# OMICS GROUP

OMICS Group International through its Open Access Initiative is committed to make genuine and reliable contributions to the scientific community. OMICS Group hosts over **400** leading-edge peer reviewed Open Access Journals and organizes over **300** International Conferences annually all over the world. OMICS Publishing Group journals have over **3 million** readers and the fame and success of the same can be attributed to the strong editorial board which contains over **30000** eminent personalities that ensure a rapid, quality and quick review process. OMICS Group signed an agreement with more than **1000** International Societies to make healthcare information Open Access.

# OMICS Journals are welcoming Submissions

OMICS Group welcomes submissions that are original and technically so as to serve both the developing world and developed countries in the best possible way.

OMICS Journals are poised in excellence by publishing high quality research. OMICS Group follows an Editorial Manager® System peer review process and boasts of a strong and active editorial board.

Editors and reviewers are experts in their field and provide anonymous, unbiased and detailed reviews of all submissions.

The journal gives the options of multiple language translations for all the articles and all archived articles are available in HTML, XML, PDF and audio formats. Also, all the published articles are archived in repositories and indexing services like DOAJ, CAS, Google Scholar, Scientific Commons, Index Copernicus, EBSCO, HINARI and GALE.

**For more details please visit our website:**
**http://omicsonline.org/Submitmanuscript.php**

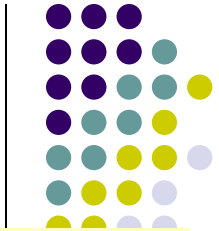
# Dr. Hadir M. Maher

Associate Professor, Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Alexandria University, Alexandria, Egypt.

Associate Professor, Pharmaceutical Chemistry Department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

Editor in **"Journal of Chromatography & Separation Techniques"**

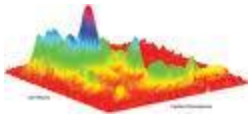
# Scientific Qualifications



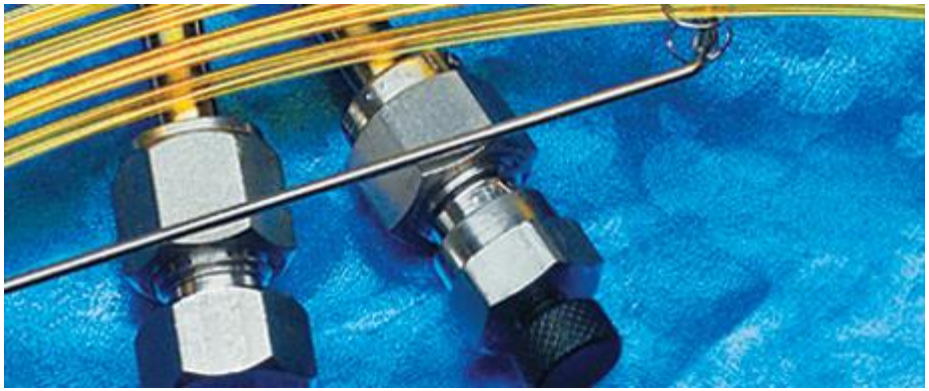
- Bachelor of Pharmaceutical Sciences, June 1999, with general grade "Excellent, High Honor" Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.
- Master of Pharmaceutical Sciences (Pharmaceutical Analytical Chemistry), April 2003, Faculty of Pharmacy, University of Alexandria. Alexandria, Egypt. Thesis entitled: "Analysis of certain anticancer drugs in their pharmaceutical preparations and biological fluids".
- Ph.D. of Pharmaceutical Sciences (Pharmaceutical Analytical Chemistry), November 2005, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt. Thesis entitled: "Analysis of some cough-cold preparations using modern spectroscopic and chromatographic techniques ".
- Post doctoral scholarship in the department of Pharmacal Sciences, Harrison School of Pharmacy, Auburn University, Auburn, Alabama, USA, beginning (August 2008 - August 2009).

# Research interest

- Analysis of drugs in their pharmaceutical products and biological fluids using different instrumental techniques including; spectrophotometry, spectrofluorimetry, electrochemical methods of analysis and chromatography. Among the chromatographic methods are: HPLC, HPTLC, CE, GC-MS and GC-IRD techniques.
- The application of chemometrics to handle different response data for an attempt to solve the problem of interfering background.



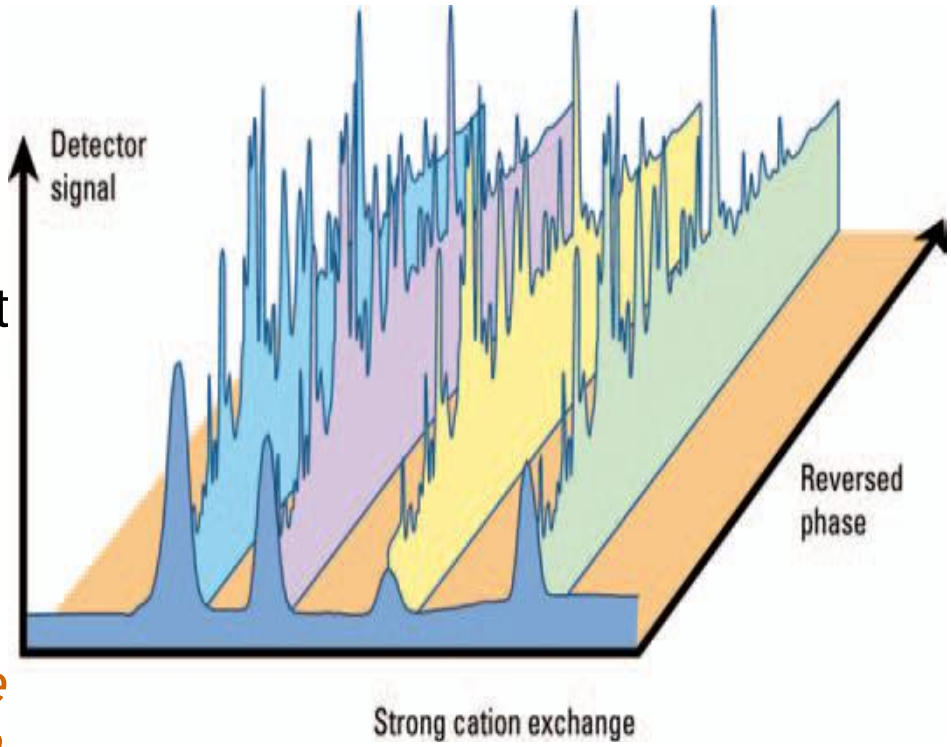
# Multidimensional chromatography



Prepared by:
**Dr. Hadir M. Maher**
Associate Professor of Pharmaceutical Analytical Chemistry
Alexandria University, Egypt

