The optimisation of GC x GC and the analysis of diesel petrochemical samples

by

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Abstract

Comprehensive two-dimensional gas chromatography (GC x GC) is a new technique with a
promising future in analytical chemistry. Researchers have already shown the advantages of this

technique to unravel complex samples consisting of hundreds of compounds. The predominant advantage of GC x GC above conventional one-dimensional gas chromatography is the greatly enhanced peak capacity. To fully utilise this enhanced peak capacity the instrumentation needs to be run at optimum conditions. The optimisation of one-dimensional gas chromatography (GC) is done on a routine basis in analytical laboratories and handbooks are available to cover these optimisation strategies. This study was aimed at providing similar guidelines for GC x GC. Since the underlying theory of GC and GC x GC are essentially the same, conventional GC optimisation strategies were the point of departure for this research. The different operational parameters in GC x GC were identified and emphasis was then placed on a method to simultaneously optimise the flow rate in both columns, taking into consideration the common practice of series-coupling of columns of different internal diameters. The influence of second-dimension stationary phase, temperature program and modulator operation on the distribution and shape of chromatographic peaks in the two dimensions is also investigated. The results obtained from this study provide a useful new approach to optimise a GC x GC system where two gas chromatographic columns of various dimensions are connected in series. The use of diesel samples in this optimisation process presented some useful applications for future research in the petrochemical industry. Examples of potential applications such as "fingerprinting techniques" and compositional analysis are also discussed.

The optimisation of GC x GC and the analysis

of diesel petrochemical samples

(Die optimering van GC x GC en die analise

van diesel petrochemiese monsters)

deur

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Ekserp

Twee-dimensionele gaschromatografie (GC x GC) is 'n nuwe tegniek met 'n belowende toekoms

in analitiese chemie. Navorsers het reeds die voordele van die tegniek uitgewys om komplekse

monsters, bestaande uit honderde komponente, te ontrafel. Die mees prominente voordeel van GC x GC bo gewone een-dimensionele GC is die verhoogde piek-kapasiteit. Om die groter piek-kapasiteit ten volle te benut moet die instrument by optimum kondisies funksioneer. Die optimering van een-dimensionele GC word gedoen op 'n roetinebasis in menigte analitiese laboratoriums en daar is handboeke beskikbaar wat die optimering van GC bespreek. Hierdie studie was daarop gemik om soortgelyke riglyne vir die optimering van die meer komplekse GC x GC daar te stel. Omdat die onderliggende teorie van GC en GC x GC essensieel dieselfde is, is konvensionele GC-optimering gebruik as uitgangspunt gebruik vir hierdie navorsing. Die verskillende operasionele parameters in GC x GC is eers geïdentifiseer, waarna klem gelê is op 'n metode wat gelyktydig die lineêre vloei in beide kolomme kan optimeer, met inagneming van die algemene praktyk om serie-gekoppelde kolomme met verskillende binnedeursneë te gebruik. Die invloed van die tweede-dimensie stasionêre fase, temperatuur-programmering en die modulator op die vorm en verspreiding van chromatografiese pieke in die twee dimensies is ook ondersoek. Die resultate verkry in hierdie studie bevestig die bruikbaarheid van 'n nuwe optimeringsmetode vir GC x GC waar kolomme van verskillende binnedeursnit aanmekaar gekoppel word. Die gebruik van dieselmonsters in die optimeringstudie het 'n paar potensiële gebruike uitgewys vir toekomstige navorsing in die petrochemiese industrie. Voorbeelde bespreek sluit hoofkomponent- en "vingerafdruk"- analises in.

Contents

List of Tables

List of Figures

Chapter 1

Introduction

Table of Contents

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 emmisions.

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

Table of Contents

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].

