

Comprehensive GC×GC

or

When one separation is not enough

What

Comprehensive GC×GC is a separation technique, in which sample components are first separated on one column and then all components of the sample pass through a second column with a different stationary phase and thus different retention mechanism.

Why

Comprehensive GC×GC can be used when resolution obtained on a single separation column is insufficient. This usually applies to complex mixtures of compounds with a broad range of physical-chemical properties. The second dimension allows us to separate compounds that co-elute from the first column.

How to comprehend the “comprehensive”?

To understand what comprehensive GC×GC means you have to imagine an onion. Those of you who lack imagination can fetch a real onion (warning: real onion can cause eye irritation ;-). You can also look at **Figure 1**.

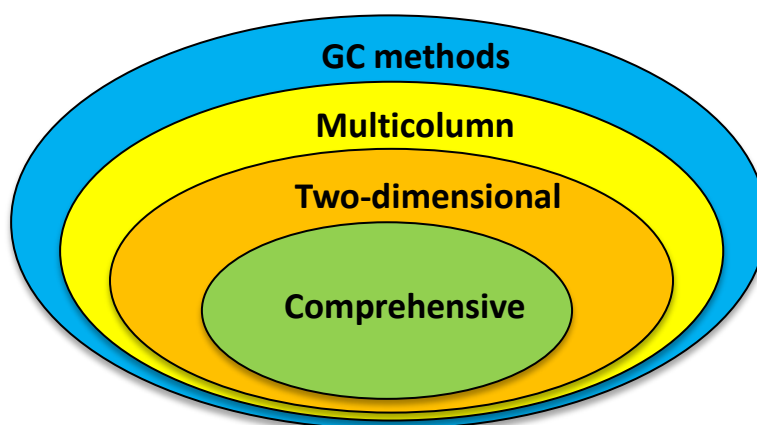


Figure 1 Categorization of GC methods.

1. The whole onion represents **all gas chromatography methods**. Even the simple ones where you have one column and simply separate components of your sample on this single column.

If we peel off the first layer of the onion, we remove these simple methods with one column.

2. Now we have **all multicolumn methods** left. In these methods – surprise, surprise – more than one column is used.

*If we peel off the second layer we are removing the simplest types of multicolumn methods as illustrated in **Figure 2**.*

A) Methods where you split the sample to several parts and each part passes through a different column. Thus you have several columns in parallel.

B) Methods where you connect columns in series and the sample passes sequentially through all of them.

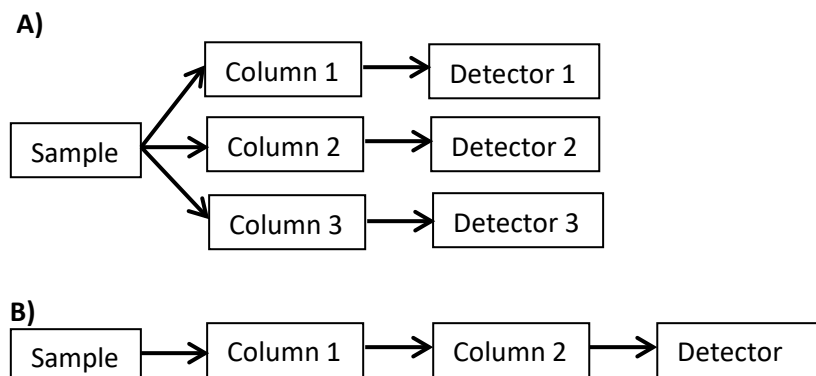


Figure 2 Multicolumn methods that are NOT two-dimensional. A) The sample is split into several columns in parallel. B) The sample passes through several columns connected in series.

3. We have **all the two-dimensional methods** left. The rules for a method to be two-dimensional are already quite strict: a) sample components are subjected to two mutually independent separation steps, b) sample components once separated must remain separated until the end of the analysis, i.e. in the second dimension you must not lose resolution of two compounds you managed to separate in the first dimension. For this reason, you cannot simply connect two different columns in series. If you do so, different selectivity of the second column will probably cause some of the compounds separated on the first column to overlap during the second separation, at least you cannot guarantee it won't happen.

If we peel off the third layer we are peeling off all methods that are two-dimensional but not comprehensive. In these methods, we usually perform separation of all sample components on the first column and then we inject only one or a few non-resolved bands eluting from the first column to the second column. A different stationary phase of the second column enables the separation of the co-eluting compounds. This is illustrated in **Figure 3**.