# What is multidimensional chromatography

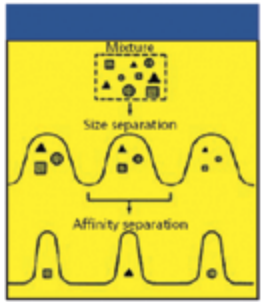
- **Multidimensional chromatography** allows separation of complex mixtures by using **multiple columns** with **different stationary phases.**
- These columns are coupled ***orthogonally***, which means that fractions from the first column can be selectively transferred to other columns for additional separation.
- *This enables separation of complex mixtures that cannot be separated using a single column.*



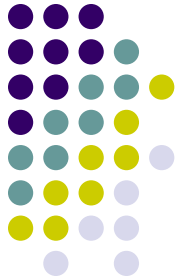
# Advantages:

- **Multidimensional methods** allow the analysis of complex samples in a few steps.
- Beside the **coupling of several columns** with the same properties for highest resolution, combinations of chemically different stationary phases are also used.
- These approaches enable

  the increase of the peak capacity and can help to avoid co-elution as can happen in a single dimension chromatography.



# Concepts of multidimensional chromatography

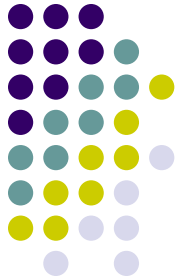


- In HPLC and GC practice different concepts are used and common:

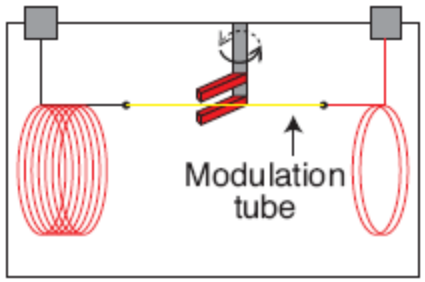
  I- *peak cutting* and the selective transfer of peaks or chromatogram sections onto a second column (heart-cut method)

  II- continuous two-dimensional chromatography (comprehensive chromatography).

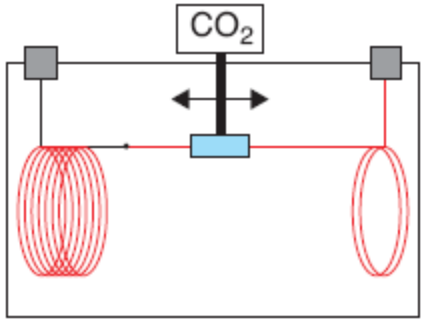
# *I-heart-cutting MDGC technique*



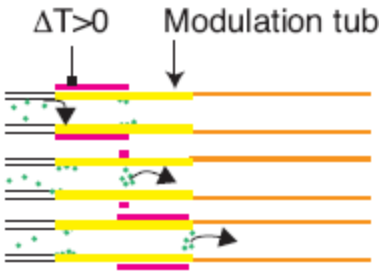
- **In the *heart-cutting MDGC technique*, one or more unresolved fractions from a first column (first dimension) are transferred to a second one having a different polarity (second dimension) where the separation of the compounds will be achieved.**
- **The heart-cut can be directly transferred to the second column or it can be trapped on a cryogenic device and transferred later.**
- **Thus, it is possible to enrich the trap with many heart-cuts of the same analyte coming from sequential injections.**

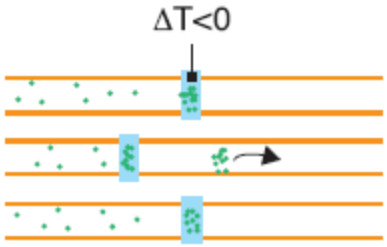


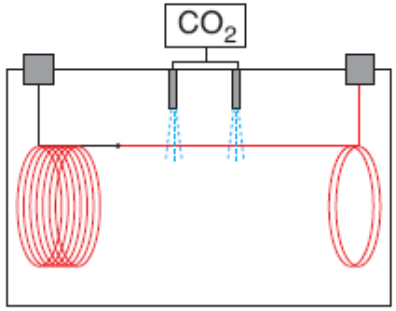

a)



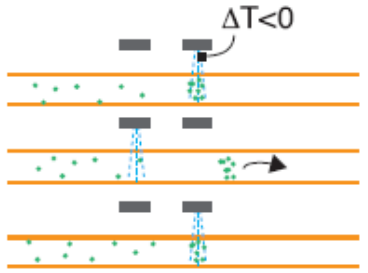

b)

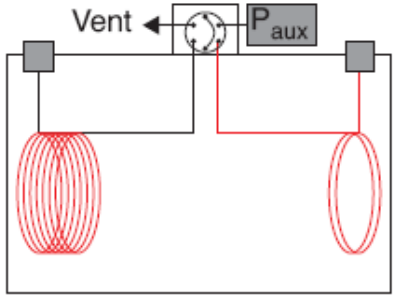


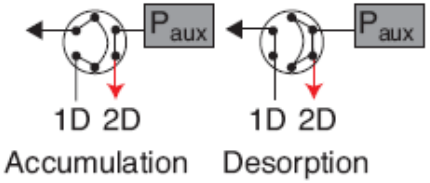




c)




d)

