfer system (modulator) located between them

- the type of interface used is linked to the specific methodology

- the function of the interface is to cut and then release continuous fractions of the primary column effluent (^{1}D) onto a fast separation column (^{2}D)

Comprehensive MD Chromatography

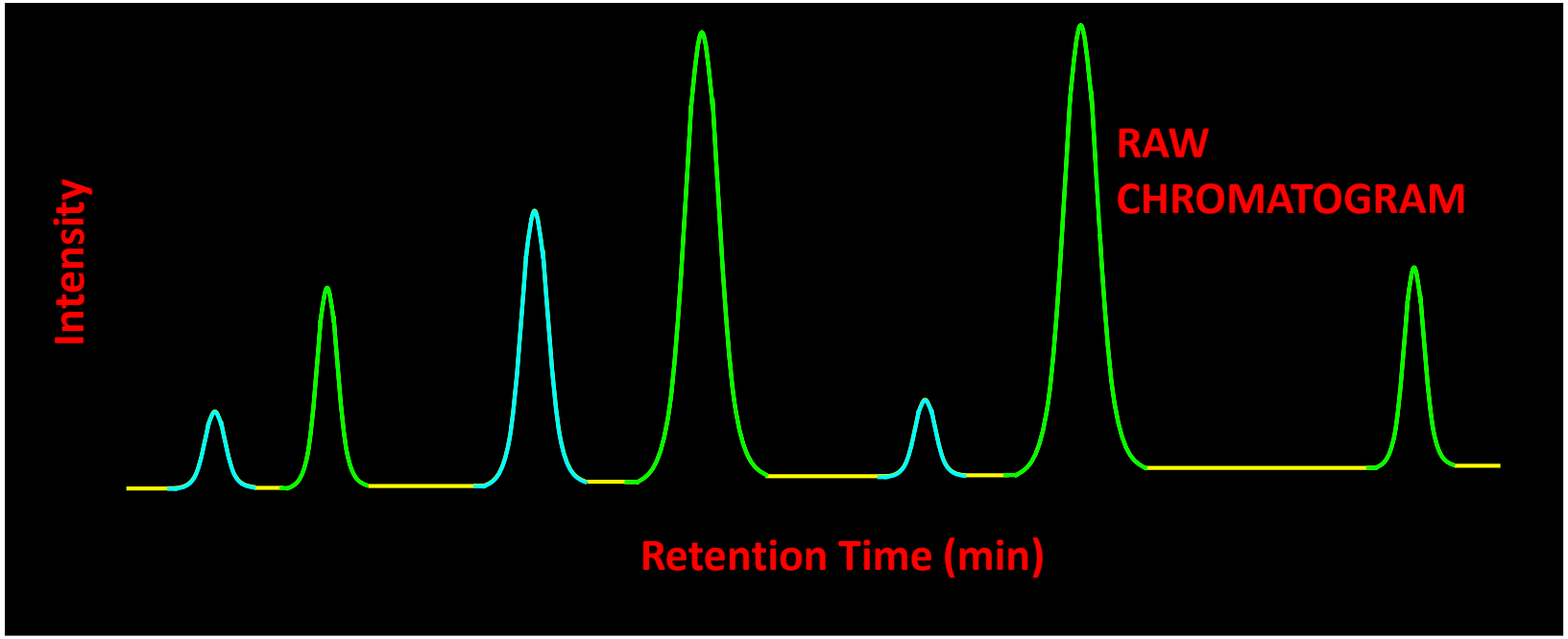
Data processing



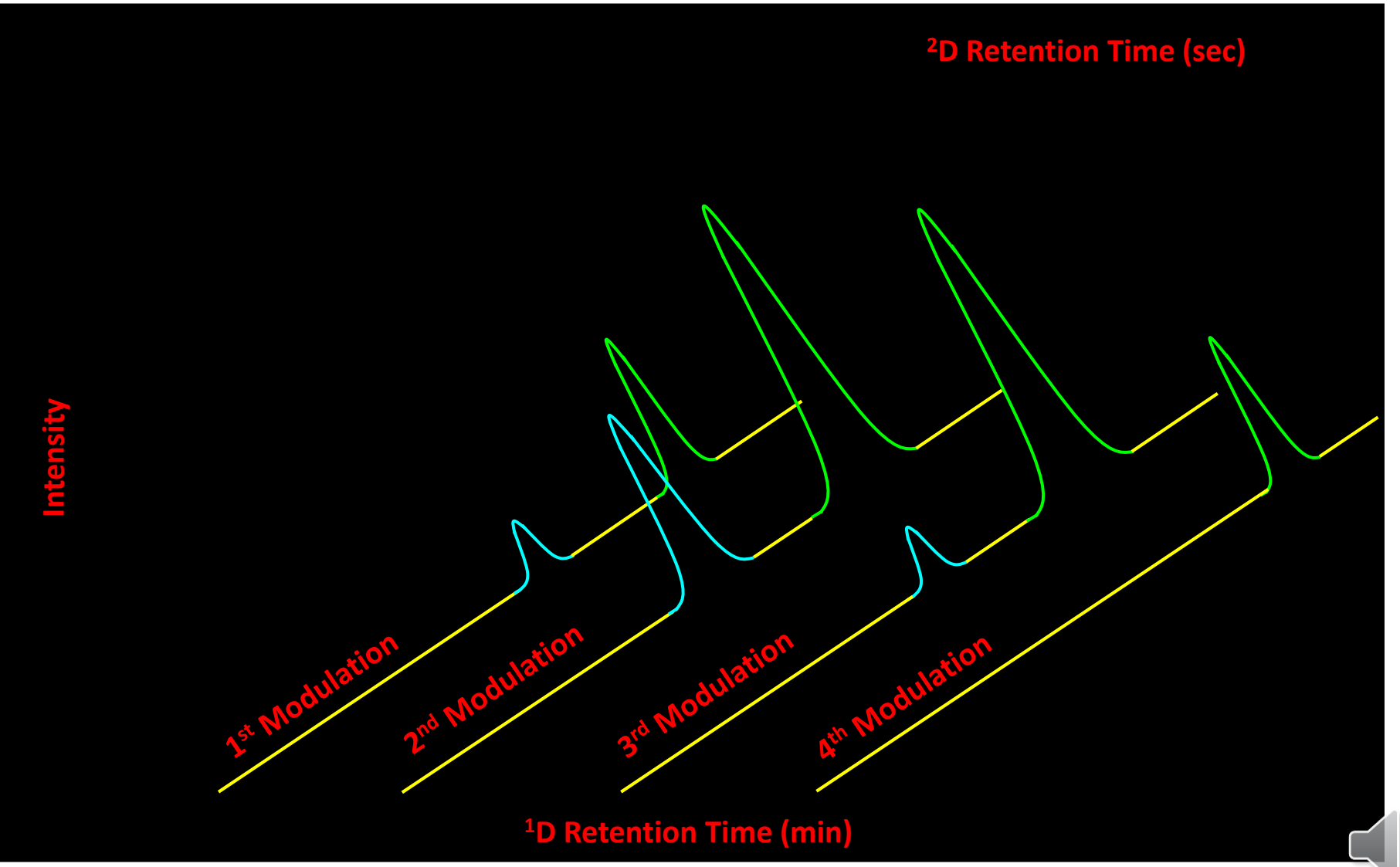
Comprehensive Chromatography Data Elaboration

- C×C produces a great amount of data which require considerable data elaboration power and dedicated software (some of them are commercially available as a complete package).

- C×C data elaboration is based on a specific procedure. The double separation is acquired by a single detector. Mathematic data manipulation has to be carried out for the generation of comprehensive chromatographic data



C×C Data Elaboration



Nomenclature and Conventions in Comprehensive Multidimensional Chromatography

Peter Schoenmakers,[a,b] **Philip Marriott**[c] **and Jan Beens,**[d]
[a]Polymer-Analysis Group, University of Amsterdam, The Netherlands,
[b]Dutch Polymer Institute, Eindhoven, The Netherlands,
[c]Department of Applied Chemistry, RMIT University, Melbourne, Australia,
[d]Faculty of Science, Free University, Amsterdam, The Netherlands.

