

*Chapter 1*  
Introduction

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## *Chapter 1*

# Introduction

### 1.1 Background

In the ever advancing age of technology the need to improve and control fuels, foods and various other products is having a more and more integrate part of daily work. Health and other control organisations are increasing pressure on industries to provide detailed analysis of their products. Many of the analytical methods used to date can provide good results but at an increasingly high cost. To achieve most of the regulatory detection levels, these instruments are pushed to their limits, requiring a support infrastructure and highly skilled manpower that can only be afford by the developed world. Developing countries find it increasingly difficult to certify their products for export and domestic use. This is already resulting in some of the countries denouncing products from these poorer countries.

Some examples:

Automobile fuels need to be carefully controlled to prevent the release of harmful exhaust gases to the atmosphere. The fuels should not only be less harmful, but also more effective, economic etc. To achieve these qualities, the composition of the fuel and the function of each of the components needs to be known and carefully studied.

All products for human consumption have to be monitored carefully. Fresh fruits and other farm grown products usually contain a variety of potentially harmful pesticides that need to be controlled. Health organisations across the developed world put a lot of pressure onto the developing countries to provide food low in pesticide levels. Each of the pesticides used now has a maximum allowed level in the food, but unfortunately most of these pesticides are complex mixtures and very difficult to detect, especially in the trace amounts found in food. The food itself contains hundreds of

compounds that will mask these pesticides in most analytical techniques used today.

In forensic science, food adulteration analysis, pollution control and in various molecular fingerprinting techniques, the need to analyse lower levels with greater confidence is also increasing. Most of the fingerprinting techniques used today are already well established but as criminals become more and more sophisticated, these techniques become less effective. Arson and horse-doping serves as examples. The same trend can also be seen in food adulteration; To gain higher profits many additives have been added to, for example, olive oil that does not influence the taste but increases the volume. These adulterations have been going on for ages, but the sophistication in this type of crime has made it increasingly difficult to detect by conventional methods.

From the above examples it should be clear that there is a critical need for improvements in complex sample analysis.

One way of improving existing techniques is to couple various instruments to one another for multidimensional analysis, as discussed in chapter 2. These techniques have already expanded the scope of analytical chemistry tremendously. The multidimensional technique used in this study is GC x GC. This instrument uses two gas chromatographic (GC) systems that separate compounds on the basis of two different molecular properties, for example boiling point and polarity. The two gas chromatographic systems are coupled in such a way that all the eluent from the first system is subjected for analysis in the second GC without losing the separation obtained by the first. This is called comprehensive two-dimensional gas chromatography and will also be discussed in more detail in chapter 2.

## 1.2 Approach

Gas chromatography is a well-used technique for various applications. In many of these applications a level of sophistication has been reached that makes any further improvements on the respective

systems too expensive to consider. With the development of the two-dimensional gas chromatograph (GC x GC) some of these existing applications can be revisited and improvements can be done at little extra costs. In some cases even a reduction in cost of the current analytical system can be anticipated.

The focus of this study was to improve techniques used for the analysis of diesel petrochemical samples. Some of the present techniques in industry, like the PIONA analysis (chapter 4), where petrochemical fractions are separated into chemical groups, can be potentially replaced by a less complicated GC x GC system. The GC x GC would also be able to do the same group analysis in a single run, resulting in significant saving of time and money. Some other applications include “fingerprinting” of diesel samples and the investigation of interesting properties observed in diesel-paraffin mixtures used in underground mining operations to decrease exhaust emissions.

These applications, however, cannot commence before the new technique is fully understood and can be routinely operated at its maximum potential. Most of this study was thus focussed on optimising the GC x GC. The optimisation of an analytical instrument, as discussed later in chapter 3, is a prerequisite for reliable analysis. Due to the absence of a guideline for GC x GC optimisation, the first aim of this study was to create such a guide and to investigate the parameters involved in optimising the separation efficiency of the instrument.

### 1.3 Presentation and arrangement

This chapter gives a brief introduction to the background and approach of the work done. In the next three chapters a background (literature overview) is given of the technique under investigation. In part 1 of the background the principles of multidimensional techniques are discussed along with the history and advantages of GC x GC. Part 2 focuses on the optimisation of gas chromatographic systems and includes the difficulties involved in optimising a comprehensively coupled column system, such as in GC x GC. Part 3 focusses on the analysis of petrochemical samples, an overview of the chromatographic methods used today and the potential of employing GC x GC in these fields.

Chapter 5 deals with the actual optimisation. Chapter 6 contains some examples of diesel analysis and in chapter 7 the conclusions are reported.

*Chapter 2*

Background: Part 1

GC x GC as a Multidimensional Technique

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## *Chapter 2*

### Background: Part 1

## GC x GC as a Multidimensional Technique

### 2.1 Multidimensional separation techniques

The search for answers in complex chemical mixtures, such as the intrinsic lubricating properties of diesel fuel, demand more detailed separation and analysis. One-dimensional techniques have been pushed to their limits (in search of these answers) and modern research has therefore been forced to change the approach to these problems. One strategy has been the introduction of multidimensional techniques. These techniques are now starting to mature and are slowly filling the information gaps left by one-dimensional techniques. More complex samples can now be separated and analysed to answer the questions of every applied scientist: “Why? What? How?”

In multidimensional separation, two or more independent separation techniques are coupled to give improved resolution and therefore a clearer picture of a sample composition. Such a system utilises the specific separation parameters of each technique to provide a better, more complete separation of an extremely complex mixture. Separation parameters are based on characteristic properties of compounds, such as their partition coefficients or density. There are numerous separation systems, each using a different property to control separation, each answering a different question. A short selection of the most common separation types is listed in table 1 [1].

<b>Table 1.</b> One-dimensional separations which might serve as building blocks for multidimensional separation techniques [1].			
<b>Type of separation</b>	<b>Abbreviation</b>	<b>Property controlling separation</b>	
Bulk displacement	BLK	Nonselective	N
Flow	FLO	Nonselective	N
Chromatographic	CHR	Partition coefficient	K
Field flow fractionation	FFF	Field interaction parameter	$\phi$
Electrophoretic	ELP	Electrical mobility	$\mu$
Isoelectric	IEL	Isoelectric point	I
Isotachophoretic	ITP	Electrical mobility	$\mu$
Dielectrophoretic	DEL	Dielectric constant	K
Sedimentation	SED	Sedimentation coefficient	s
Isopycnic sedimentation	IPY	Density	$\rho$
Magnetic gradient	MAG	Magnetic susceptibility	$\psi$
Thermal diffusion	THD	Thermal diffusion coefficient	D'
Thermogravitational	THG	Thermal diffusion factor	$\alpha$
Diffusophoretic	DIF	Interfacial energy	$\gamma$
Photophoretic	PHO	Photophoretic mobility	$\sigma$

Each of these techniques has been successfully used as an individual, one-dimensional technique. However, each of these techniques can be coupled to a second technique. This results in a separation across a two-dimensional plane. A two-dimensional (x,y) plane results when the first separation parameter separates compounds in a linear axis and the second parameter separates the compounds in an independent second axis. Some examples of combinations of table 1 to provide two-dimensional separations are ELP x PHO or THD x CHR [1]. This type of coupling between two separation systems results in up to 225 possible two-dimensional techniques [1].



Furthermore, within any single type of separation in table 1, such as chromatography, several independent, techniques can exist, yielding more coupling options. The following is a list of the main chromatographic separation techniques.

- Gas liquid chromatography (GLC)
- Gas solid chromatography (GSC)
- Supercritical fluid chromatography (SFC)
- Reverse phase liquid chromatography (RP LC)
- Normal phase liquid chromatography (NP LC)
- Size exclusion liquid chromatography (SE LC)
- Gradient elution liquid chromatography (GE LC)
- Thin layer liquid chromatography (TLC)

Any two of these chromatographic techniques can be coupled to one another to provide some 81 additional two-dimensional separation systems [1]. By adding more parameters to an existing two-dimensional system, separation would be further enhanced, but it would also increase the complexity of the system.

## 2.2 Principles of comprehensively coupled techniques

In order to understand the principles of comprehensively coupled techniques, it is necessary to examine the associated terminology. The terms of *comprehensive coupling*, *orthogonality*, *peak capacity* and *modulation* are discussed in the following sections.

### 2.2.1 Comprehensive coupling

Comprehensive coupling of two separation techniques is obtained if they are coupled (typically in-line) to one another in such a way that every single compound subjected to the first separation is transferred to the second dimension and that the full separation of each technique is preserved.

### 2.2.2 Peak capacity ( $n$ )

The effectiveness of most separations is measured by its peak capacity, which is defined as the maximum number of peaks or zones that will fit into the available separation space [2]. Guiochon [3] described the enhancement of peak capacity in chromatographic systems. From his work, it can be deduced that the maximum peak capacity of a two-dimensional system is described approximately by the multiplicative law.

$$n_2 \sim n_y \times n_z \sim n_1^2$$

Where  $n_2$  represents the peak capacity of the two-dimensional system and obtained by comprehensively coupling of the two distinct one-dimensional separations of peak capacity  $n_y$  and  $n_z$ . A peak capacity of  $n_1^2$  is obtained for the special case when  $n_y = n_z = n_1$ .