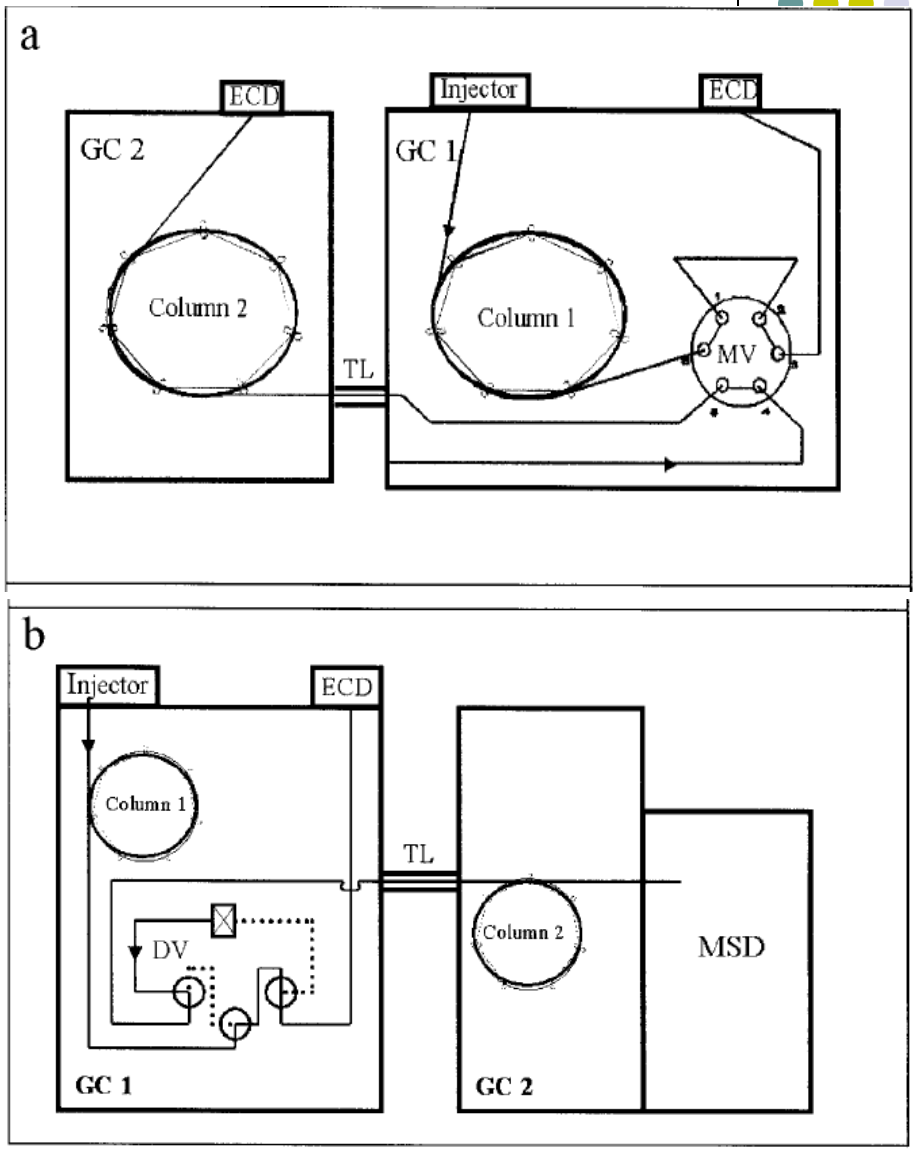



# Different modulation systems

a) Sweeper modulator
b) Longitudinally modulation system
c) Dual jet $CO_2$ modulator
d) Valve-based modulator

# Experimental Design

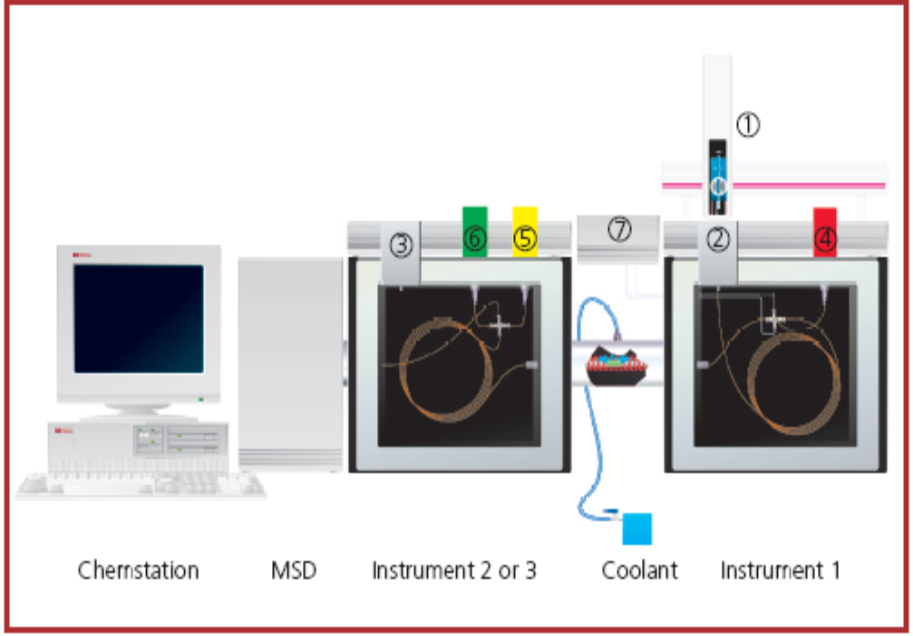
- **Basically, to perform MDGC, one GC oven, two columns, two detectors and one switching system are needed. The disadvantage of this simplest configuration is that if a temperature program is needed to separate different peaks of one sample, the program will be the same for both columns, losing peak capacity.**
- **To solve this problem one can install a cryogenic trap inside or outside the GC. In this case, analytes will be trapped there for some time and will be released later to the second column.**



# Experimental Design

- **Another possibility is to work with two independent ovens. One GC with two ovens can be used. Or, two GCs are connected together by means of a heated transfer line.**
- ***When working with two ovens*, it is not necessary to use a cryogenic trap. In this case, the heart-cut from the first column is sent to the second oven where the temperature is lower and then the analytes will be trapped at the beginning of the column until the temperature program starts.**

**Figure 1:** Schematic of the GC–GC system. 1 = injector for either oven; 2 = PTV inlet on instrument 1; 3 = PTV inlet on instrument 2 or 3; 4 = monitor FID on instrument 1; 5 = sulphur chemiluminescent detector; 6 = nitrogen chemiluminescent detector; 7 = pneumatics unit for column switching device.

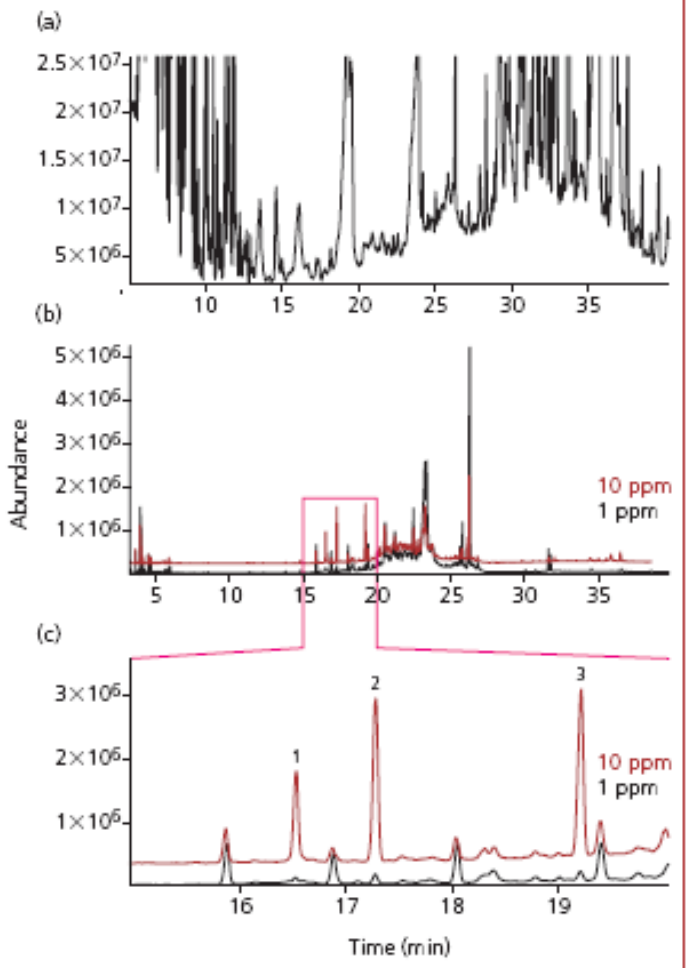


# RECENT APPLICATIONS IN MULTIDIMENSIONAL CHROMATOGRAPHY

## OP pesticides in Lemon oil

- **Figure 3 compares the scan TIC traces for direct injection to the HP5 column only (no heartcutting) of the lemon oil spiked with 10 mg/L (ppm) of the OP mix, with a similar TIC trace but after three heartcuts from the DB17 precolumn.**