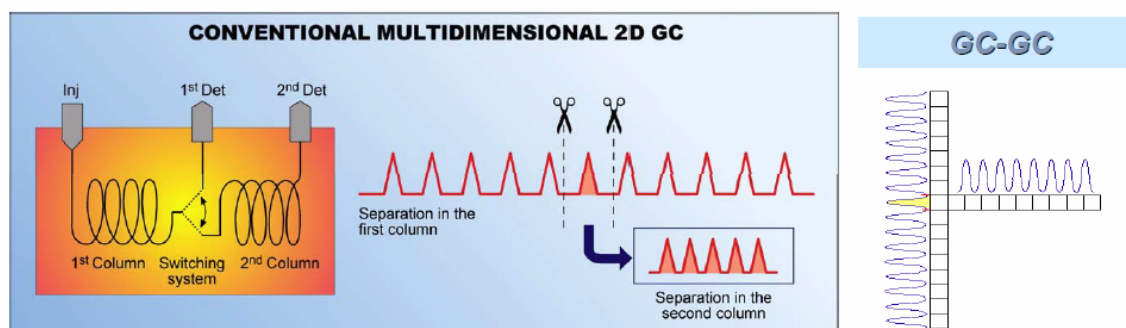


Figure 3 Two-dimensional but NOT comprehensive GC separation. Reprinted from Cavagnino D.: 4th Thermo Scientific High-Resolution GC/MS meeting on POPs, Barcelona 2010.

4. Finally, what is left are the **comprehensive two-dimensional GC×GC methods**. This is a special case of two-dimensional methods and the rules for a method to be in this elite group are: a) ALL components of the sample are subjected to two independent separations, b) equal percentages of all sample components pass through both columns and eventually reach the detector, c) separation (resolution) achieved in the first dimension is practically maintained (in practice the loss of resolution from the first dimension should not exceed 10%). Comprehensive GC×GC is illustrated in **Figure 4**.

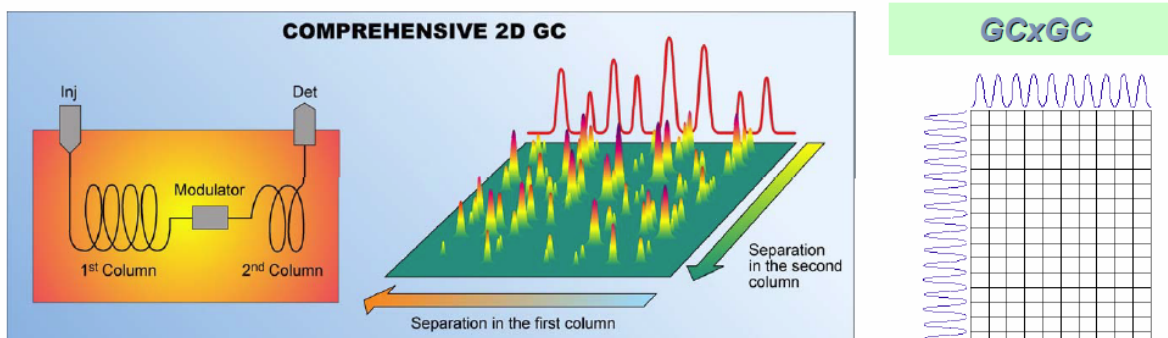


Figure 4 Comprehensive two-dimensional GC separation. Reprinted from Cavagnino D.: 4th Thermo Scientific High-Resolution GC/MS meeting on POPs, Barcelona 2010.

5. Now you can throw the onion peels away. We will stick with comprehensive GC×GC methods.

How it works

In comprehensive GC×GC, **the first separation is done on a classical column** (e.g. 30 m × 0.25 mm) and the effluent from the first column is being injected to **the second column for fast separation** (e.g. 1 m × 0.1 mm column) in very short intervals. The second column has a different stationary phase which allows separation of compounds not separated on the first column. The second column is very short which shortens the separation time. It also has a low inner diameter which causes high flow rates and thus even faster separations. The sampling of the effluent from the first column to the second column is called **modulation**. Due to the very fast second separation, a fast detector is required in GC×GC instruments.

Terminator, predator, and modulator

You will probably be disappointed but we are going to talk only about the one that does not fit in this triad, **the modulator**. The principle of **modulation** is shown in **Figure 5**. Sample components eluting from the first column are retained in the modulator for a very short time interval and then injected to the second column, where their very fast second separation is performed. While the second separation is being performed, other compounds eluting from the first column are being collected and retained by the modulator. When the second-dimension separation of the previous fraction is finished, compounds collected in the modulator are injected into the second column and separated there.

Figure 6 illustrates how the data obtained from GC×GC are processed to transform the record from the single detector into a three-dimensional chromatogram.