### 2.2.3 Orthogonality

In order to get separation in a two-dimensional system, two separation parameters are required. The parameters should be mutually independent to separate compounds over the full separation plane created by the coupled technique. One such a system is the GC x GC, discussed in section 2.3 in detail, where the first dimension separates components on a volatility basis according to boiling point, while the second dimension separates the components further on a polarity basis, giving a two-dimensional chromatogram with independent axes. Orthogonality thus simply indicates that the two separation systems are totally independent of each another. For closely related separation mechanisms, the components of a mixture will tend to elute on the diagonal between the two separation axes, i.e. again in a single dimension.

## 2.2.4 Modulation

The process of collecting narrow elution fractions from the first separation system and transferring them to the second is called modulation. To achieve comprehensive coupling of two techniques, the modulation frequency has to be high enough as to prevent loss of first dimension (D1) separation. This in turn, limits the time available to the second dimension (D2) separation, as successive separations have to be performed at the same frequency. The time interval between successive second dimension injections is known as the modulation period.

## 2.3 GC x GC

### 2.3.1 Definition

The abbreviation GC x GC [4] is used when two GCs are connected in a *comprehensive orthogonal* manner. This means that two columns are *coupled in-line* comprehensively so that the separation in each column is preserved.

Most GC x GC separations start with a non-polar column, where compounds are separated according to volatility parameters and the second column uses selective molecular interactions (polar or structural interactions) to separate the compounds further. Gas chromatographic interaction is dependent on temperature, thus, although the second dimension is based on steric interactions, it still exhibits strong temperature dependency, resulting in the two dimensions not being completely orthogonal to each other. This problem can be solved by running the second column at temperatures close to the elution temperatures of compounds from the first column.

There are four major advantages to GC x GC [5,6,7]

- It provides highly detailed, interpretable images of complex samples
- Peaks can be grouped to represent different chemical classes, creating a viable alternative for group types analysis.

- It provides superior resolution, high peak capacity and increased sensitivity relative to conventional GC, which allows accurate determination of different components
- It can provide boiling-point distribution for different classes of compounds in one run.

### 2.3.2 Modulators in GC x GC

#### Basic principle [4,6,7]

The modulator is the heart of the GC x GC system. The modulator is situated between the two serially coupled columns. The eluents from the first column are captured, refocused and reinjected into the second column. The reinjected sample is then subjected to further separation on the second column.

The period between the reinjected samples, which is constant, is known as the modulation period. Statistically, in order to preserve the separation of the first dimension, each first-dimension peak needs to be analysed as several segments. Thus, to obtain a comprehensive separation, the modulation period must be as short as possible. The modulator therefore needs to perform on a rapid, repeatable basis. The typical modulation period is in the order of two to ten seconds, providing five to six secondary analyses per first dimension peak.

The second dimension thus has a very short time period to separate components further and is therefore run under fast gas chromatographic conditions. However, the absence of fast temperature programmed second-dimension columns for rapid separation, limits the column to isothermal conditions. One of the prerequisites of fast GC is narrow sample injection, in order to obtain well separated chromatograms with the best possible peak capacity.

#### Evolution [6]

A number of modulators have been developed that satisfy the requirements discussed above. These modulators can be grouped into two major categories, those based on thermal modulation and those based on mechanical (valves and diaphragms) modulators. The thermal modulators consist of a

variety of elegant and simple basic principles. The development of these modulators is also the history of the GC x GC.

The ability of a column with a thicker stationary phase to slow down the movement of chromatographic peaks was the basis of most of the early modulators. The first modulator was reported in 1991 by Phillips and Liu [4]. This novel GC x GC system had a thermal desorption modulator in-between the two gas chromatographic columns. The modulation was achieved by means of a resistively heated trap that repetitively heats a segment of column with a thicker stationary phase. The modulator was able to separate complex samples but it was rather unstable [4]. This led to the design of other modulation systems, which utilizes the same phase ratio effect. The most notable of these are the slotted heater (sweeper) modulator [8] and the segmented resistively heated modulator [9].

The next evolution in modulator development occurred when, instead of using the phase ratio to slow down the movement of the sample, the researchers switched to cryogenics to condense or "freeze out" the sample. This led to the current generation of modulators [10].

The use of valves and diaphragms, in GC x GC modulation, occurred in parallel to thermal modulators. These modulators are based on GC-GC reinjections (discussed later) where only up to 70% of the sample is reinjected giving an almost comprehensive separation [10].

During the last ten years research was done in the improvement and development of modulators, with the most important modulators briefly described below .

### 2.3.2.1 Thermal modulation

#### The slotted heater-thermal modulator [8]

This modulator (fig. 1), developed by Phillips *et al* produced some of the first two-dimensional chromatograms. The modulator is based on thermal modulation.

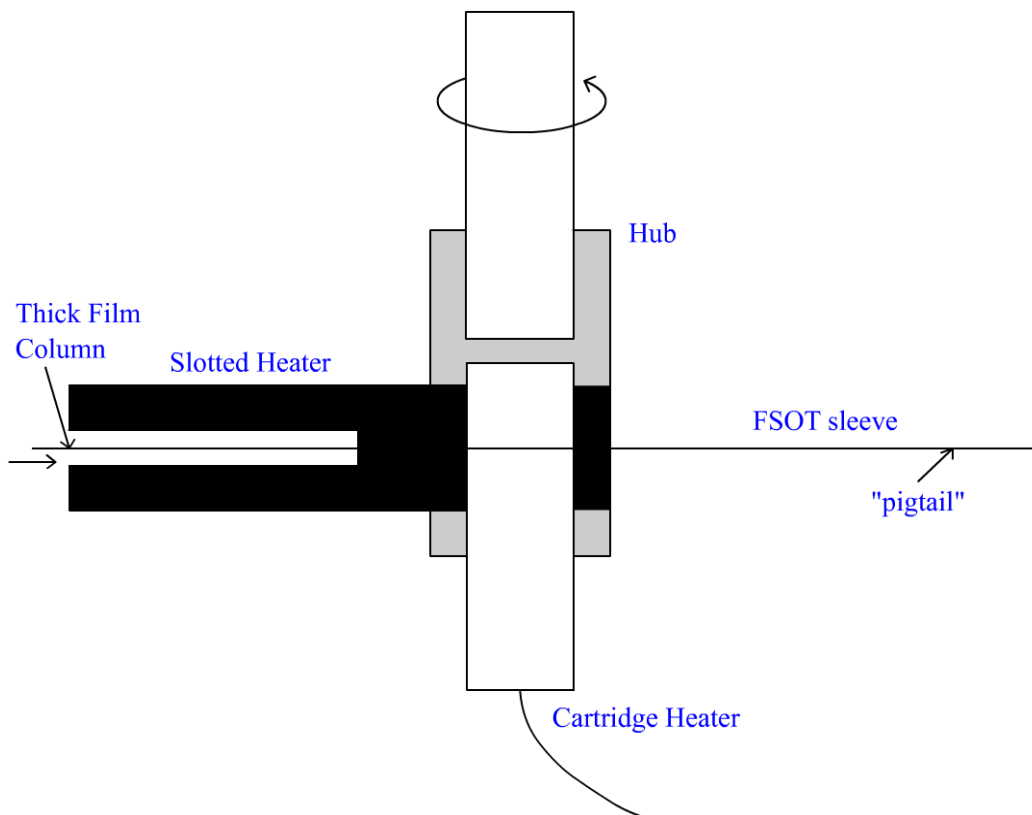
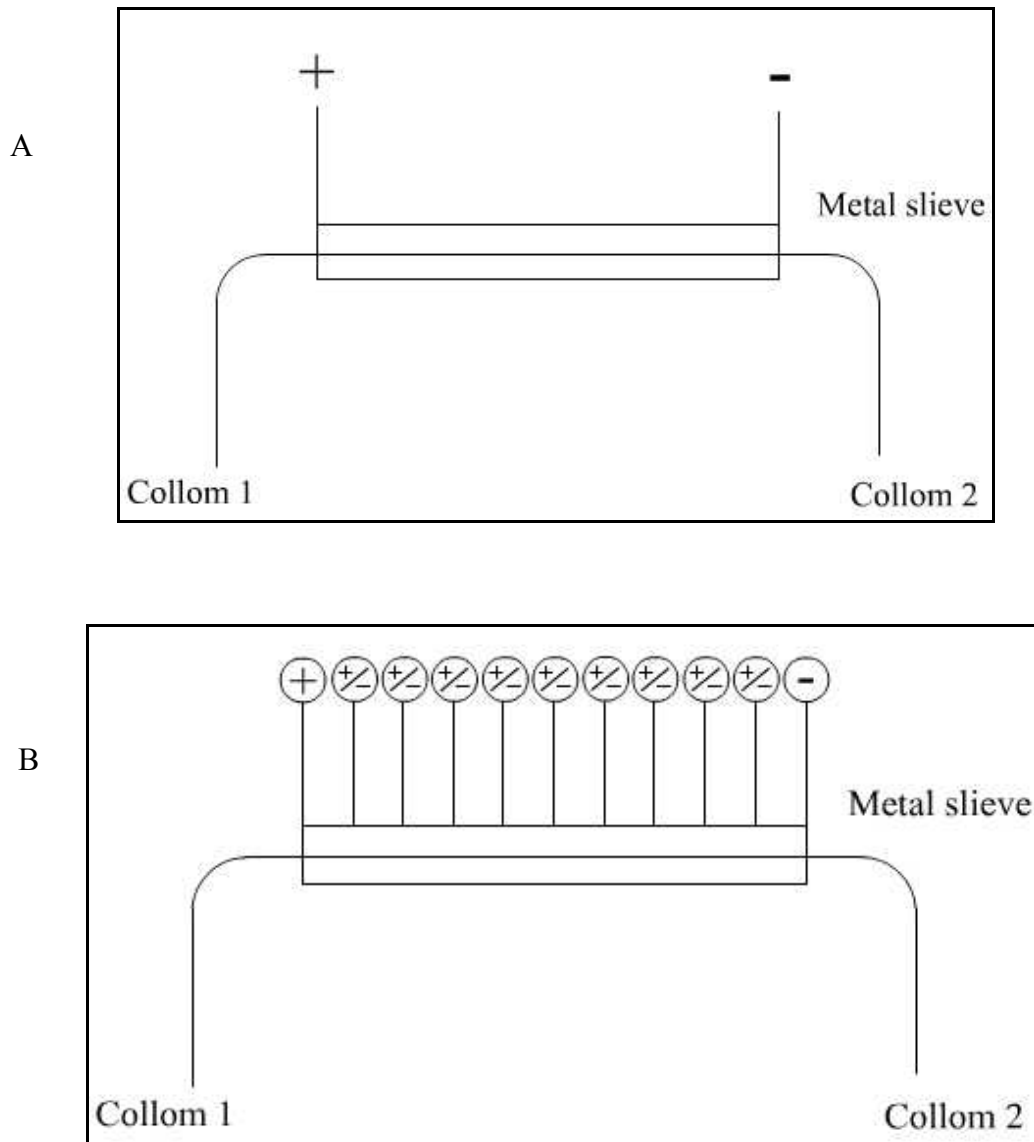