Comprehensiveness

Therefore, a two-dimensional separation can be called comprehensive if

1. Every part of the sample is subjected to two different separations
2. Equal percentages (either 100% or lower) of all sample components pass through both columns and eventually reach the detector
3. The separation (resolution) obtained in the first dimension is essentially maintained.

Table 1: Examples of abbreviations involving the multiplex (×) sign.

Abbreviation	Full term
GC×GC	Comprehensive two-dimensional gas chromatography
GC×GC–FID	Comprehensive two-dimensional GC with flame-ionization detection
GC×GC–MS	Comprehensive two-dimensional GC with flame-ionization detection
LC×LC	Comprehensive two-dimensional liquid chromatography
LC×SEC	Comprehensive two-dimensional (liquid × size-exclusion) chromatography
LC×GC	Comprehensive two-dimensional (liquid × gas) chromatography
SFC×GC	Comprehensive two-dimensional (supercritical-fluid × gas) chromatography
GC×GC×GC	Comprehensive three-dimensional gas chromatography
LC–GC×GC	On-line liquid chromatography–Comprehensive two-dimensional gas chromatography
SFC–GC×GC	On-line supercritical-fluid chromatography–Comprehensive two-dimensional gas chromatography

Nomenclature and Conventions in Comprehensive Multidimensional Chromatography – An Update

LCGC Europe
Volume 25, Issue 5, pg 266–275

May 01, 2012
By Philip J. Marriott [1], Ze-ying Wu [2], Peter Schoenmakers [3]

Table 1: Examples of abbreviations involving the multiplex (×) sign.

Abbreviation	Location
GC×GC	Comprehensive two-dimensional gas chromatography
GC×GC–FID	Comprehensive two-dimensional GC with flame-ionization detection
GC×GC–MS	Comprehensive two-dimensional GC with mass spectrometry detection
GC×GC–'X'	Comprehensive two-dimensional GC with 'X' detection
LC×LC	Comprehensive two-dimensional liquid chromatography
LC×SEC	Comprehensive two-dimensional (liquid × size-exclusion) chromatography
LC×GC	Comprehensive two-dimensional (liquid × gas) chromatography
SFC×GC	Comprehensive two-dimensional (supercritical-fluid × gas) chromatography
GC×GC×GC	Comprehensive three-dimensional gas chromatography
LC–GC×GC	On-line liquid chromatography — comprehensive two-dimensional gas chromatography
SFC–GC×GC	On-line supercritical-fluid chromatography — comprehensive two-dimensional gas chromatography
SFC×SFC	Comprehensive two-dimensional supercritical-fluid chromatography
CE×CE	Comprehensive two-dimensional capillary electrophoresis
LC×CE	Comprehensive two-dimensional (liquid chromatography × capillary electrophoresis)
LC×GC×GC	Comprehensive three dimensional (liquid × gas × gas) chromatography
LC×LC×CE	Comprehensive three dimensional (liquid×liquid) chromatography×capillary electrophoresis
DGC×DGC	Comprehensive dynamic GC×GC
IC×LC	Comprehensive two-dimensional (ion×reversed-phase liquid) chromatography
Generic abbreviations for the technique. Whilst these have been noted in the literature, no agreement has not been adopted on their use, and so they are presented without comment.	
C2DC; CMDC; C2DS; CMDS	Comprehensive two-dimensional chromatography; Comprehensive multidimensional chromatography; Comprehensive two-dimensional separation; Comprehensive multidimensional separation

Suggested Literature on Multidimensional Chromatography

- (Seeley *J. Chromatogr. A, 962, 2002, 21)*

Band Broadening factor (sampling time and sampling phase) is negligible for modulators with duty cycles less than 1.

- (Carr and co-workers, Anal. Chem 80, 2008, 461; Anal. Chem 80, 2008, 8122)

Average ^{1}D Broadening factor (b) (sampling time and the standard deviation of the ^{1}D peaks prior to sampling).

- (Carr and co-workers, J. Chromatogr. A 1218, 2011, 64-73)

Quantitative estimation of ^{1}D under-sampling according to different variables (column length, flow rate, eluent composition)

- (Carr and co-workers, J. Chromatogr. A 1255, 2012, 267-276)

Evaluation of "effective" LC × LC peak capacity relative to the accessible area of the 2D separation space by using fractional coverage metrics