Table 1. One-dimensional separations which might serve as building blocks for multidimensional separation techniques [1].			
Type of separation	**Abbreviation**	**Property controlling separation**	
Bulk displacement	BLK	Nonselective	N
Flow	FLO	Nonselective	N
Chromatographic	CHR	Partition coefficient	K
Field flow fractiation	FFF	Field interaction parameter	φ
Electrophoretic	ELP	Electrical mobility	μ
Isoelectric	IEL	Isoelectric point	I
Isotachophoretic	ITP	Electrical mobility	μ
Dielectrophoretic	DEL	Dielectric constant	K
Sedimentation	SED	Sedimentation coefficient	s
Isopycnic sedimentation	IPY	Density	ρ
Magnetic gradient	MAG	Magnetic susceptibility	ψ
Thermal diffusion	THD	Thermal diffusion coefficient	D`
Thermogravitational	THG	Thermal diffusion factor	α
Diffusophoretic	DIF	Interfacial energy	γ
Photophoretic	PHO	Photophoretic mobility	σ

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.

$$\boldsymbol{n_2} \; \sim \; \boldsymbol{n_y \, x \, n_z} \; \sim \; \boldsymbol{n_1^{\,2}}$$

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

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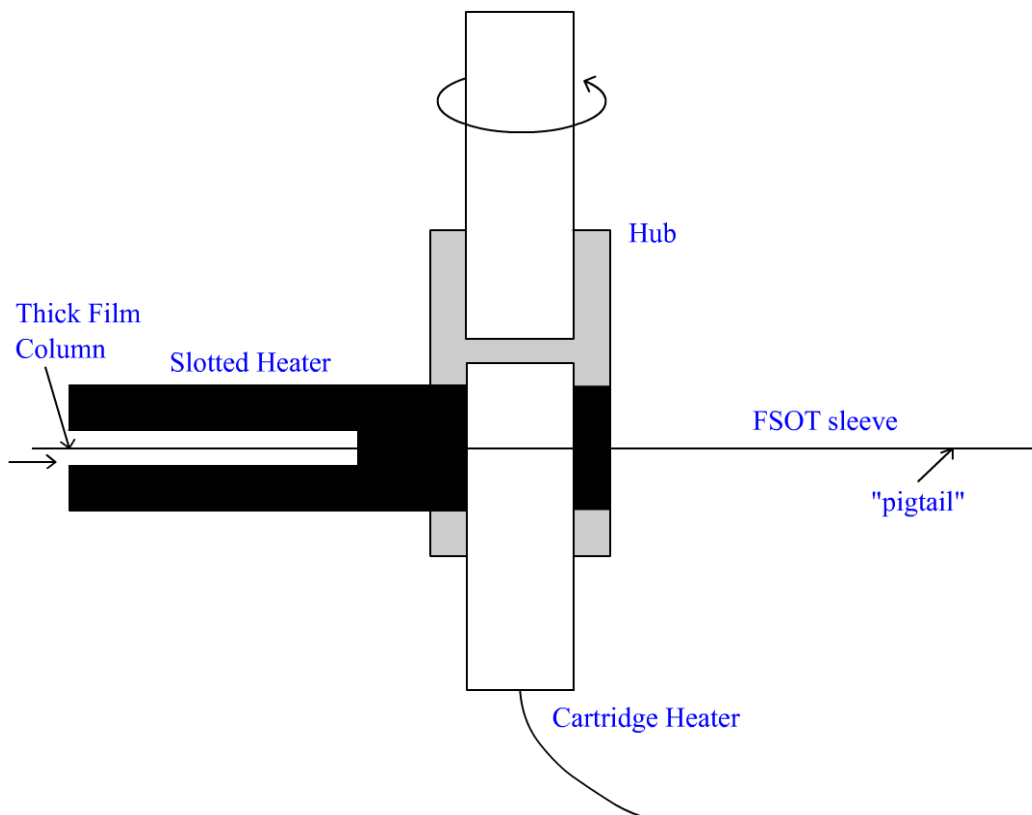
Fig. 1 Slotted thermal heater developed by Phillips [8]