Fig. 1 Slotted thermal heater developed by Phillips [8]

The modulator works in the following way: The eluent from the first column is collected on a short segment of thick-film capillary column and then released into the second column by the application of a moving heat “wave” from the rotating heater.

Further developments of thermal modulators (fig. 2) are the resistively heated modulator and the segmented resistively heated modulator [9], which employ similar principles as the preceding modulator and are therefore not discussed further. The heat “wave” in the latter system is achieved by electrical rather than mechanical means, resulting in a much more robust and reliable modulator.

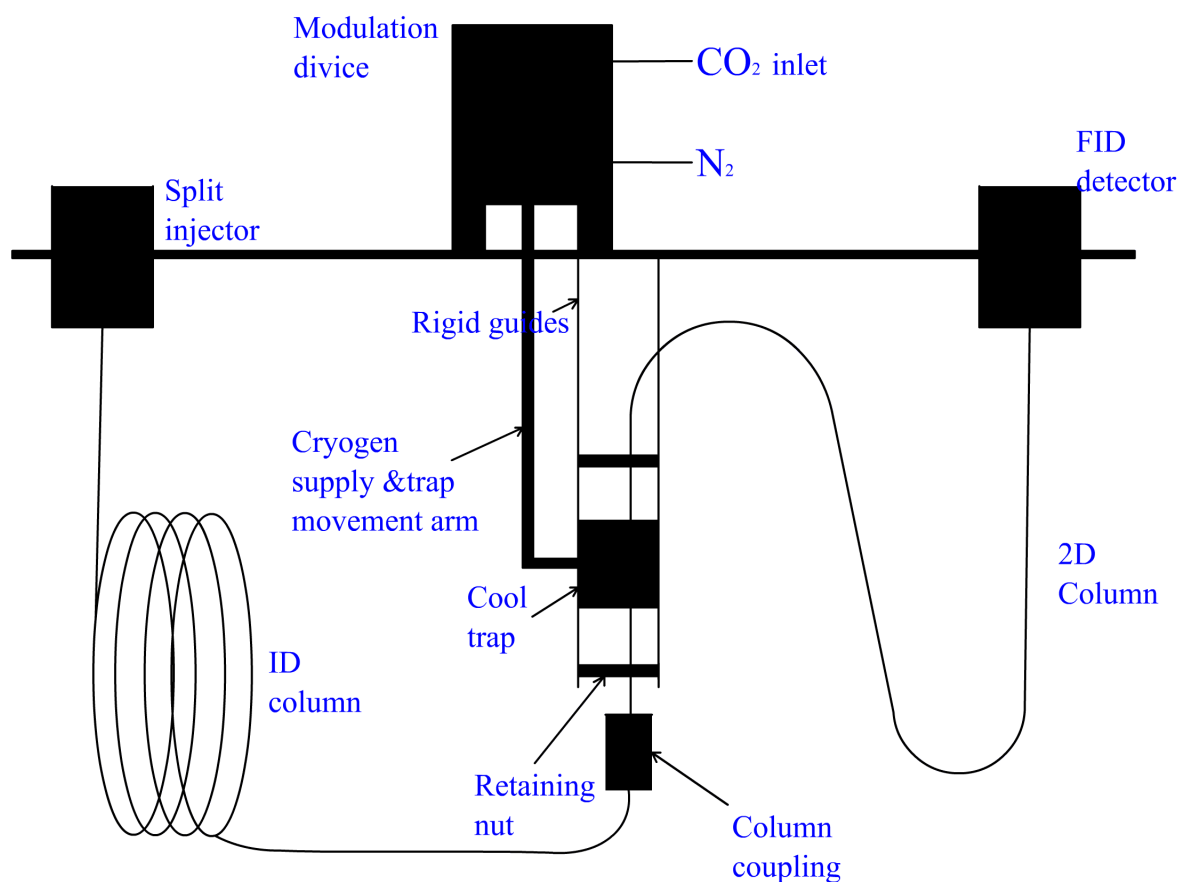


*Fig. 2 The two different resistively heated modulators, (A) is designed by Philips et al [4] and (B) designed by Burger et al [9]*

## 2.3.2.2 Cryofocussing modulation

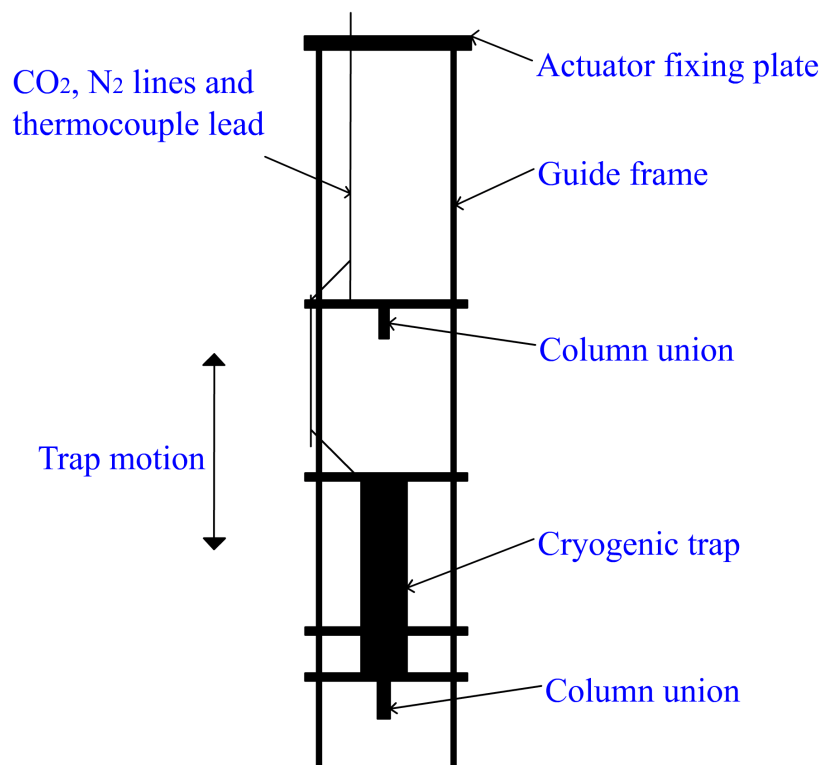
**A) Longitudinal cryogenic modulator [10] (fig. 3,4)**

This modulator, the first generation of cryogenic trap modulators, works on the principle of trapping eluents from the first column by cooling down the column, thereby slowing down the movement of compounds in the column. Upon reheating, the eluent is remobilised and starts to move through the second column.