**Figure 3:** (a) Scan TIC after injection of lemon oil with 10 mg/L OP pesticides to HP5-MS column only; (b) Scan TIC after injection of lemon oil with 1 and 10 mg/L OP pesticides to DB-17MS precolumn with three heartcut transfers to HP5-MS column.




**Peaks:** 1 = methyl parathion, 2 = fenchlorphos, 3 = chlorpyrifos.

# Recent applications

## Spicy citrus aroma

- **The group of peaks eluting near 7 minutes contained a spicy citrus aroma.**
- The 6-port valve was then turned to the **multidimensional configuration** and a second Twister stir bar was analyzed with **a heart cut in the 7.9-8.5 minute** region to transfer the group of peaks to the cold trap at the head of the second (main) column. Heating the cold trap to start the second dimension separation gave a good separation of peaks, allowing identification of decanal as the compound responsible for the spicy citrus aroma. In addition, seven more trace aroma compounds could be detected in this same heart cut fraction.

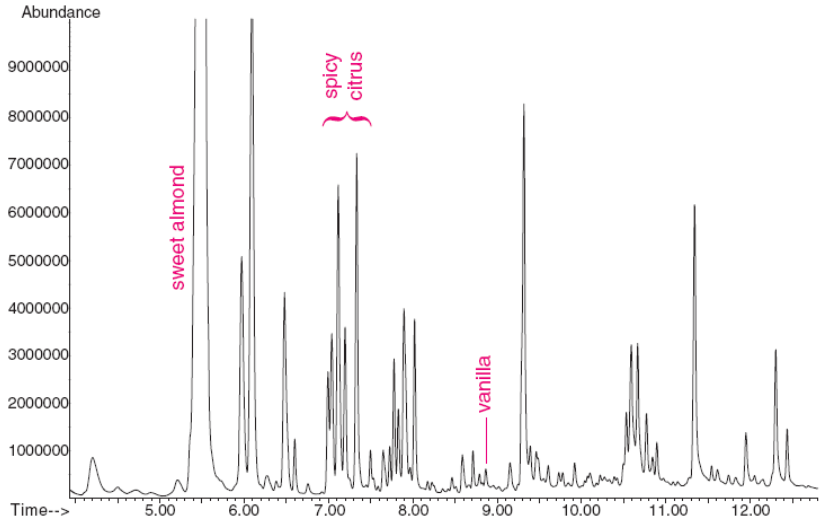



**Figure 2.** Single dimension separation of Amaretto flavor extract on a 30m x 0.25mm x 0.25µm DB-5MS column with main fragrance regions identified.

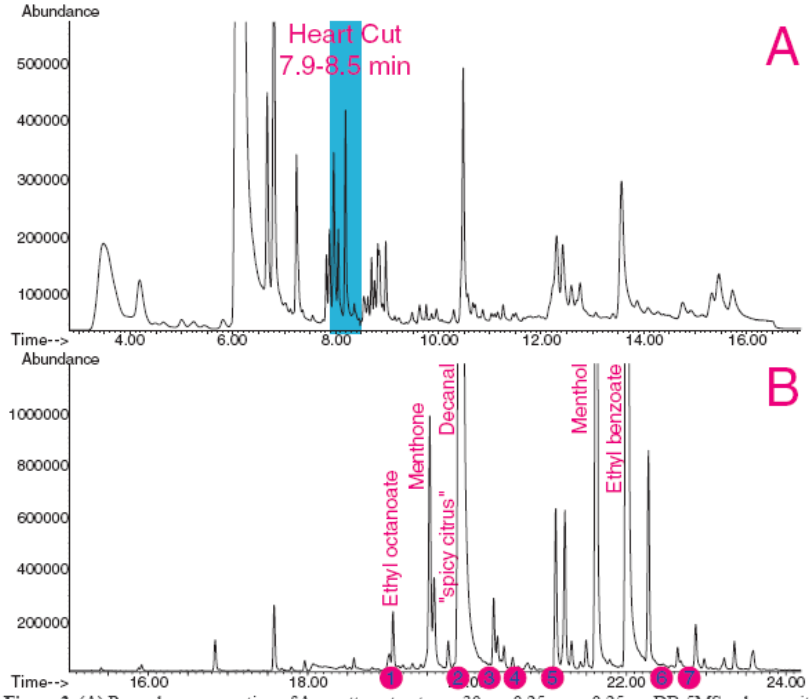



**Figure 3.** (A) Pre-column separation of Amaretto extract on a 30m x 0.25mm x 0.25µm DB-5MS column with heartcut region (7.9-8.5 minutes) highlighted. (B) Main column separation of heartcut region with major "spicy citrus" aroma identified as decanal. Descriptors for additional aroma regions are listed in Table 1.