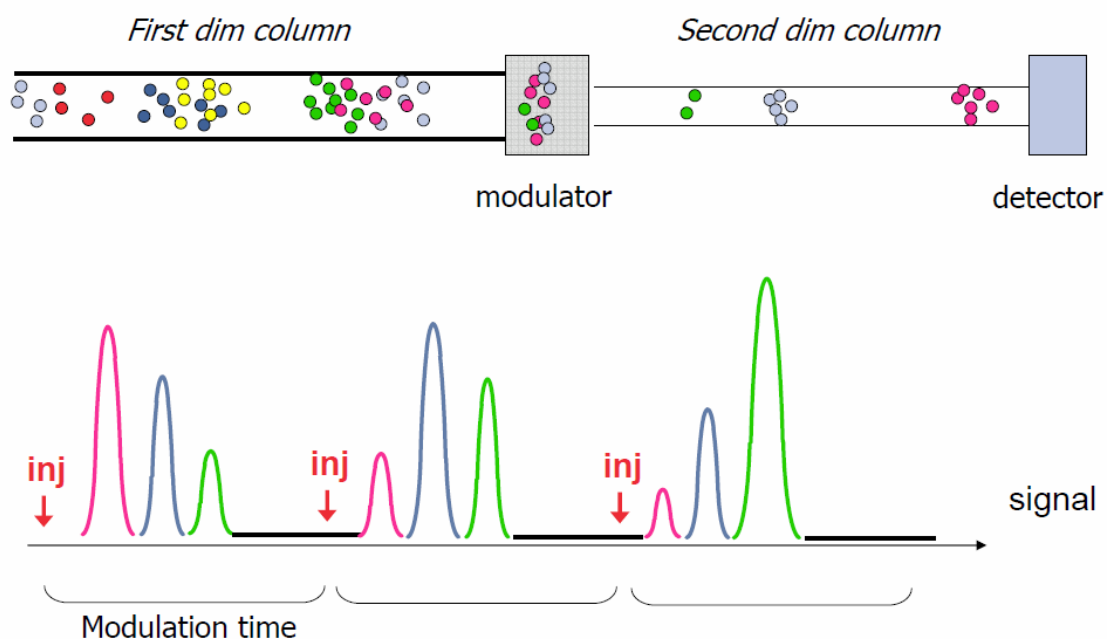


Figure 5 Principle of modulation. Reprinted from Cavagnino D.: 4th Thermo Scientific High-Resolution GC/MS meeting on POPs, Barcelona 2010.

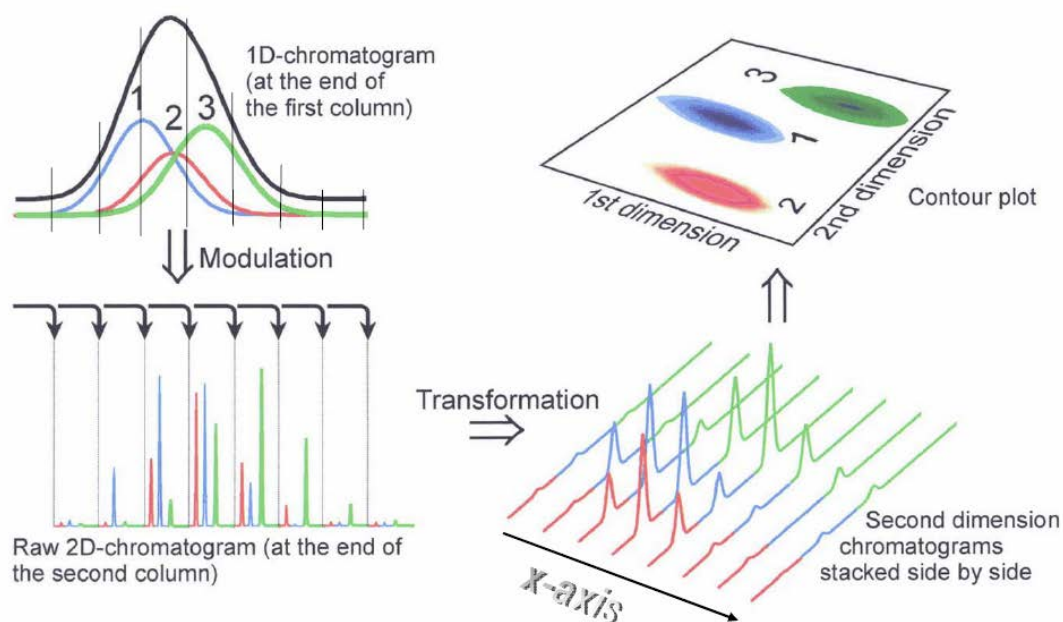


Figure 6 GC×GC data processing. Reprinted from Cavagnino D.: 4th Thermo Scientific High-Resolution GC/MS meeting on POPs, Barcelona 2010.

Modulation period

The time interval during which effluent from the first column is collected by modulator before it's injected to the second column is called **modulation period**. The modulation period must be carefully chosen because we are tiptoeing between Scylla and Charybdis here.

First of the two monsters is the **modulation period being too short**. The modulation period must be long enough to allow the second dimension separation to be completed while the next fraction of effluent is collected in the modulator. If the period is too short, the next fraction of the effluent is injected into the second column before all the analytes from the previous separation elute. Then these late eluting analytes appear at the beginning of the next modulation period as "wrap-around".

The other monster is the **modulation period being too long**. The longer the modulation period, the bigger is the loss of resolution from the first column. If the modulation period is too long, peaks eluting from the first column can be undersampled. If the modulation period is very long then two peaks that were completely separated in the first column can be collected back together in the modulator and injected together in one zone to the second column.

Therefore, the modulation period should be as short as possible but long enough to avoid the wrap-around effect.