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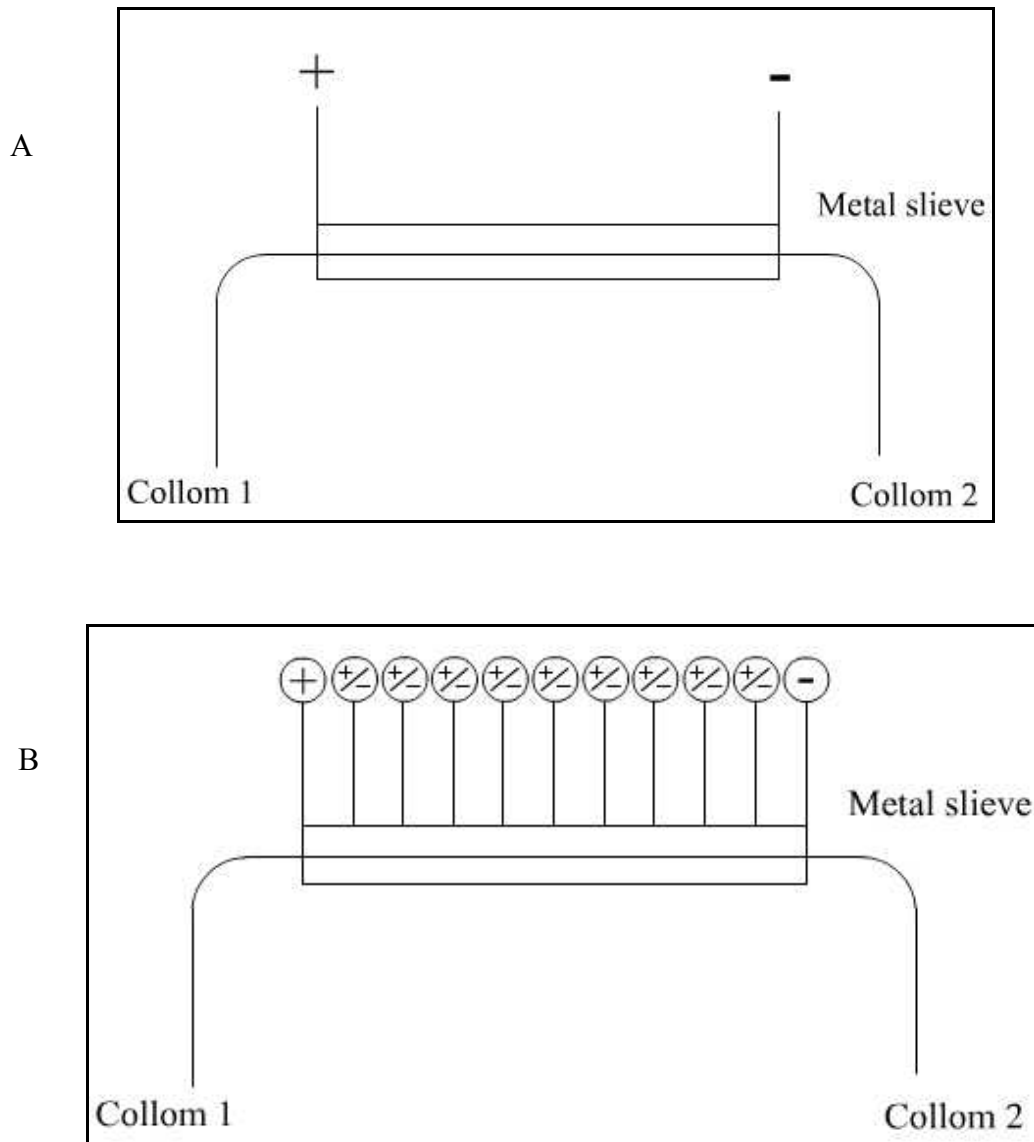
Fig. 2 The two different resistively heated modulators, (A) is designed by Philips et al [4] and (B) designed by Burger et al [9]