*Fig. 3 Longitudinal modulator developed by Marriot [10]*



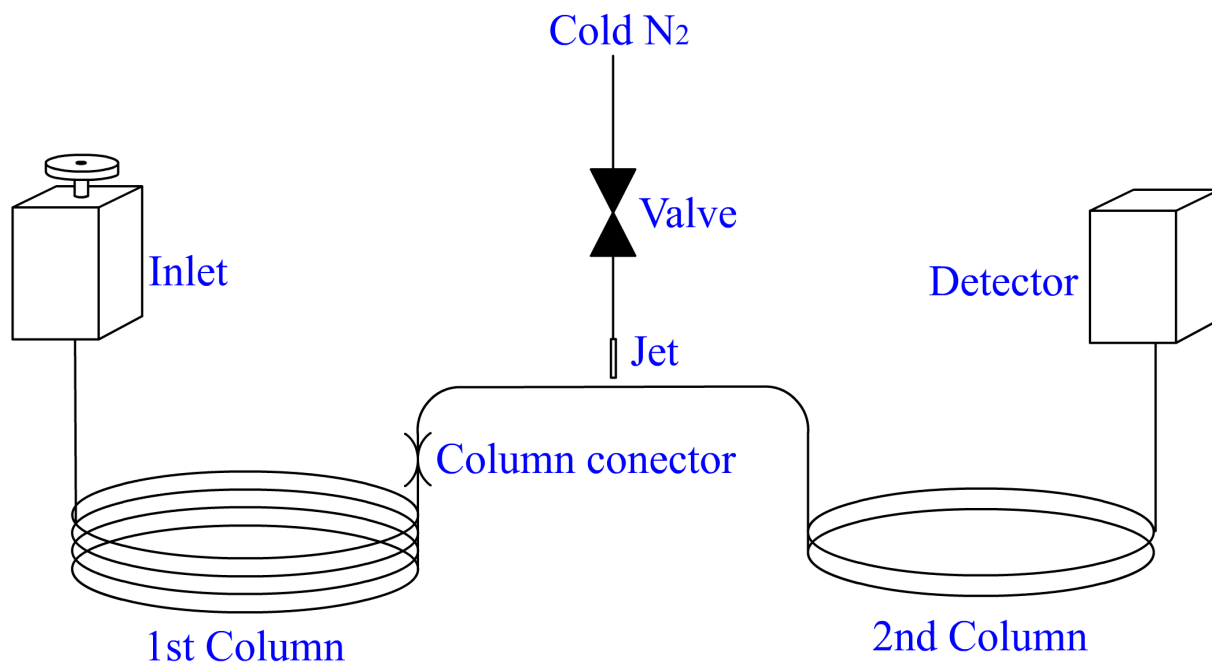


*Fig. 4 The trap assembly of the LMCS [10]*

The modulator operates in the following way: When the modulator is at the top position, a narrow zone containing the eluents from the first column is cold-trapped and focussed in the inlet of the second column. When the modulator moves to the bottom position the cold spot in the second column is rapidly heated by the oven and the eluents are remobilised for separation in the second column. The modulation chamber is cooled down to 100°C below the oven temperature by evaporative cooling of liquid CO<sub>2</sub> expanding at the orifice at the end of the supply tubing. The temperature is controlled by activating and deactivating the carbon dioxide solenoid valve. This modulator has proved to be very simple and robust, producing narrow peaks (100 ms, peak base width) and can be operated at high oven temperatures.

**B) Jet-cooled thermal modulator [11]**

This type of cryofocussing modulator uses no moving parts, its simplicity making it extremely robust and versatile. A whole range of new modulators was inspired by the first model developed in Ledford's group (fig. 5).

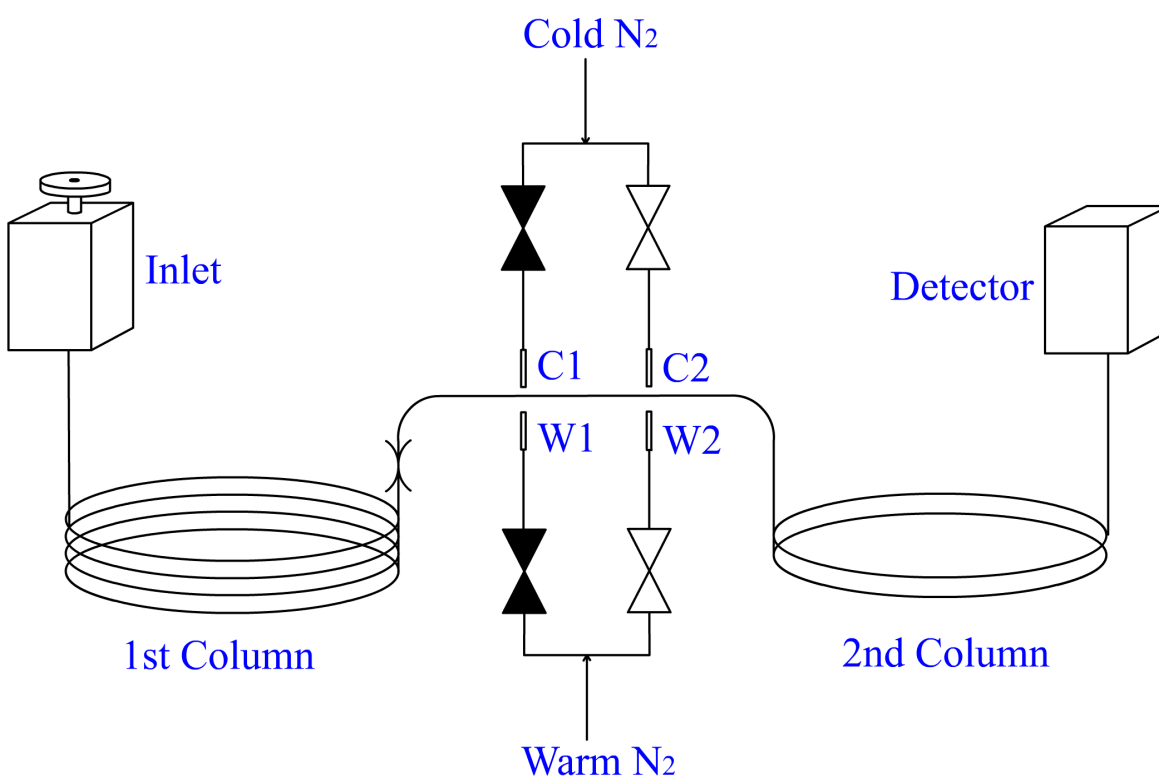


*Fig. 5 The single-jet thermal modulator by Ledford[11]*

In this modulator, the modulation tube is kept at 100°C below oven temperature by means of a pre-cooled gas jet. The eluents in the cryotrap were then remobilised by switching off the cold jets and allowing the oven to heat up the cold zone. Ledford and Billesbach [11] pointed out that the design could be improved by using two jets capable of pulsing cold and hot gas.

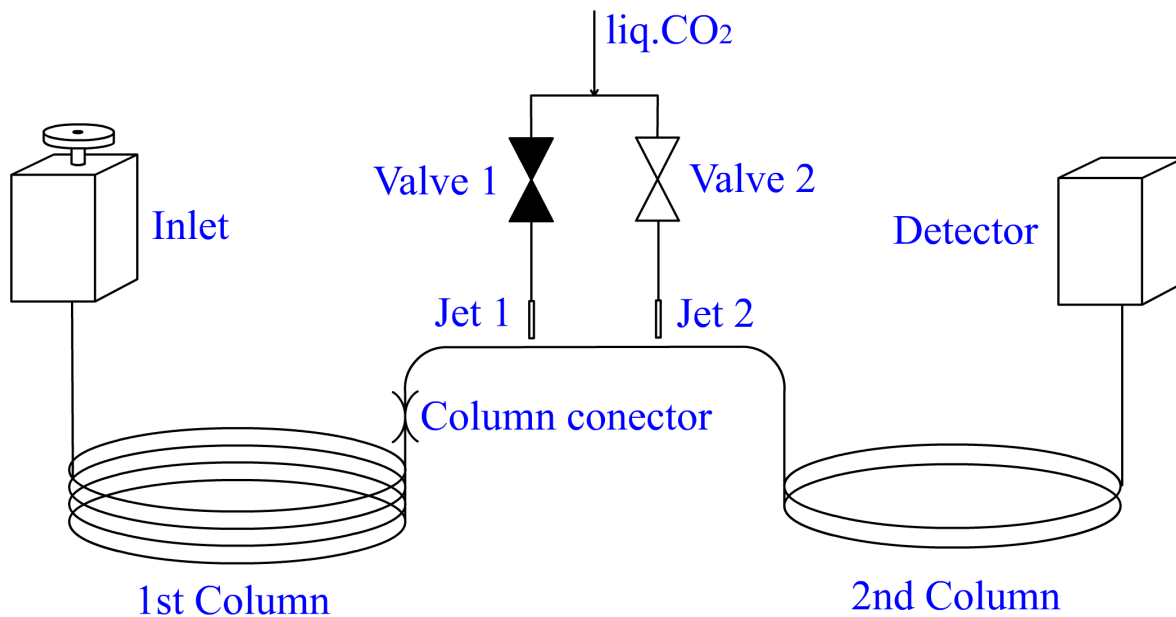
**C) Nitrogen jet-cooled thermal modulator [12] (fig. 6)**

This further refinement of the jet-cooled modulator, developed by Ledford and co-workers, operates in a simple fashion, making it an ideal modulator for precision work. The modulator has two cooled jets for trapping and two warm jets for remobilisation. The nitrogen in the cooled jets is cooled in a nitrogen exchanger, while the nitrogen for the heated jets is heated through a cartridge-heated block in front of the modulator. This is also the modulator used in this research and will be dealt within more detail later.



*Fig. 6 Nitrogen cryogenic thermal modulator with four jets developed by Ledford [12]*

The concept of Ledford was pursued by Beens and co workers [13] (fig. 7).



*Fig. 7 Duel stage jet modulator of Beens and co workers [13]*

The above modulation works on the same concept as Ledford's but uses two CO<sub>2</sub> jets to cool down the modulation tube. The arrangement of the jets is in such a way that no heat pulses are required, instead it uses the temperature of the oven to remobilise the sample.