Choose your favorite modulator

Modulators can be categorized by two criteria. The first criterion is the principle of modulation – we have **thermal modulators** and **pneumatic modulators**. Thermal modulators are based on fast cooling that leads to the condensation of analytes. Thanks to that the analytes stay in the modulator and are no longer carried by the mobile phase gas. In pneumatic modulators, valves are used to stop and resume the flow not only of the analytes but of all mobile phase.

The second criterion is the mode of operation – there are **single-stage** modulators where modulation occurs in one part of the modulator. **Dual-stage modulators** (or two-stage modulators) switch between two events occurring in series in two different parts of the modulator. **Twin-stage modulators** work with two reciprocal events.

Some examples of modulators

There are many types and variations of modulators today. As the purpose of this material is not to provide a complete list of them, let's have a look at some examples to get the idea of what kinds of technical solutions can be used.

Thermal modulators

The first example is the **dual-stage thermal-desorption modulator** published by Z. Liu and J. B. Phillips (J. Chromatogr. Sci. 26 (1991) 227). They used two columns connected in series. The head of the second column was looped outside the GC oven and coated with conductive gold paint. The part of the column that was outside the oven had a low temperature, which caused the analytes to condense and stay immobile in the modulator. The painted part of the column was divided into two segments that were alternately heated by electric current pulses. The heating evaporated the

analytes, which led to their mobilization and injection to the second separation column. The function is illustrated in **Figure 7**.

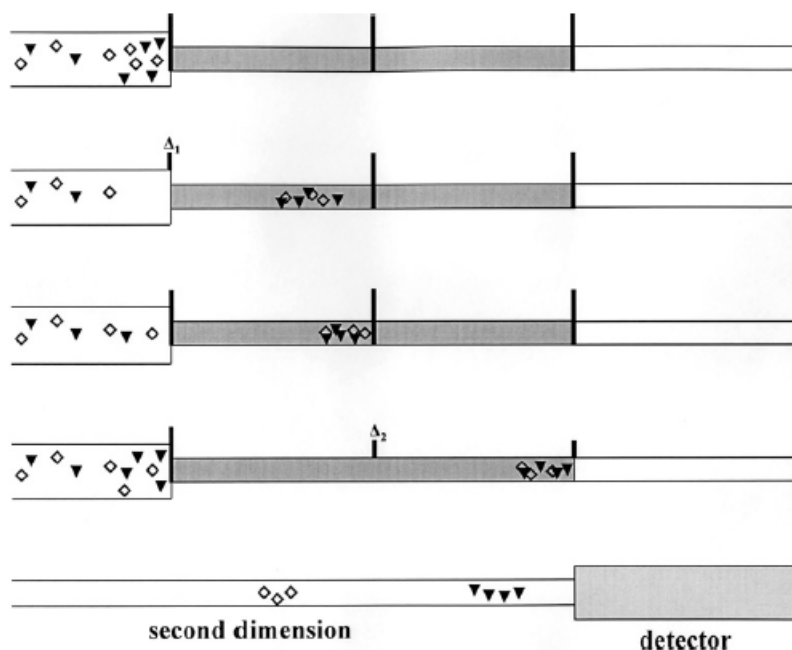


Figure 7 Function of the dual-stage thermal-desorption modulator. Reprinted from Z. Liu and J. B. Phillips: *J. Chromatogr. Sci.* 29 (1991) 227.

Another example is the **dual-jet CO₂ modulator**, published by Beens et al. (*J. Chromatogr. A* 919 (2001) 127). Its function is shown in **Figure 8**. In this modulator, two cool jets with pressurized liquid CO₂ are placed inside the GC oven. They alternately cool two segments of the capillary connecting both separation columns. Cooling traps the analytes in the modulator. When the jet is switched off, the heat of the oven re-heats the capillary and analytes are thus evaporated and injected into the second column.

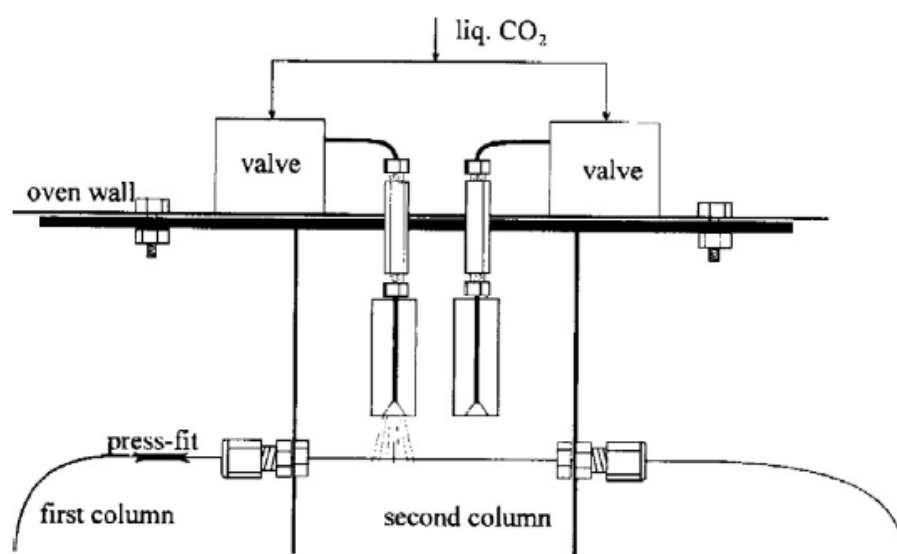


Figure 8 Function of dual-jet CO₂ modulator. Reprinted from Beens et al.: *J. Chromatogr. A* 919 (2001) 127.