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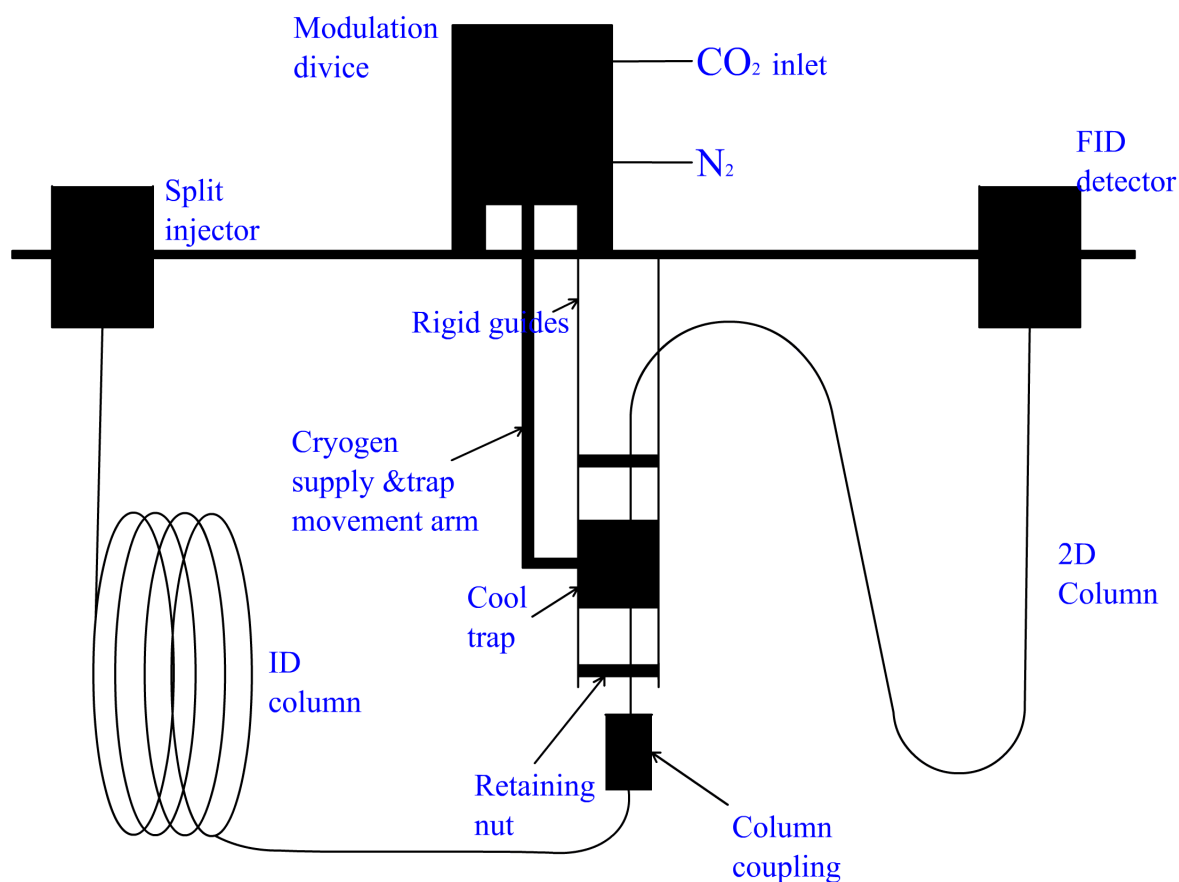
Fig. 3 Longitudinal modulator developed by Marriot [10]

CO2, N2 lines and
thermocouple lead

Actuator fixing plate

Guide frame

Column union

Trap motion

Cryogenic trap

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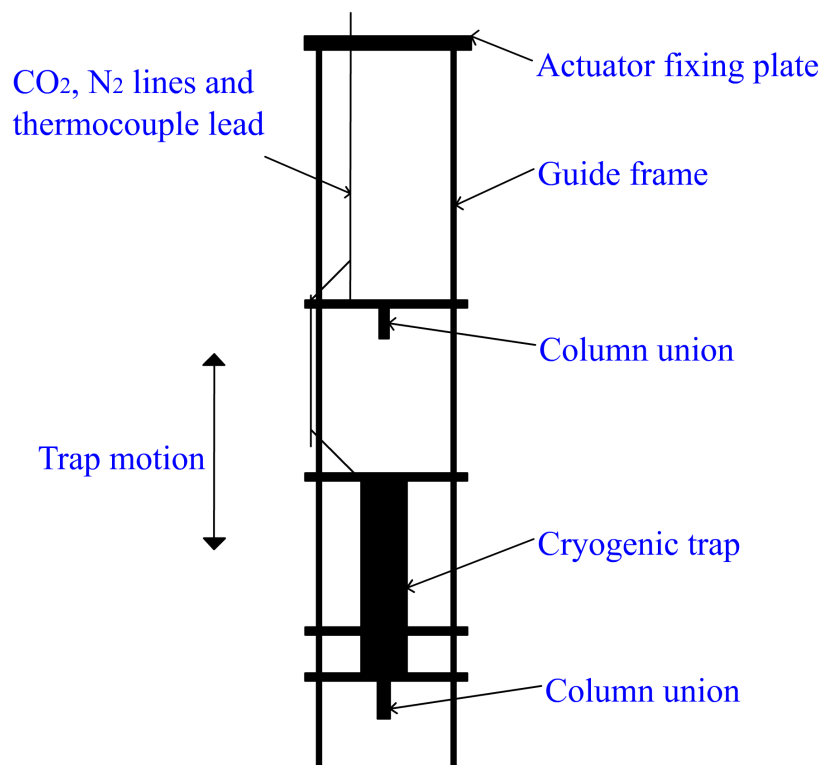
Fig. 4 The trap assembly of the LMCS [10]