# Identification of sulfur compounds in Sauerkraut juice extract

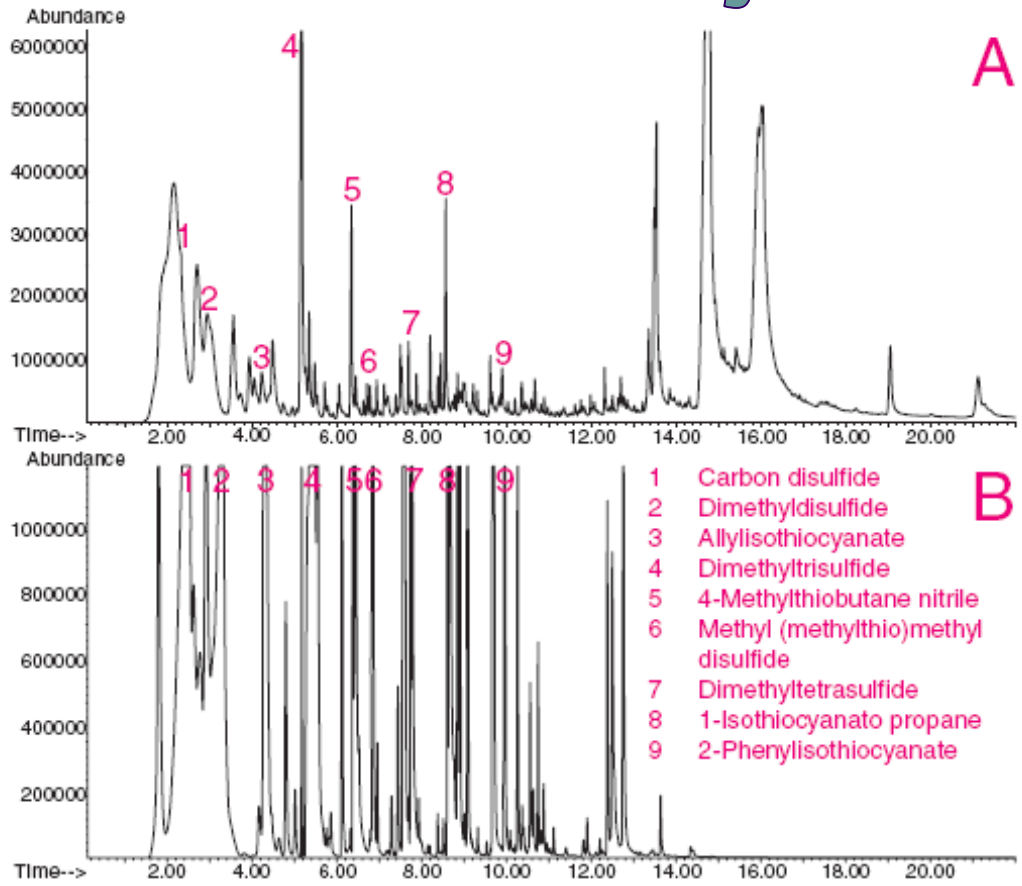



**Figure 4.** Single dimension separation of commercial sauerkraut juice extract with TIC (A) and PFPD (B) response. Peaks corresponding to main organosulfur compounds are numbered.

# Sauerkraut juice extract

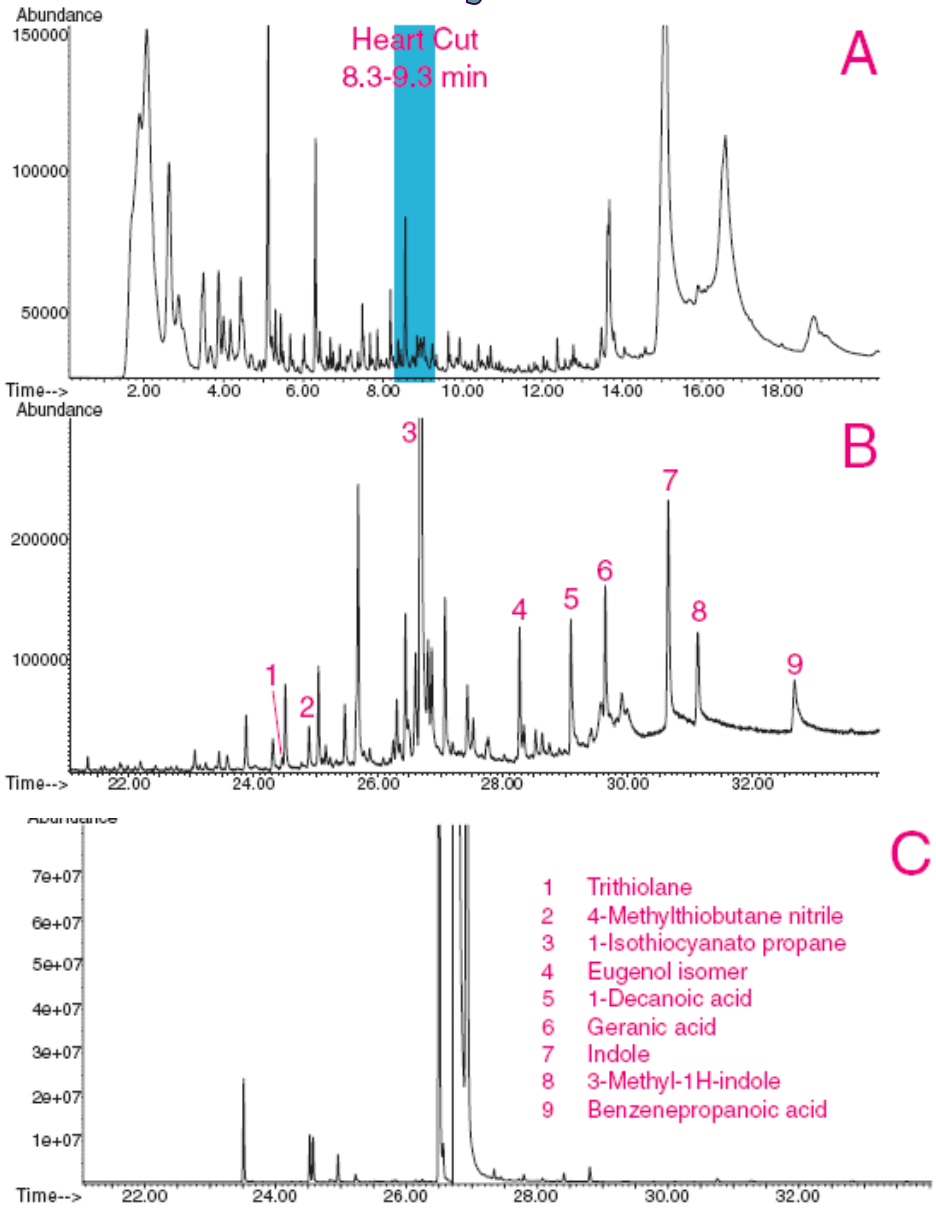



**Figure 5.** Pre-column separation of sauerkraut juice extract with heartcut region (8.3-9.3 minutes) highlighted. Main column separation TIC (B) and PFPD response (C) of heartcut region with three sulfur and six additional compounds identified.