The last example of a thermal modulator is the **dual-stage loop-type modulator** that uses two jets, one with hot and the second one with cold nitrogen. Dual-stage operation is achieved by looping the capillary. Thanks to the loop there are two segments of the capillary being cooled and heated even though there is only one hot and one cold jet. The setup can be seen in **Figure 9**.

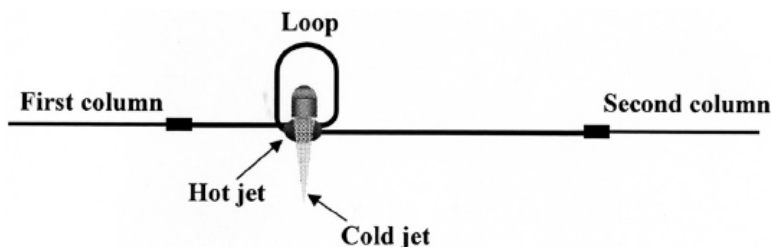


Figure 9 Dual-stage loop-type modulator. Reprinted from Ledford E. B. et al.: Pittcon 2002, New Orleans, LA, USA.

Pneumatic modulators

An example of a **two-stage valve pneumatic modulator** is shown in **Figure 10**. Two stages are alternating during the operation of the modulator. During the accumulation period (which takes 80% of all the modulation period) the effluent is directed by the valve to the sample loop. During the injection period, the valve is switched to the other position and the sample components collected in the sample loop are flushed by 20 times higher flow rate to the second separation column. During this period of injection to the second column, the effluent from the first column is directed to waste. Due to that, not all sample components necessarily pass through both columns but regarding a short modulation period and a very short injection period, the analysis is still considered comprehensive.

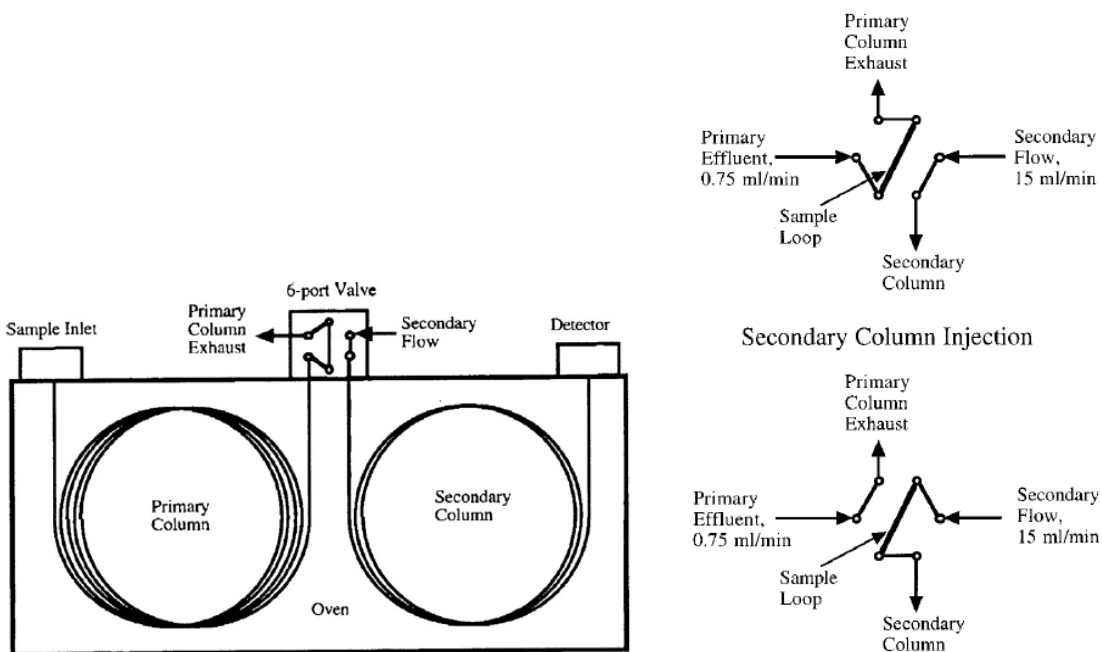


Figure 10 Two-stage valve pneumatic modulator. Reprinted from Seeley J. V. et al.: *Anal. Chem.* 72 (2000) 4345.