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_2 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).
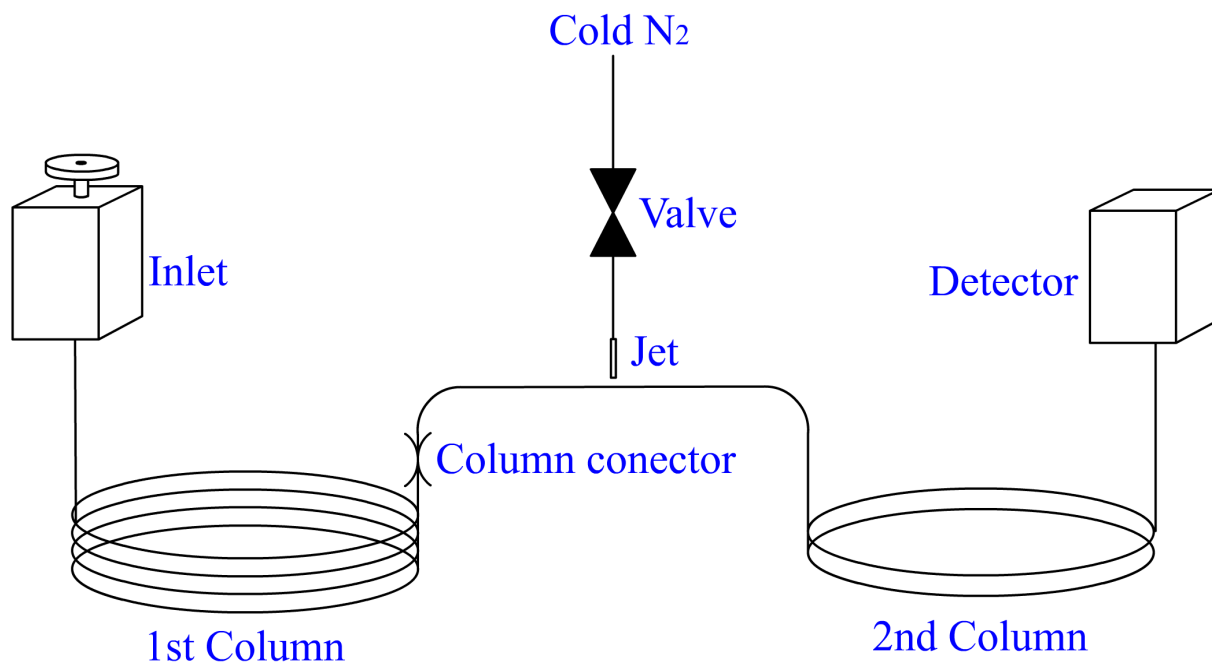
Fig. 5 The single-jet thermal modulator by Ledford[11]

In this modulator, the modulation tube is kept at $100\,^\circ$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.