## 2.3.2.3 Diaphragm-valve modulators

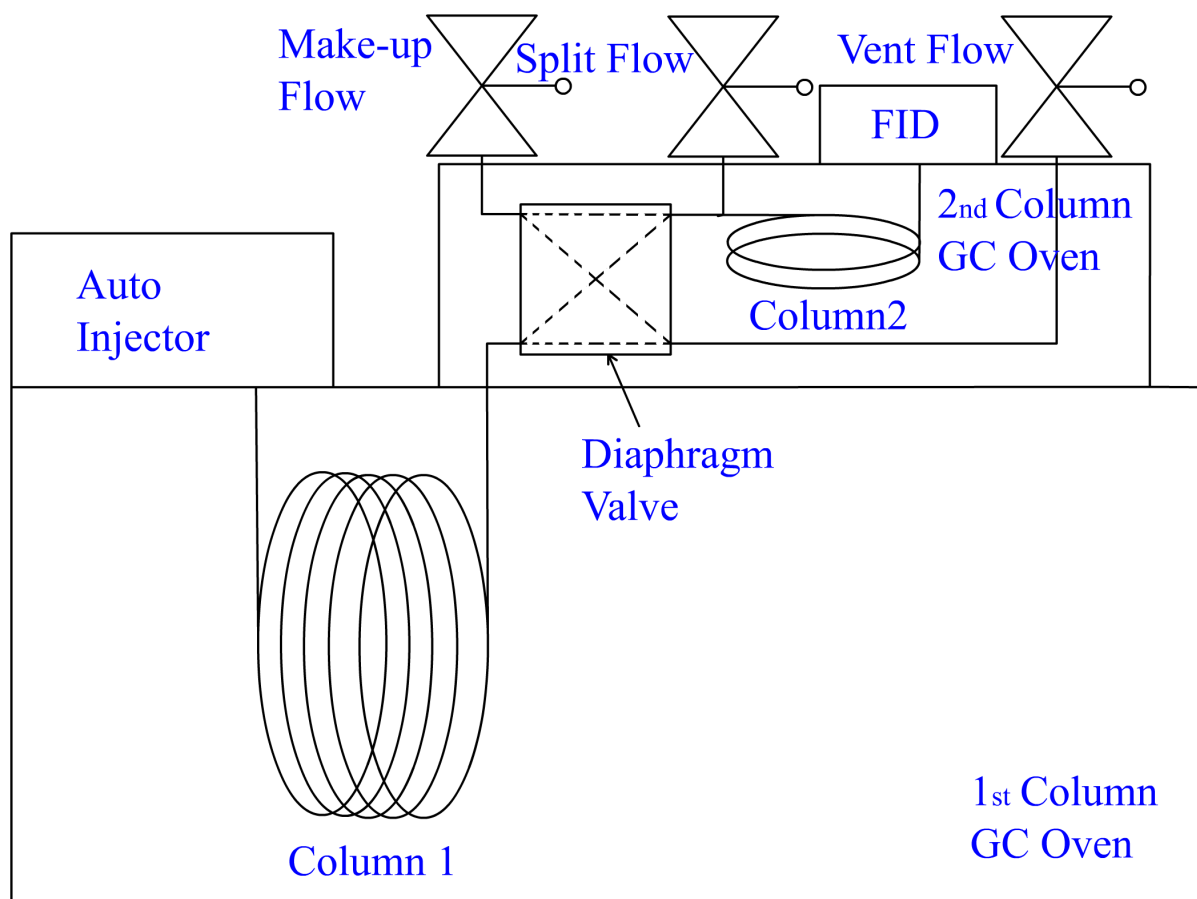


Fig. 8 Schematic diagram of a diaphragm-valve modulator by Synovec [14]

The modulator by Synovec and co workers (Bruckner CA) [10] works with a fast switching six port valve. The fast switching creates pulses of the sample to be analysed on a separate GC column. Only 10 to 20% of the sample injected reaches the detector, thus reducing the sensitivity. The modulator cannot operate at temperatures above 200°C thus the use of the valve is limited to samples of medium volatility.

## 2.3.2.4 Comparison of Modulators

The different modulators are compared by Beens *et al* [15] to one another in table 2 according to the

following parameters:

- Focussing effect; the method employed for trapping the eluents from the first column before remobilisation or reinjection.
- Bandwidth; this parameter indicates the width of the remobilised peak. To obtain better chromatographic separation and peak capacities the reinjected band of peaks should be as narrow as possible.
- Sensitivity enhancement; this occurs due to refocusing of the eluents from the first column. The compounds eluting during the modulation period (2 to 10 s) are trapped in the modulator, causing a concentration effect. These concentrated eluents are then remobilised in the second dimension as a narrow band (10 to 300 ms).
- Comprehensiveness of the modulator indicates whether the whole sample is subjected to both dimensions of separation and whether the separation of both columns is preserved.

Type	Focussing effect	Bandwidth Injection pulse (ms)	Sensitivity enhancement percentage %	Comprehensiveness
Dual-stage heated	Phase ratio	16-20	10-20	yes
multi-stage heated	Phase ratio	300	1-2	yes
Sweeper	Phase ratio	60	5-15	yes
LMCS	Cryo CO <sub>2</sub>	20-50	5-15	yes
Moving direct spray	Cryo CO <sub>2</sub>	20-50	5-15	yes
Four jets cryo	Cryo N <sub>2</sub>	<10	10-40	yes
Two jets cryo	Cryo CO <sub>2</sub>	<10	10-40	yes
Single jet cryo	Cryo CO <sub>2</sub> / N <sub>2</sub>	<10	10-40	yes
Rotating cryo	Cryo CO <sub>2</sub>	50	5-15	yes
Diaphragm valve	Valve	50	1	no
Differential flow	Valve	50	20	almost

The cryogenic jet modulators have the narrowest injection pulses and the biggest peak enhancement of all the modulators available. The cryogenic jet modulators thus outperform the other designs and is a popular choice where the cost of gas and liquid nitrogen can be ignored.

### 2.3.3 Detectors in GC x GC

For GC x GC peak detection, a fast detector is needed. The peak widths on the second dimension are in the order of 20 ms [16] at the head of the modulator and 100 ms to 200 ms at elution. Thus, to have a representative number of detection points per peak ( for instance, at least ten points per peak) the sampling rate of the detector should be at least 100 Hz. The most common detector used in almost all GC x GC separations is the FID (flame ionisation detector) due to its speed (easily allowing sampling at 200 Hz). Any other GC detector which has a similar sample rate could also be used.

While mass spectrometry, as in all other GC applications, could enhance the identification of peaks, most mass spectrometers lack a satisfactory sampling rate, with the exception of the TOFMS (time-of-flight mas spectrometer) which can have a sampling rate of up to 500 Hz [17].

### 2.3.4 Interpretation of GC x GC separation

In order to interpret a GC x GC chromatogram, it is essential to understand the separation of compounds in different dimensions. The assumption of random distributions in one-dimensional chromatography [18,19] provided valuable information in the understanding of peak distributions in a chromatographic axis. However, peak distribution is not truly random: All the components in the mixture have definite structures and must be directed to definite locations in retention space based on these structures [20].

Giddings [21] showed theoretically that the key property of a separation method, which determines whether or not it can show the inherent structure of a mixture being separated, is the method's *dimensionality* that should match the *dimensionality* of the mixture. The *dimensionality* of a mixture is the number of independent variables in which the members of the mixture can be separated. When a mixture is then separated according to these independent variables (by a system of matching dimensionality), each type of compound will separate to a unique location on the separation plane(chromatogram). However, as indicated above, the compounds are composed of molecules with discrete structures that are related, the compounds must thus distribute over the dimensional separation space (chromatogram) to discrete locations which are also related to each other [21].

To explain the Giddings theory, separation in GC x GC is used as an example: If a mixture is separated into one dimension, such as the boiling point fractions in petroleum samples, the alkanes and the aromatics with similar boiling points will overlap and thus insignificant ordering of compounds occurs. If the variable “boiling point” is changed to “polarity” the same overlap does not occur but a new overlap is created by different “boiling point” fractions, thus the separation or ordering is still insignificant. The mixture is simply not sufficiently well ordered in any one dimension, it requires at least two matching independent variables to uniquely separate the compounds of the mixture.

GC x GC chromatograms (volatility x polarity separation, method dimensionality of two) of petroleum fractions are highly ordered, indicating that these samples have a *dimensionality* of two (can be classified by either volatility or polarity) , for most of their components [19]. Ordered chromatograms have the potential advantage of being more interpretable than disordered ones. The pattern of peak placement is highly informative by itself and may make it possible to identify most or all of the components of a given mixture.



### 2.3.5 GC x GC vs GC - GC

GC x GC is a term used to describe a comprehensive two-dimensional technique where the entire sample separated by the first dimension column is subjected to separation by the second column. GC - GC, in contrast, is not a comprehensive technique, as it works on the basis of taking specific sections of the elution profile from the first dimension and subjecting those discrete sections to further separation on a second column.

Chromatographic resolution of complex samples can be improved by increased peak capacity, for example by increasing the length of the column. Resolution, however, only increases by the square root of column length and is finally limited by pressure drop requirements of the carrier gas. It could thus be a very expensive exercise to increase peak capacity on a single column when mixtures are truly complex. Two-dimensional chromatography offers a new way of increasing peak capacity. Many variations of two-dimensional chromatography exist. The “heart cut” method where a segment of the elution of the first column is injected into a second column of different stationary phase, provides good separation of compounds but is very time consuming if repetitive injections have to be made in order to analyse all first dimension sections also on the second dimension. This could take weeks for a complex sample.