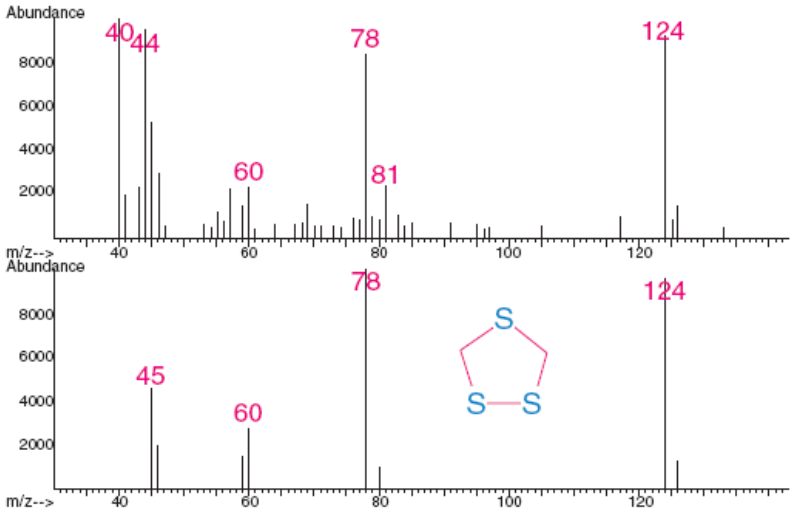



**Figure 6.** Wiley138 library match for trithiolane detected in sauerkraut juice.

# Soy sauce

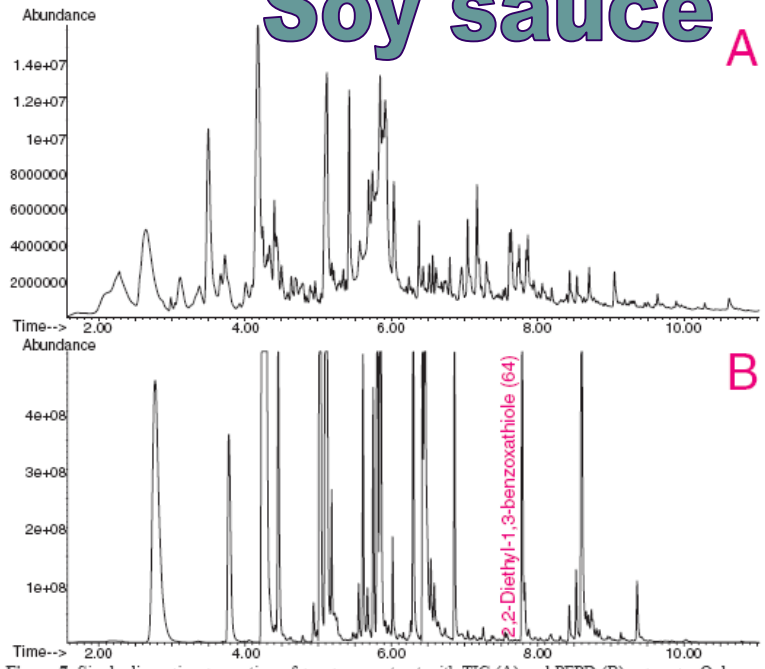



Figure 7. Single dimension separation of soy sauce extract with TIC (A) and PFPD (B) response. Only one organosulfur compound could be tentatively identified.

- Figure 7 shows the first dimension separation of a Twister extract of soy sauce run with fast heating (50C/min).
- The 6-port valve was then turned to the **multidimensional configuratior** and a second Twister stir bar was analyzed with **a heart cut in the 5.5 5.92 minute region** containing sever sulfur compounds. Heating the cold trap at the head of the main column t start the second dimension separatio (Figure 8) gave a good separation of peaks, all well resolved from the benzoic acid interference.

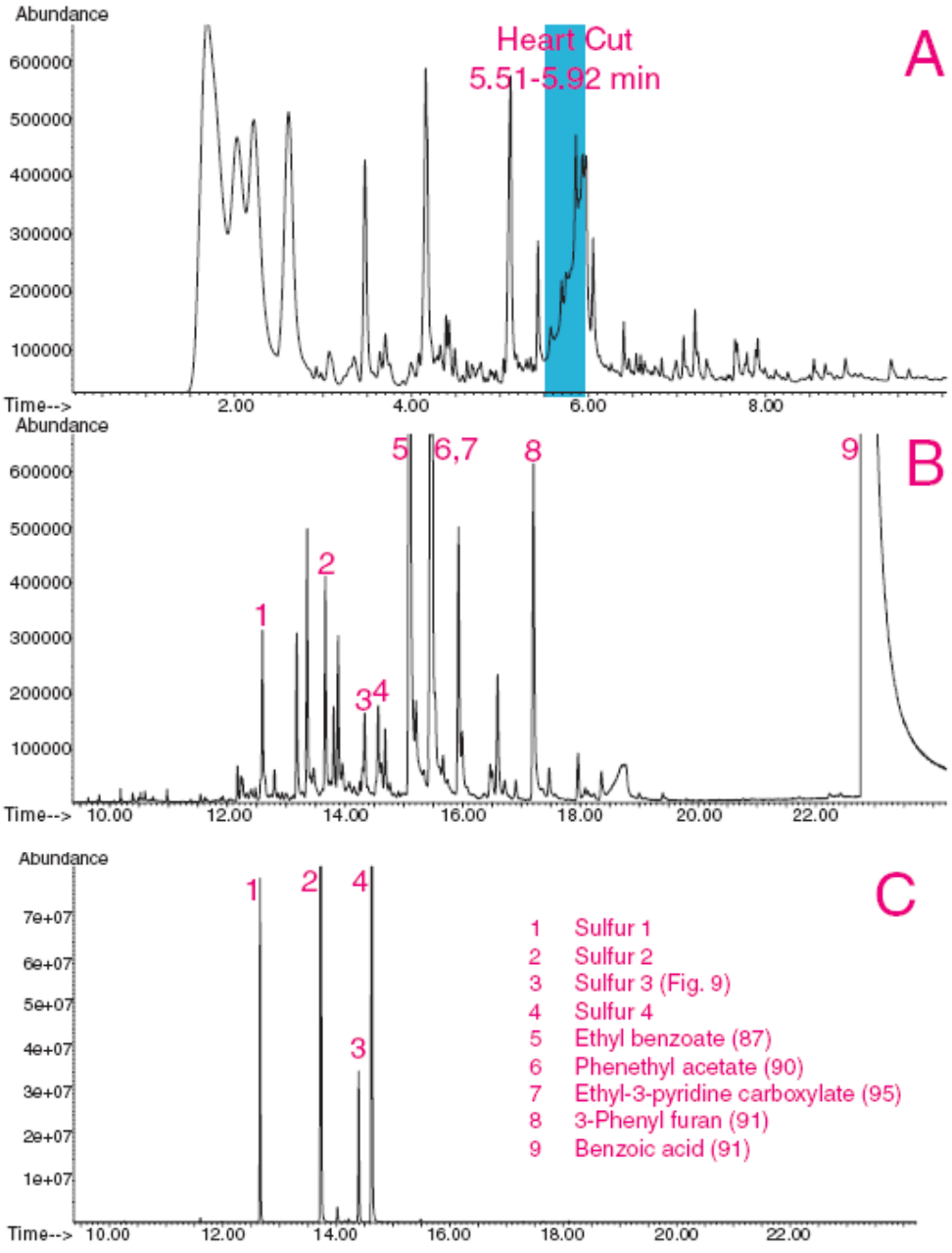
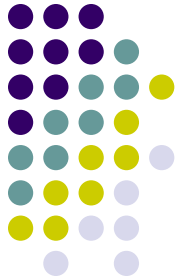



Figure 8. Fast pre-column separation of soy sauce extract with heartcut region (5.51-5.92 minutes) highlighted. Main column separation TIC (B) and PFPD response (C) of heartcut region with three sulfur and six additional compounds identified.