Cold N_2

Inlet

Detector

C1   C2

W1   W2

1st Column

2nd Column

Warm N_2
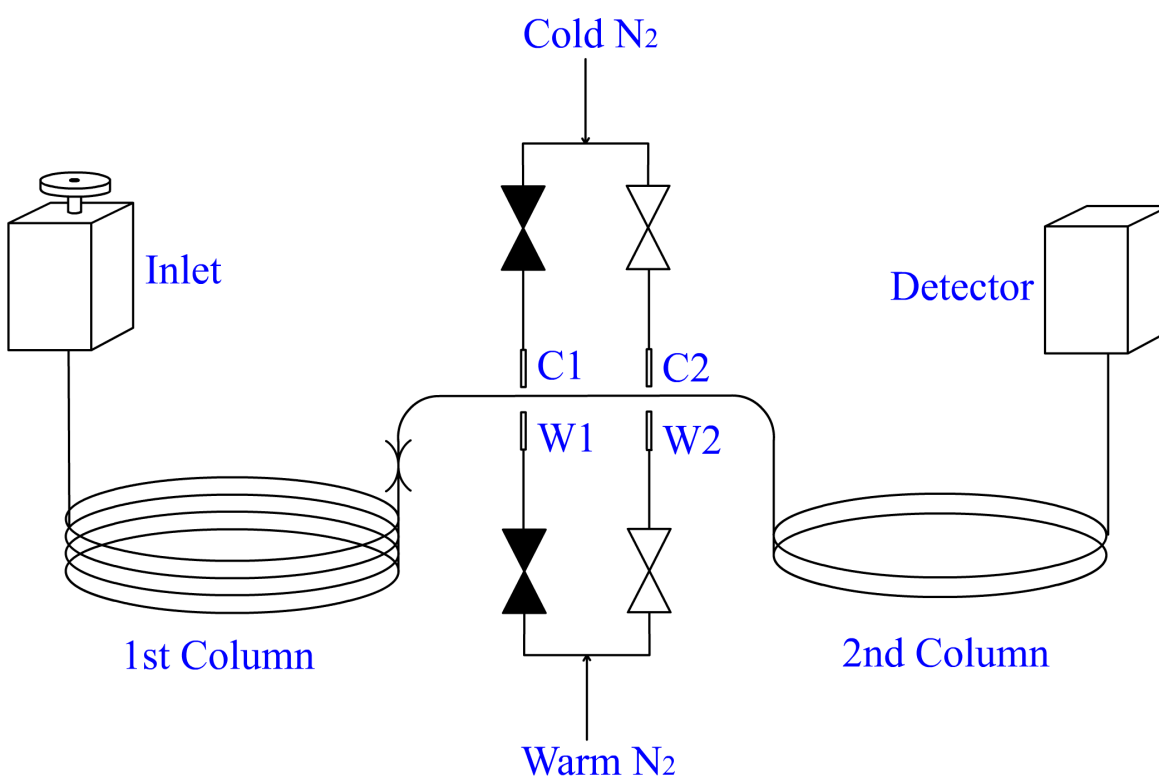
Fig. 6 Nitrogen cryogenic thermal modulator with four jets developed by Ledford [12]