The peak capacity of a GC - GC system (single heart-cut) is only the peak capacity of the first column plus the peak capacity of the second dimension column. In the case of GC x GC the total time of analysis is decreased and provides a full overview of the sample in two dimensions with the overall peak capacity equal to the first dimension's peak capacity multiplied by the peak capacity of the second dimension. Due to the high speed requirement of the second-dimension of GC x GC it does not have the same peak capacity as the second dimension in GC - GC. The overall peak capacity generated per time scale is, however, much higher. The overall peak capacity of GC x GC can be further improved by lengthening the columns or slowing down the first-dimension temperature program, but this unfortunately will result in a much longer total analysis time.

*Chapter 3*

Background: Part 2

GC x GC optimisation

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### *Chapter 3*

## Background: Part 2

# Optimisation of a GC x GC system

### 3.1 Introduction

The analytical requirements will dictate the type of optimisation required by a particular chromatographic separation. Some optimisation goals are: resolution, sample capacity and speed of analysis. When all the components of a very complex mixture need to be determined, chromatographic separation is optimised for maximum resolution in order to measure the maximum number of peaks. Another type of optimisation has as goal the fastest analysis time. This type of chromatographic separation is often required in process control where fast response times are essential. Faster analysis times often results in decreased resolution.

GC x GC provides us with a means to minimise the speed/resolution trade-off. Because of its greatly increased peak capacity, it can be used for quality control processes where many compounds need to be resolved, in a time-efficient manner.

The GC x GC system consists of two different columns, the first column is run under normal GC conditions and the second under fast GC conditions. Therefore, optimising the two columns involve two separate sets of conditions. The optimisation, however, is complicated by the fact that the two columns are serially connected to each other. This results in changes to the one set of conditions having an effect on the other.

Each column can be studied individually before considering the additional constraints of a coupled system. In this chapter, the parameters of each chromatographic system (normal GC and Fast GC) are discussed, followed by the new parameters required by joining the two systems.

## 3.2 One-dimensional GC considerations

In our study we aimed at obtaining a maximum resolution in the separation. From the resolution equation some important conclusions can be drawn regarding the column stationary phase, plate number and temperature [22,23].

$$R = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k}{k + 1} \right) \quad [22,23]$$

- N = the theoretical number of plates, defined by the length and diameter of the column.
- $\alpha$  = the selectivity factor is the ratio of the retention factors of two compounds (A and B) on a column.  $\alpha = k_B / k_A$
- k = the retention factor, defined by the partitioning of a compound between the mobile and stationary phase.

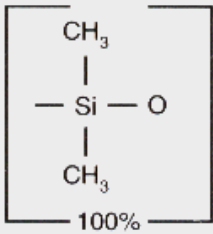
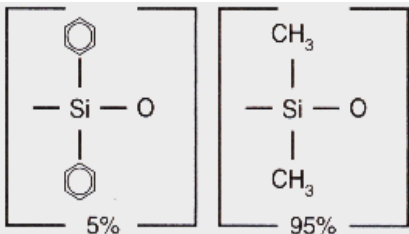
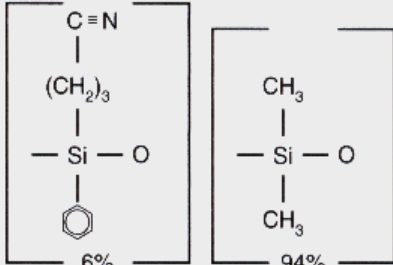
The most obvious way to improve column separation is to increase the column length or to reduce the diameter of the column, thus increasing the number of plates (N). By reducing the column diameter the plate height (H) is reduced and more theoretical plates are possible per column length ( $H = L/N$ ) [22,23].

By changing the column stationary phase, film thickness or temperature the k terms can be changed. The selectivity,  $\alpha$ , is changed by changing the stationary phase and, to a lesser extent, by the temperature.

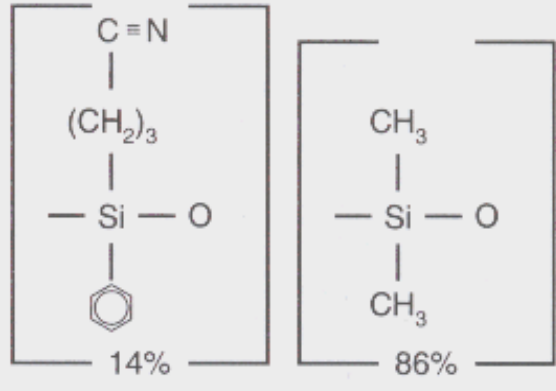
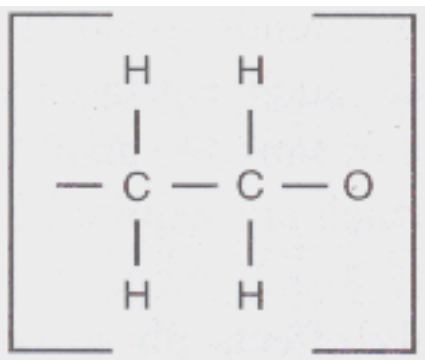
### 3.2.1 Column stationary phase

The type of column used is a very important parameter in the design of a GC x GC system. In a GC x GC, two orthogonal GC columns are used [4]. For example, it is assumed that if two solutes co-elute on a non-polar column they will have the same or similar boiling points. This forces their separation to be a function of their polarity, or other interaction with the stationary phase. The choice of a stationary phase depends on the selectivity and separation required. For practical

reasons the first-dimension stationary phase is generally chosen to perform a volatility separation. The most common of these stationary phases is listed in table 3 [ 24].

Table 3 Different non-polar stationary phases [24]		
100% dimethyl polysiloxane	5% diphenyl - 95% dimethyl polysiloxane	6% cyanopropylphenyl 94% dimethyl polysiloxane
 <p>100%</p>	 <p>5%      95%</p>	 <p>6%      94%</p>
non-polar	non-polar	slightly polar

The selection of a second-dimension column is more critical. Separation on the second column is dictated by the selective interaction of the sample with the stationary phase. Therefore, the choice of second dimension column will be based on the sample and what selectivity is required [22,23]. The most common of these stationary phases is listed in table 4 [ 24]. For example, in this study, two second dimension columns were used. The first column had a poly-ethylene glycol stationary phase,  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{CH}_2\text{CH}_2\text{OH}$ . This stationary phase contains hydroxyl groups that can undergo hydrogen bonding with oxygenated compounds, making it an ideal column for the resolution of oxygenated compounds (particularly alcohols) and other polar solutes [25].

Table 4 Second-dimension polar stationary phases [24]	
14% cyanopropylphenyl 86% dimethyl polysiloxane	poly(ethylene glycol) (Carbowax or PEG)
	
intermediately polar	polar

The second column, a polycyanopropylphenyl stationary phase, contains a polar cyano functional group. This cyano group has a strong dipole moment which provides strong orientation and induction interactions with dipolar solutes and unsaturated hydrocarbons [22,23]. This phase is mostly used for the resolution of saturated and unsaturated hydrocarbons.

### 3.2.2 Column dimensions

Although a number of different column lengths and sizes have been used and discussed in GC x GC literature [4,6,7,10,11], the underlying principle governing the selection is constant. For the two columns to work in a comprehensive way, without losing efficiency in either of the two, the second column must run at speeds much faster than that of the first column. In general, this speeding up of separation (in fast GC) is obtained by increased linear flow rates [26]. Unfortunately, since the columns are connected in series, without additional carrier gas sources, the linear flow rate is governed by the first column's requirements. However, by coupling a column with a smaller inner

diameter to the first column a faster linear flow rate of the second column and thus faster separation speed is advised. The most common choice of columns is a 250  $\mu\text{m}$  inner diameter column coupled to a 100  $\mu\text{m}$  column.

### 3.2.3 Linear flow rate

High linear flow rates speed up the separation of compounds, but will lower the efficiency due to slow radial equilibrium resulting in peak broadening [27]. Too low linear flow rates also affect peak broadening due to solute diffusion in the column axis. The Van Deemter-Golay equation [22,23,27] shows (Fig. 9) the relationship between band broadening and the linear flow rate.