# Steps to trace compound identification by heartcut GC-GC



- ***The major steps to trace compound identification by heartcut GC-GC are:***

1-Introduce sufficient mass of the compounds of interest into the column to obtain adequate response at the selective detector (ODP or PFPD) and the MSD.

2- Identify the regions of the chromatogram in which target compounds elute using a selective detector.

3- Reconfigure the GC for **heartcut GC-GC**.

- ***Heart cut regions*** containing target compounds and interfering matrix components onto a second, orthogonal GC column to separate matrix interferences from the compounds of interest.

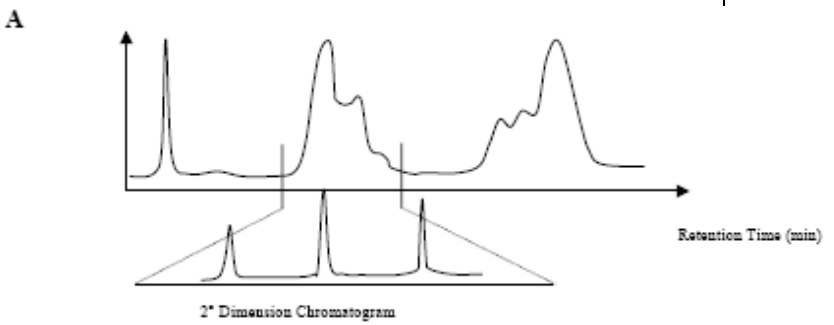
4- Identify the resolved components using selective detection and MSD in parallel.

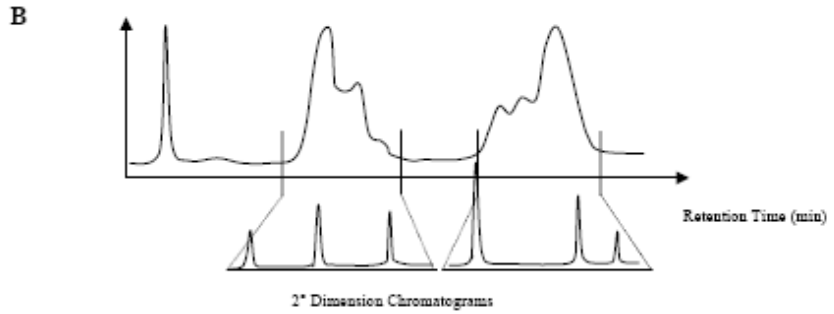
# II- Comprehensive two-dimensional chromatography

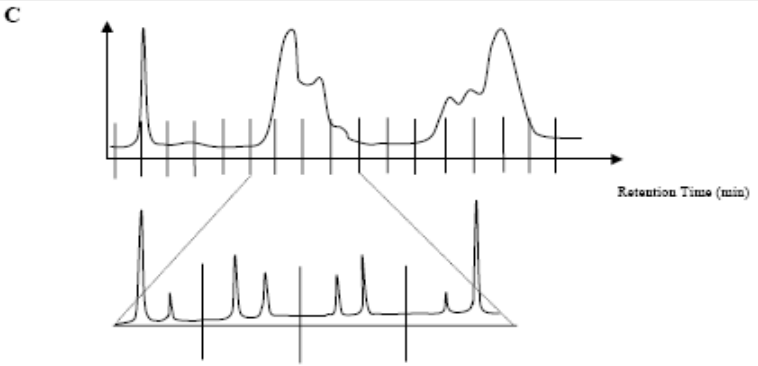
- In ***comprehensive two-dimensional gas chromatography (GCxGC)***, the entire sample, and not only fractions, are separated on two different columns. The columns are shorter, typically 15 to 30 meters for the first and only 1 to 2 meters for the second. The short length of the second column will enable very fast separations while collecting the fractions from the first column. The most important component of the system isthe 'modulator', which will accumulate the fractions coming from the first column on a short segment of column, and then release it quickly into the second one. There are different kinds of modulators but in general the mechanisms involve alternate cryo-focussing and thermal desorption of the analytes trapped.

- 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.
- The abbreviation GC×GC is suggested for a comprehensive two-dimensional gas chromatography system with two parallel second dimension columns. According to the guidelines given above for use of the word "comprehensive," this is applicable to such a system, provided that the sample is distributed across the two second-dimension columns without any discrimination.

# Multidimensional against heartcut GC




The concept of multidimensional GC. (A) single heart-cut GC analysis, in which a portion of the effluent from the primary column containing analytes of interest is diverted to the second dimension column and subjected to additional separation over an extended period of time. (B) dual heart-cut GC analysis, in which two regions with coelutions are diverted to the second dimension column, with less time to perform each separation. (C) comprehensive two-dimensional GC analysis, in which the sizes of the sequential heart-cut fractions are very small, and the time to develop each sequential second dimension chromatogram is very short