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

liq.CO_2

Valve 1   Valve 2

Inlet

Detector

Jet 1   Jet 2

Column conector

1st Column   2nd Column
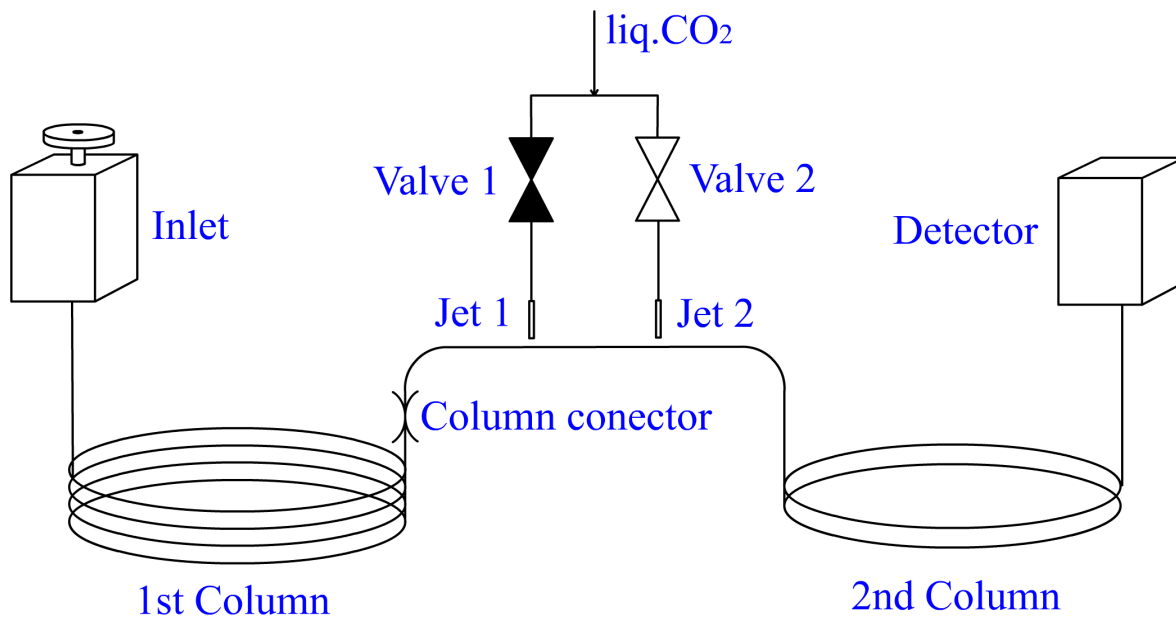
Fig. 7 Duel stage jet modulator of Beens and co workers [13]

The above modulation works on the same concept as Ledford's but uses two CO_2 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
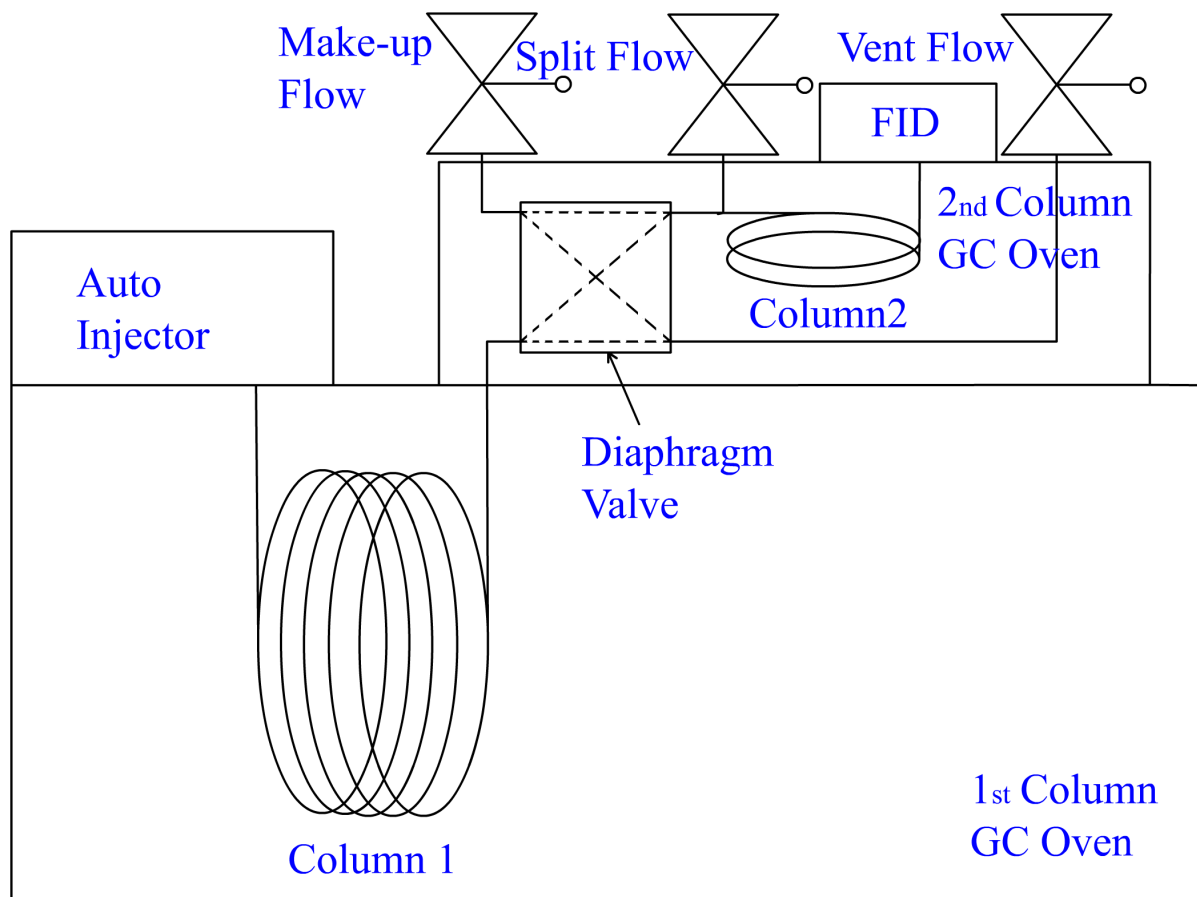
Fig. 8 Schematic diagram of a diaphragm-valve modulator by Synovec [14]