In **Figure 11** you can see an example of a **twin-stage in-line valve modulator**. In the OFF position, the effluent from the first separation column fills the loop 1 and the content of the loop 2 is flushed to the second column. In the ON position, effluent from the first column is accumulated in loop 2 and loop 1 is flushed to the second column.

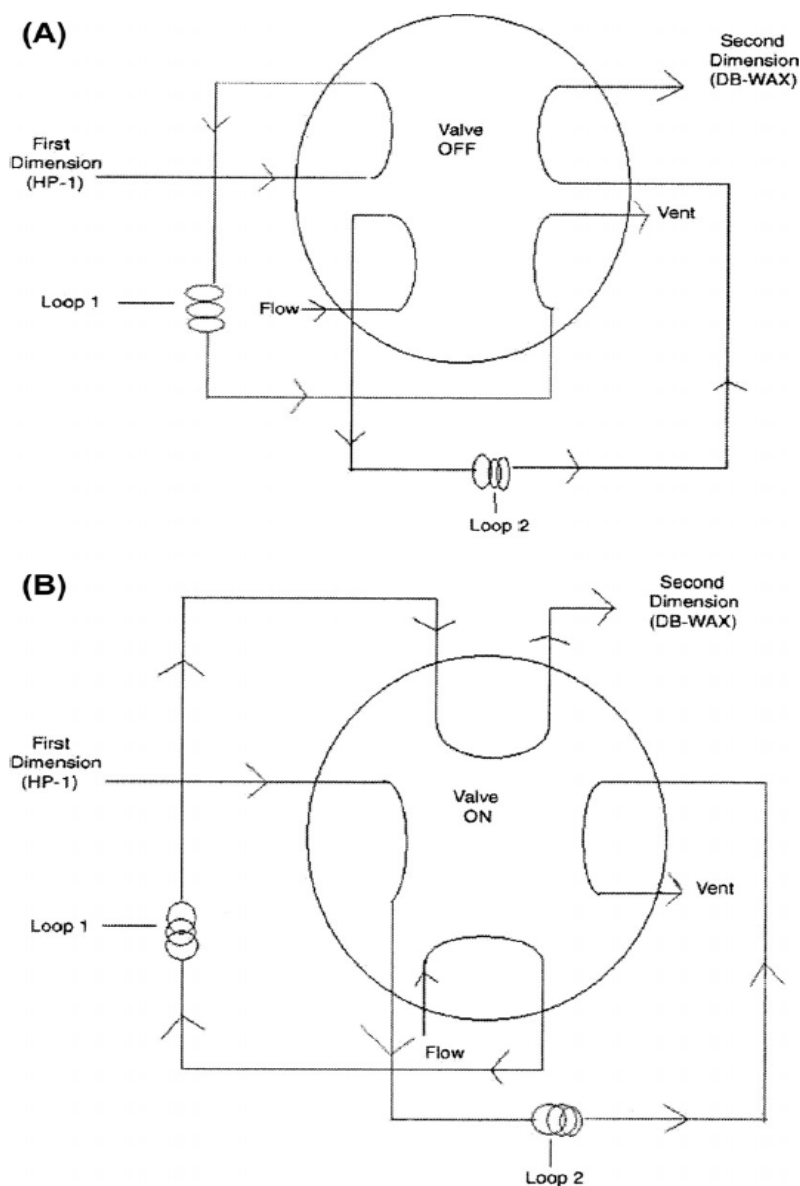


Figure 11 Twin-stage in-line valve modulator. Reprinted from Diehl J. W., Di Sanzo F. P.: *J. Chromatogr. A* 1080 (2005) 157.

Another example of a pneumatic modulator is the so-called **Dean's switch** in **Figure 12**. In this modulator, auxiliary flow is always faster than flow from the first column. A solenoid valve is used to switch between the bypass and inject modes. In the bypass mode, fast auxiliary flow overrules the flow from the first column and forces the effluent from the first column to go to the lower branch where the effluent is collected. When the valve is switched to the inject mode, the auxiliary flow flushes the effluent cumulated in the lower branch to the second column.