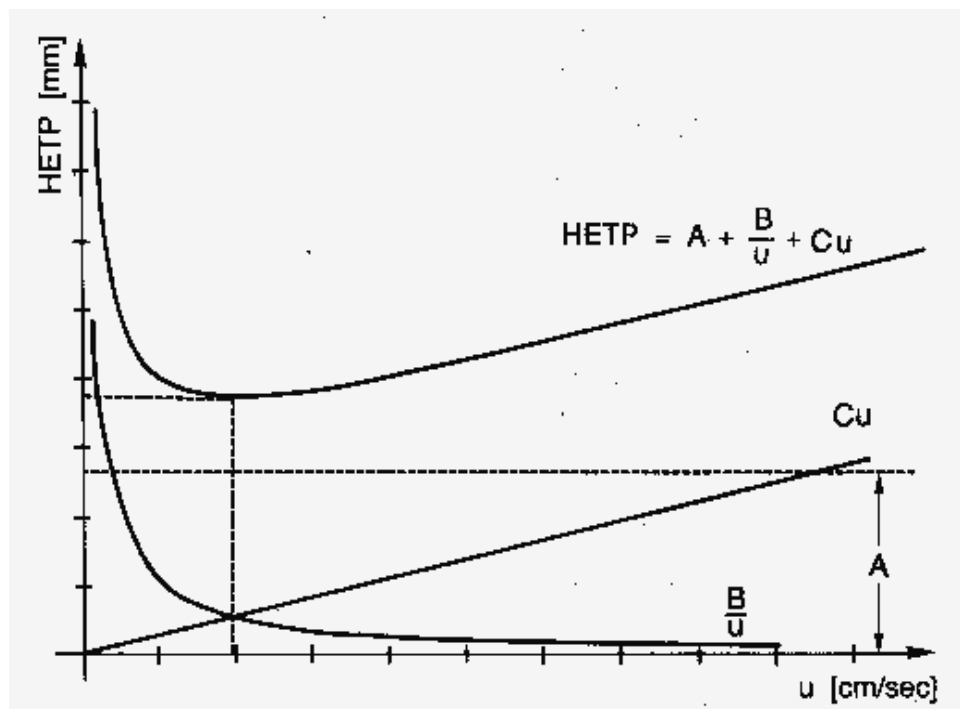


Fig. 9 Influence of Linear flow rate,  $u$ , on peak broadening during chromatographic separation (Efficiency) measured as HETP [27]

In the Van Deemter-Golay equation, band broadening is expressed as HETP, the sum of the following terms [22,23,27]:

$C_u =$  The dominant cause of band broadening at high flow rates is the resistance to mass transfer, preventing the existence of an instantaneous equilibrium between solute, stationary phase and mobile phase. The term increases in proportion to linear flow rate,  $u$ .

$A =$  Different pathways (packed columns only) have different lengths in the same column. This packed column parameter is flow independent.

$B/u =$  Longitudinal diffusion (diffusion of solute in the axial direction), increases with the time spent in the column and is therefore inversely proportional to the linear flow rate.

The van Deemter-Golay equation is widely accepted by chromatographers around the world and defines the strategy for optimising flow rates.

### 3.2.4 Temperature considerations

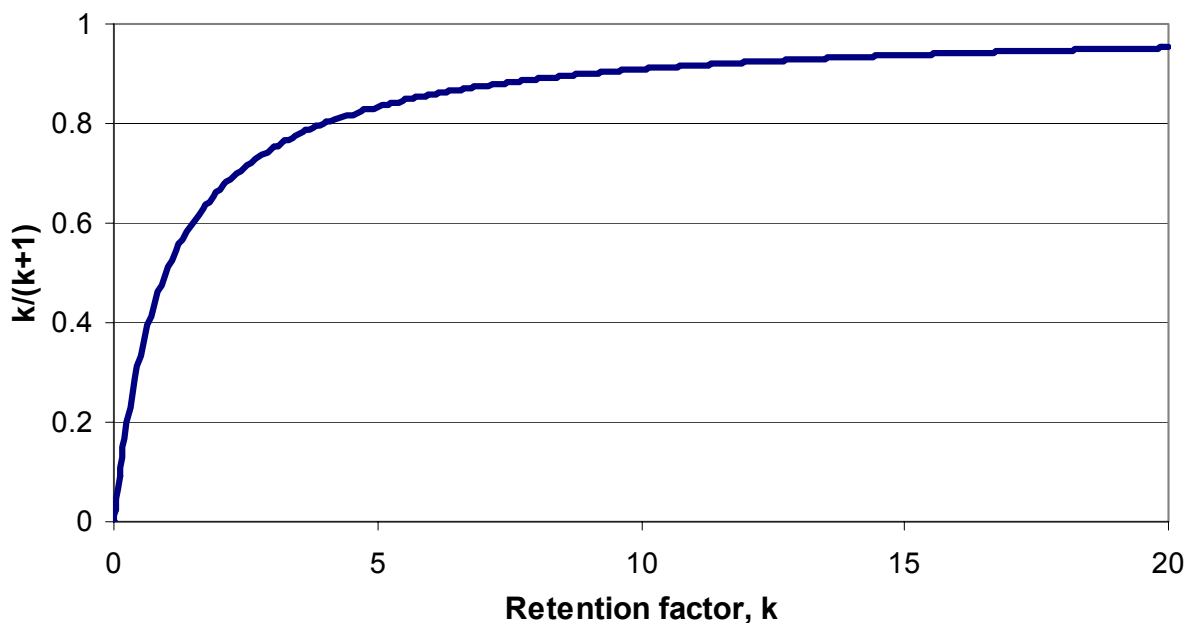
Next to selecting the stationary phase, temperature is the most important parameter in a GC system to be optimised [22,23,27]. Temperature has an influence on retention of analytes ( $k$ ) and to a lesser extent selectivity between analytes ( $\alpha$ ). Temperature thus needs to be optimised for practical considerations such as analysis speed, sample type and aim of analysis [22,23,27].

In the scope of a GC x GC system some more considerations need to be addressed. High separation efficiency is required from the first column which needs to be preserved in the rest of the system. Since the modulator combines already separated segments during the trapping stage, which is the length of time required by the second separation, the peaks eluting from the first column should be as wide as possible, without losing first-dimension ( $^1D$ ) resolution. This can be achieved by slowing down the temperature ramp used in the first dimension.

The second consideration in GC x GC temperatures is that the modulation period is often shorter than the separation time needed in the second-dimension ( $^2D$ ). This problem is addressed by



increasing the temperature in the second dimension. The increase in temperature speeds up the elution, but with a trade-off to resolution between compounds in the early part of the chromatogram. This well-known dilemma in chromatography is referred to as the general elution problem [23]. The graph of  $k/(k+1)$  against  $k$  in figure 10 explains, in terms of the resolution equation in paragraph 3.2, how the resolution drastically drops when  $k$  approaches zero. (If  $k/(k+1) \approx 0$ ,  $R \approx 0$ ).



*Fig. 10 The graph of  $k/(k+1)$  against  $k$  [23]*

Ideally a fast temperature-programmed GC could be used in the second dimension, but up to date there is no such development.

### 3.2.5 Fast isothermal GC

The second column in GC x GC runs under fast isothermal GC conditions. The principles and theory of fast GC were established in the 1960s [28,29,30]. These are well summarised by figure 11 showing the reduction in minimum plate height ( $H_{\min}$ ) and simultaneous increase in optimum linear flow rate ( $u_{\text{opt}}$ ) with reduction in diameter [31].

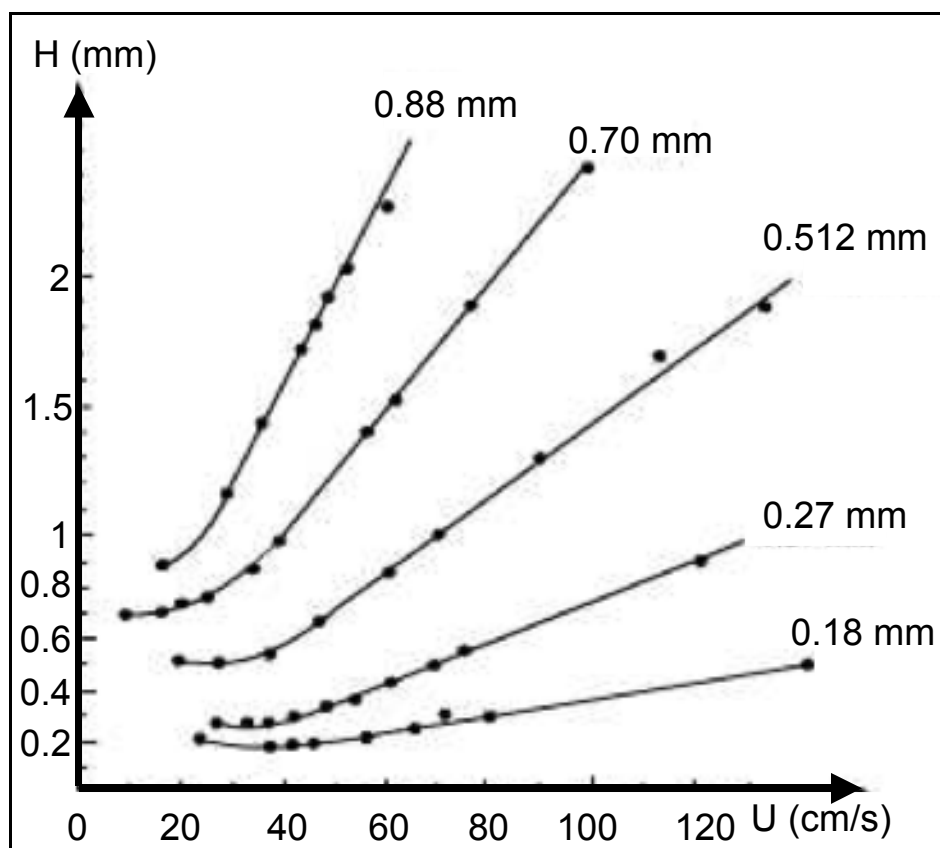


Fig. 11 The change in the Van Deemter curve with change in column inner diameter [31]

In terms of the Van Deemter equation, both changes are a result of the smaller  $C_u$  term, due to smaller diffusion distances causing faster equilibrium [22,23,31]. Reducing the column internal diameter results in drastic reduction in analysis times due to (a) the shorter column required to obtain a given  $N$  value and (b) the higher optimum linear flow rate. Fast temperature programming is used whenever the general elution problem occurs (i.e. when a whole range of compounds needs separation in a single run).