# Examples of comprehensive multidimensional chromatography

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

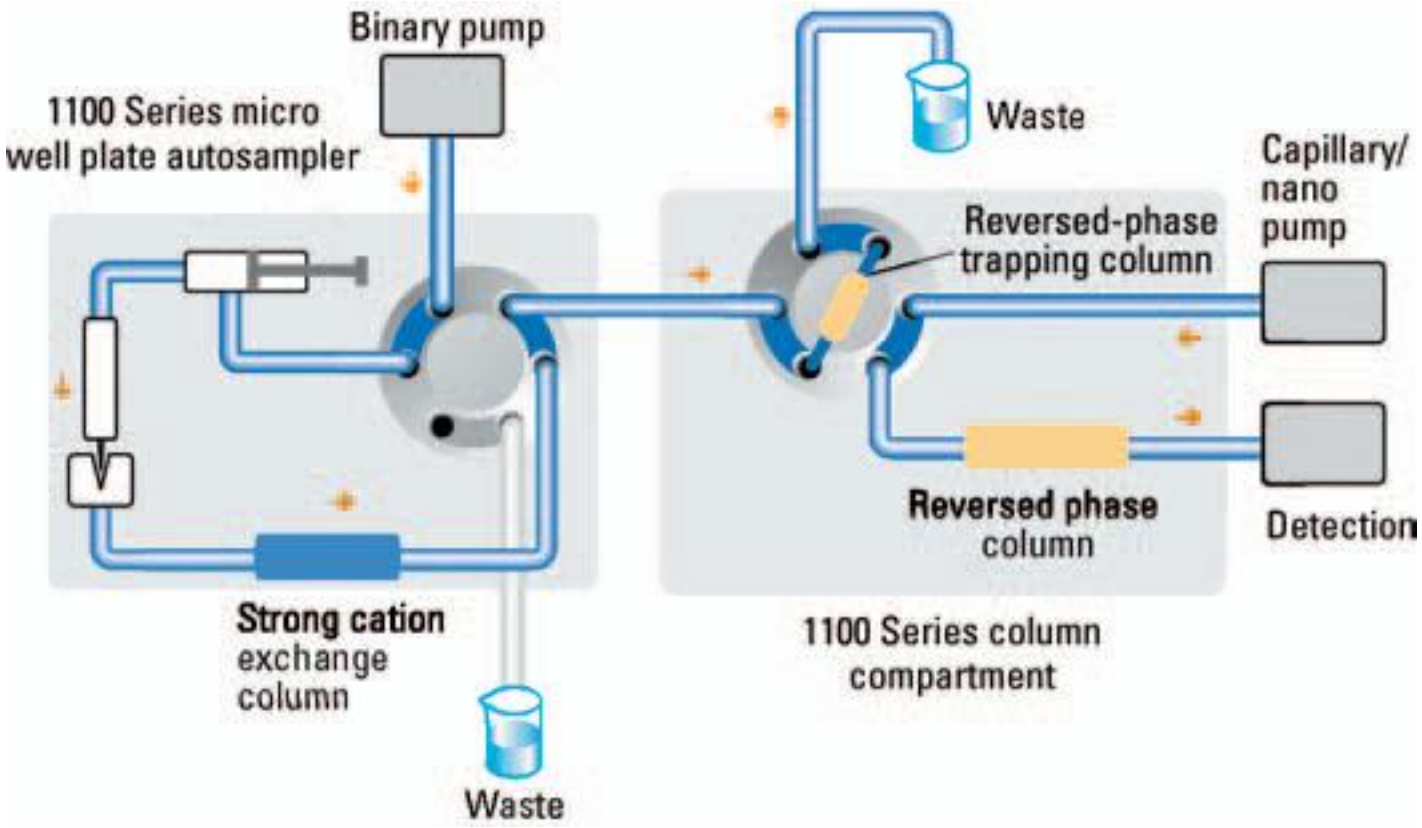
| Abbreviation | Full term |
|---|---|
| GC×GC | Comprehensive two-dimensional gas chromatography |
| GC×GC–FID | Comprehensive two-dimensional GC with flame-ionization detection |
| GC×GC–MS | Comprehensive two-dimensional GC with flame-ionization detection |
| LC×LC | Comprehensive two-dimensional 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 |

# Common applications for multidimensional LC are:


- Proteins and peptides
- Drug isolation from urine and plasma
- Polysaccharides
- Homopolymers, oligomers, copolymers
- Surfactants
- Polycyclic aromatic hydrocarbons
- DNA fragments

**proteomics,where complex protein digests are separated by multi-dimensional liquid chromatography instead of using the two dimension gel electrophoresis.**

# Determination of peptide mixtures by two-dimensional LC

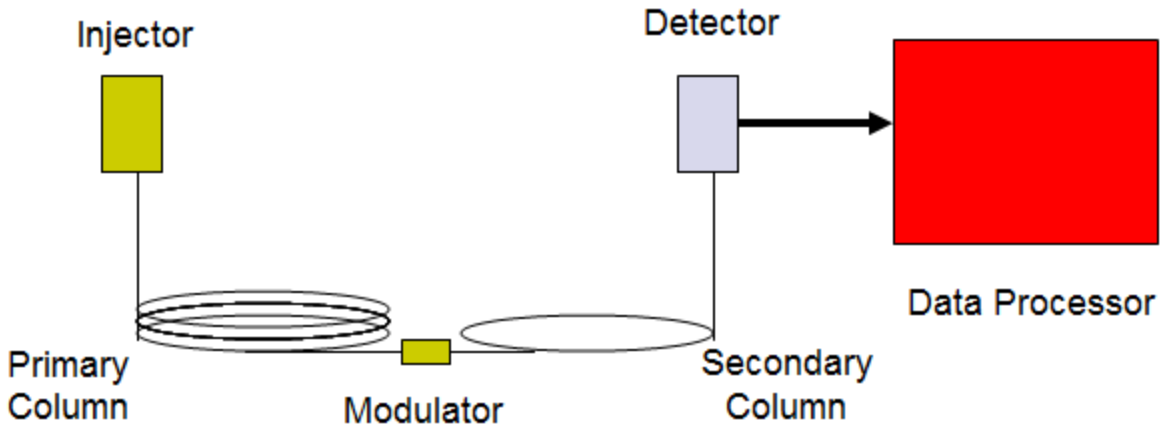


# Comprehensive LC (LC x LC)

- **Comprehensive LC** (also called **LC x LC**) couples two liquid chromatography based separation methods and enables the separation of complex samples as well as the identification of co-eluting peaks.
- **Different separation mechanisms** should be chosen in order to achieve maximum possible resolution.
- **two binary gradient systems** enable maximum flexibility in choosing the separation method and mechanism of detection after the second dimension only minimizes the transfer volume between the separation steps
- for the sample transfer a switching valve with two sample loops is used and operated in continuous mode
- **all types of LC detectors and mass spectrometers** can be used

# Comprehensive GC (GC×GC)

- It is based on **two columns of different polarities.**
- The key component of GC x GC is the **modulator** connecting both columns. It focuses the compounds from the first column and injects them into the second column.
- **Correct representation of the very narrow peaks.**



# LC-GC coupling

- For complex samples it may not be sufficient to couple two GC columns o different polarity to characterize all components. In this case, **LC can be coupled to GC**. Whereas **LC separates compounds by their polarity or size**, compounds in **GC using non-polar columns are separated by their boiling points.**
- These different separation mechanisms can be used for the separation of co-elutions in various applications, e.g. characterization of olive oil or polymers or the determination of pesticides in comple matrices.
- Transfer of the fractions to the GC is performed via a special syringe using the AOC-5000 autosampler.

# OMICS Group **Open Access Membership**

OMICS publishing Group Open Access Membership enables academic and research institutions, funders and corporations to actively encourage open access in scholarly communication and the dissemination of research published by their authors.

For more details and benefits, click on the link below:

http://omicsonline.org/membership.php