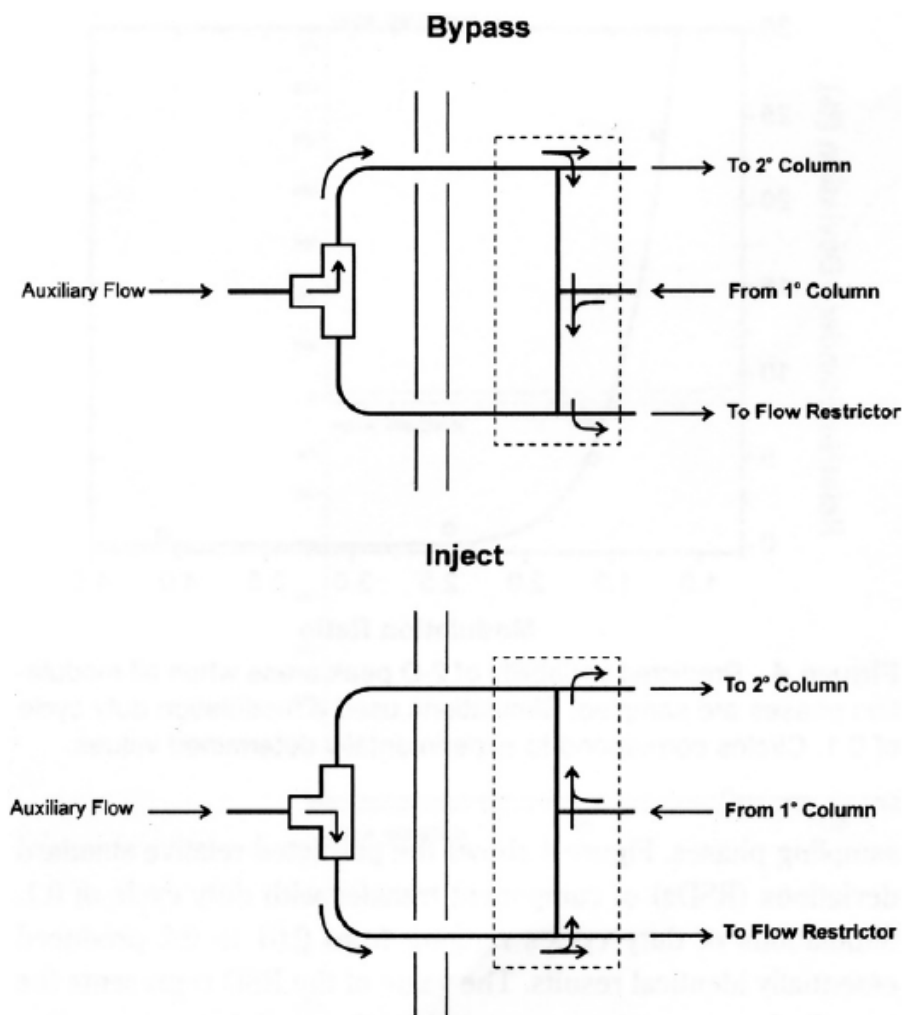


Figure 12 Dean's switch. Reprinted from Seeley J. V.: *Anal. Chem.* (2007) 1840.

Peak capacity in GC×GC

Peak capacity is an important parameter of a method because it tells us how many peaks we can theoretically fit into our chromatogram, i.e. how many compounds we can separate in one run. Let's suppose that the peak capacity of a method using a single column is 1000.

- If we connect two columns in series like in **Figure 2B** we get peak capacity of 2000 because the peak capacities of both columns are adding up.
- If we do two-dimensional non-comprehensive GC like in **Figure 3**. We do separation on one column with a peak capacity of 1000 and one part of the effluent we inject to another column with a peak capacity of 1000 so in total, we get a peak capacity of 2000.

- C) If we do comprehensive GC×GC as shown in **Figure 4** we have the first column with a peak capacity of 1000. The second column has a significantly smaller capacity because the separation must be very fast, so let's say the peak capacity is 30. Then we have a peak capacity of $1000 \times 30 = 30\,000$. Of course, this is a theoretical number. Real peak capacities are lower. Sometimes they are unnecessarily lowered by sub-optimal experimental conditions such as too long modulation periods.

Orthogonality

One of the conditions of the comprehensive GC×GC is that the sample is separated on two columns with different retention mechanisms. Ideally, these retention mechanisms should be independent, which means the retention on the two columns should be **orthogonal**. Orthogonal two-dimensional separation is a separation in which retention times in the two dimensions are mutually statistically independent.

Examples of orthogonal combinations of stationary phases are:

- Nonpolar + polar stationary phase
- Absorption + adsorption-based stationary phase
- Chiral + achiral stationary phase

The degree of orthogonality is determined by the percentage of available separation space occupied by analyte peaks. The usable space in the GC×GC chromatogram is shown in **Figure 13**. If there is a high degree of correlation, i.e. separations are not orthogonal, the peaks are distributed along the diagonal, which is equivalent to one-dimensional separation. The lower limit of usable space is marked by the dead time of the second column, only wrap-around peaks can appear below the dead time. The upper limit of the usable space is determined by the most retained peaks on the second column.

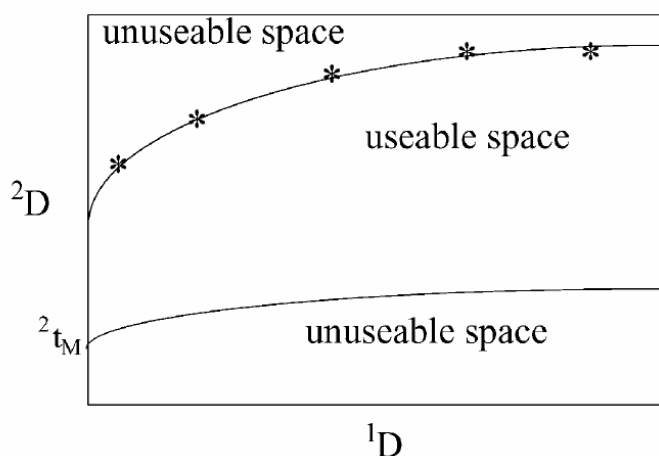


Figure 13 Usable and unusable space of a GC×GC chromatogram. Reprinted from Ryan D. et al.: *J. Chromatogr. A* 1071 (2005) 47.