### 3.2.6 Injection techniques

The injection bandwidth is probably just as important as optimising temperatures or flow rates. The resolution, and thus efficiency, of peaks is greatly dependant on the peak widths. Thus it is very important that peak broadening is only due to the van Deemter parameters discussed in section (3.2.3) and not due to a bad injection.

In a temperature-programmed GC a bad injection is masked by thermal focussing on the cool column at the start of the run (the compounds only starts moving after a specific temperature is reached). In an isothermal GC there is no such a thing as thermal focussing at low initial temperatures and all compounds start moving at the same time. Therefore much work was done on perfecting injection techniques for fast GC [32].

### 3.3 Combining the two columns

When combining the two columns new aspects in the optimisation process need to be addressed. The two columns are connected in series, implying that a change on one column would have an effect on the other. These kinds of changes include column lengths (3.2.2), carrier gas flow (3.2.3), pressures (3.2.5.1) and temperature programming (3.2.4) [33].

When combining two columns the interface responsible for linking the two, called the modulator (2.3.3), also needs to be optimised. The modulator in this study, the nitrogen jet-cooled modulator, needs to be optimised for modulator temperature, gas flows of heating and cooling gases, duration of heating and cooling pulses and duration of modulation period.

### 3.4 Conclusions

Optimising a single column by now is trivial and is done routinely around the world. By adding a second dimension, we increase the complexity of the optimisation and new strategies need to be developed.

*Chapter 4*

Background: Part 3

The analysis of petrochemical samples

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## Chapter 4

### Background: Part 3

# The analysis of petrochemical samples

## 4.1 Introduction

Petrochemical samples are extremely complex in nature, not only do they contain many thousands of compounds but they also show characteristic bulk behaviour, dependent on their quantitative and qualitative sample composition. The analysis of these samples thus not only requires the identification of all the individual components but also to the charting of compositional patterns associated with different bulk characteristics. It is mostly these characteristic properties of the samples that are of economic interest to manufacturers. The traditional methods of characterising petrochemical samples involved direct bulk measurement of viscosity, density, pour point, flash point, etc. These methods provide a fast, simple answer to many of the bulk characteristics, but require many analyses and are not always informative enough. The intensive studies of petrochemical analysis lead to the development of many analytical techniques, including chromatography. Some of these developments are discussed in the following sections.

## 4.2 Gas Chromatography (GC)

The separation potential of gas chromatography was already predicted by Martin [33] in the 1950's. Squalane [34] coated metal capillaries were first used for the characterisation of hydrocarbon mixtures [5]. These columns were used until the early 1980s, but although they produced good “repeatable” results [35], they were limited to temperatures below 90°C. Glass capillaries were first reported by Grob *et al* in 1969 [36,37], but due to their fragility they were only used by a small

group of scientist. After the development of the fused-silica column by Dandeneau and Zerenner in 1978 [38], the use of capillary columns became widely accepted. During the same time period, advances such as cross-linking polymeric films in-situ and chemically bonding stationary phases to the silica surface increased the temperature limits of the columns [5].

The detailed-hydrocarbon analyser (DHA), a 100m long column designed to separate straight-run hydrocarbon fractions is, to this date, the ultimate use of separation power in linear capillary chromatography within the petrochemical industry [5]. This approach virtually identified all hydrocarbons in petrochemical fractions up to C<sub>9</sub>, but samples that contained substantial quantities of olefins could not be separated into their individual components [5].

#### 4.2.1 Simulated distillation (*SimDist*)

Distillation is one of the most important techniques in the petrochemical industry. Distillation data are used for the characterisation of feedstock, products and for process control. Boiling-range distributions were among the first tests documented by the American Society for Testing and Materials (ASTM) in 1921, and this distillation test is still used today [5].

The similarity between a non-polar column separation and distillation data made chromatography an attractive, faster alternative for distillation testing. By running standards, usually a series of n-alkanes, the boiling points of which are accurately known, the retention-time axis can be converted into a boiling-point scale. This approach first achieved formal status in 1973 as ASTM D2887 [5]. The method covered diesel, fuel oil, and light lubricants, with boiling points up to 540°C (n-C<sub>44</sub>). The invention of capillary columns further extended the temperature range of the hydrocarbons analysed. The first reported simulated distillation use of capillary columns by Luke and Ray in 1985 [39] separated hydrocarbons up to a boiling point of 650°C (n-C<sub>70</sub>), but it was fast followed by Trestianu *et al.* [40] with their High-Temp *Simdist* method capable of eluting compounds up to C<sub>120</sub> at 430°C and up to C<sub>140</sub> in the final isothermal hold. These new developments eclipsed the use of normal distillation as, even under reduced vacuum conditions, alkanes above C<sub>60</sub> cannot be distilled [5].

#### 4.2.2 PIONA analysis

The complexity of petrochemical products and processes lead to the development of the selectively coupled column systems, which are still in use today, the PIONA. The first of these coupled systems was a GC-GC-GC system, the PNA[41], which could separate oil fractions up to 200°C into distinct chemical classes, the Paraffins (P), the Naphthenes or cyclic paraffins (N) and the Aromatics (A). The system has been improved over the years and the number of separation steps increased, resulting in a PIONA analyser [42] with GC-GC-GC-GC-GC couplings. It could now also separate and group the Iso-alkanes (I) and the alkenes or Olefins (O). This technique is known as a coupled column technique, but the entire sample is not submitted to every individual separation step. The only similarity between this technique and modern comprehensive techniques is that the entire sample eventually reaches the detector [5].

#### 4.3 GC x Selective Detector

In the petroleum industry, the separation of compounds is sometimes insufficient, therefore a number of selective detectors have been developed for their analysis and characterisation. The most common of these detectors is [5]:

- the Flame-Photometric Detector (FPD) designed for sulphur detection
- the Nitrogen-Phosphorus Detector (NPD)
- the Atomic Emission Detector (AED) for various elements
- the Chemiluminescence Detector for sulphur detection
- the oxygen Flame-Ionisation Detector (OFID) adapted to detect oxygenates
- the Mass Spectrometer (MS), which is the most versatile detector.

The petroleum industry pioneered the development of the mass spectrometer. The first commercial MS was sold to a petrochemical industry (Atlantic Refining Company in 1946 for the analysis of hydrocarbon fractions in the gasoline boiling range) [43]. The first mass spectrometers worked on a direct injection basis and were limited, as compared to GC, in that they could not detect isomers



[5]. The coupling of GC to MS became a logical next step in the development of GC- detectors to improve complex mixture detection [44].

#### 4.4 GC x GC

As discussed before, the potential of GC x GC to increase peak capacity beyond that of conventional capillary GC by about 50 times, already provides an attractive alternative to researchers in the petrochemical industry. Although the peak capacity is a big factor, the biggest attraction lies within the method's capability of arranging compounds in chemical classes, greatly simplifying identification and target analysis [45,46]. The sweeper modulated system is somewhat limited in application, as it cannot focus highly volatile compounds, while non-volatile "heavy" compounds require temperatures exceeding current column and modulator capabilities [5]. The optimum performance range of the sweeper, C10-C16 hydrocarbons, is, however, of immediate practical interest because the sophisticated methods (PIONA and DHA) developed for gasolines and naphthas are unsatisfactory in this range [5]. The recent introduction of the jet-cooled modulator [6,7] and independent temperature control of the first and second oven, are increasing the volatility range of compounds that can be analysed by GC x GC [47].

#### 4.5 GC x GC coupled to a Selective Detector

Selective detectors will surely increase the scope and dimensions of GC x GC, therefore it is just a matter of time before detectors, such as the Atomic Emission-Detection (AED), are coupled to the system. The most important *criterion* for GC x GC detectors, as described in chapter 3, is their response speed. With this in mind, the only MS which can be coupled to GC x GC is the TOFMS. The initial steps of combining the two has already been taken [48], the GC x GC - TOFMS has been used for the quantification of aromatic compounds and sulphur containing compounds in petroleum samples.

## 4.6 Other applications of GC x GC

Although the GC x GC is a good separation tool to separate complex mixtures of a hundred or more components, it is even more useful in the separation of less complex mixtures, with a dimensionality of two or three, due to its ordering capabilities. Common further applications are discussed below.

### 4.6.1 Fast screening

GC x GC has the ability to give a quick overview of a mixture, thus providing fast information to identify or classify a mixture. The fingerprinting technique used in classifying and identifying complex mixtures, for example quality control of essential oils, is demonstrated in the research done in parallel by co-workers from our laboratory [49]. The second dimension can also be tuned to unravel overlapping peaks for the analysis of trace components [50].

### 4.6.2 Environmental samples

Most environmental analyses have focussed on individual target substances and not the overall mixture. This provides a limited understanding of the true situation. Gaines *et al.* used GC x GC to determine trace oxygenates and aromatics in water samples [50]. Furthermore, this research group developed a method for the quantitative determination of benzene, toluene, ethyl benzene and xylene (BTEX) and total aromatic content of gasoline based on GC x GC [50]. They also used GC x GC as an excellent tool to identify oil-spill sources of slightly weathered marine diesel fuel from surface water [51].

## 4.7 Conclusions

The technique of GC x GC is still relatively new, it was only a decade ago that Phillips [4] introduced it to the world, but since its introduction it has taken the world by storm. The capabilities of the technique are simply astounding. Its ability to unravel very complex mixtures into structured chromatograms, or to reveal hidden trace peaks in complex matrices, has so far captured the imagination of researchers. These remarkable capabilities remain untapped and will only be unlocked with proper optimisation.