Different degrees of orthogonality are well manifested in **Figure 14** which is taken from the article published by Ryan D. et al. (*J. Chromatogr. A* 1071 (2005) 47). The authors separated a mixture of analytes covering a wide range of polarity and volatility. The second column was always polar. Various first columns were used. The polarity of the first column was increasing from Figure 14A to

Figure 14E. In panels A and B good orthogonality is observed (analytes cover a large part of the usable space). In C and D the first column is already quite polar and thus similar to the second column. Worse orthogonality is observed here. In the case of panel E, the stationary phase of the second column is the same as that of the first column. As can be expected, analytes lie very close to the diagonal and the separation is practically one-dimensional.

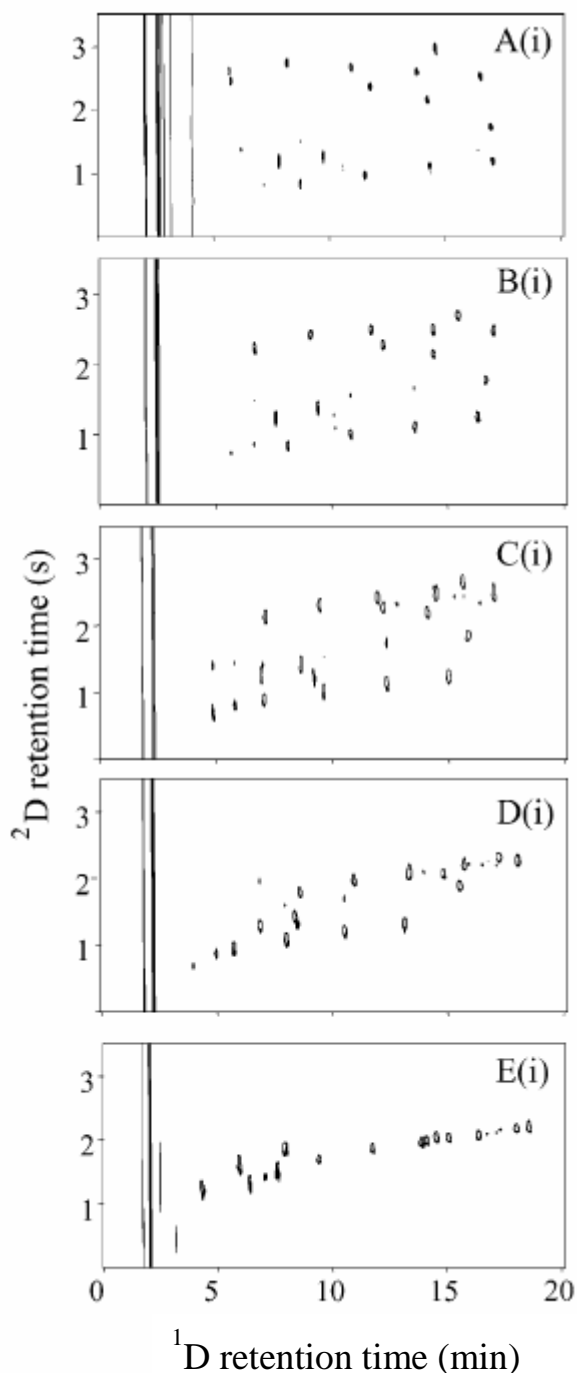


Figure 14 GC×GC separation of a set of analytes with the varied first column. The second column was always polar, the polarity of the first column increases from A to E.

Tasks

- 1) There is a technique called heart-cut gas chromatography (or heart-cutting gas chromatography). Look up the principle of this technique in literature. To which category from Figure 1 this heart-cut GC belongs and why?
- 2) As was mentioned in the text, the modulation period should be as short as possible to avoid loss of resolution achieved on the first column. Look at the chromatograms in **Figure 14** and estimate how long modulation period was used in this case. Can you tell from the chromatograms whether the modulation period was too long and could be shortened or too short and should be prolonged? Support your answers by appropriate evidence from chromatograms.
- 3) Go through the article by Goldstein A. H. et al.: J. Chromatogr. A 1186 (2008) 340. Was the modulator thermal or pneumatic? Was it a single-, dual- or twin-stage modulator? How are the analytes trapped in the modulator and how are they injected into the second column?
- 4) If we have a GC×GC system with the following columns:
1st column: 30 m × 0.25 mm with 5%-phenyl-95%-methylsiloxane stationary phase
2nd column: 1.5 m × 0.1 mm with 5%-phenyl-95%-methylsiloxane stationary phase
Will the two separations be orthogonal and why?