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.

Table 2 Comparison of modulators [15]				
Type	Focussing effect	Bandwidth Injection pulse (ms)	Sensitivity enhancement percentage %	Comprehensive
Duel-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_2	20-50	5-15	yes
Moving direct spray	Cryo CO_2	20-50	5-15	yes
Four jets cryo	Cryo N_2	<10	10-40	yes
Two jets cryo	Cryo CO_2	<10	10-40	yes
Single jet cryo	Cryo CO_2/ N_2	<10	10-40	yes
Rotating cryo	Cryo CO_2	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

Table of contents

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].

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

N   = the theoretical number of plates, defined by the length and diameter of the column.

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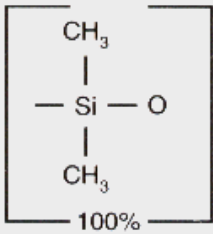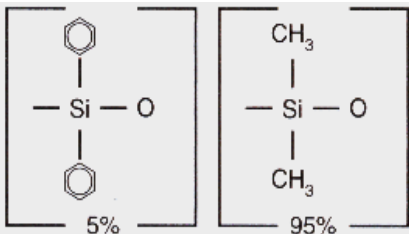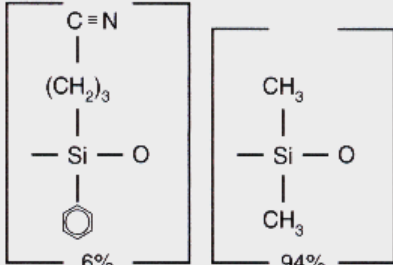
The most obvious way to improve column separation is to increase the column length or to reduce the diameter of the column, thus increasing the number of plates (N). By reducing the column diameter the plate height (H) is reduced and more theoretical plates are possible per column length (H = L/N) [22,23].

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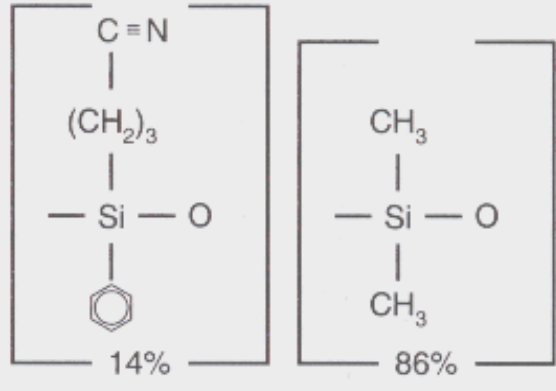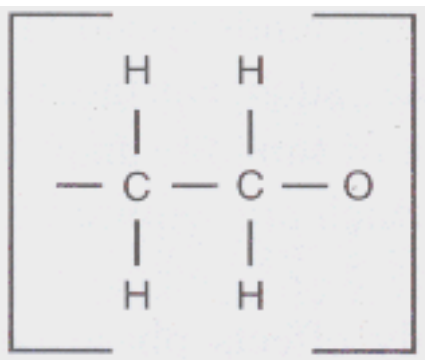
3.2.1   Column stationary phase

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
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, $HO(CH_2CH_2O)_nCH_2CH_2OH$. 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 μm inner diameter column coupled to a 100 μm 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.
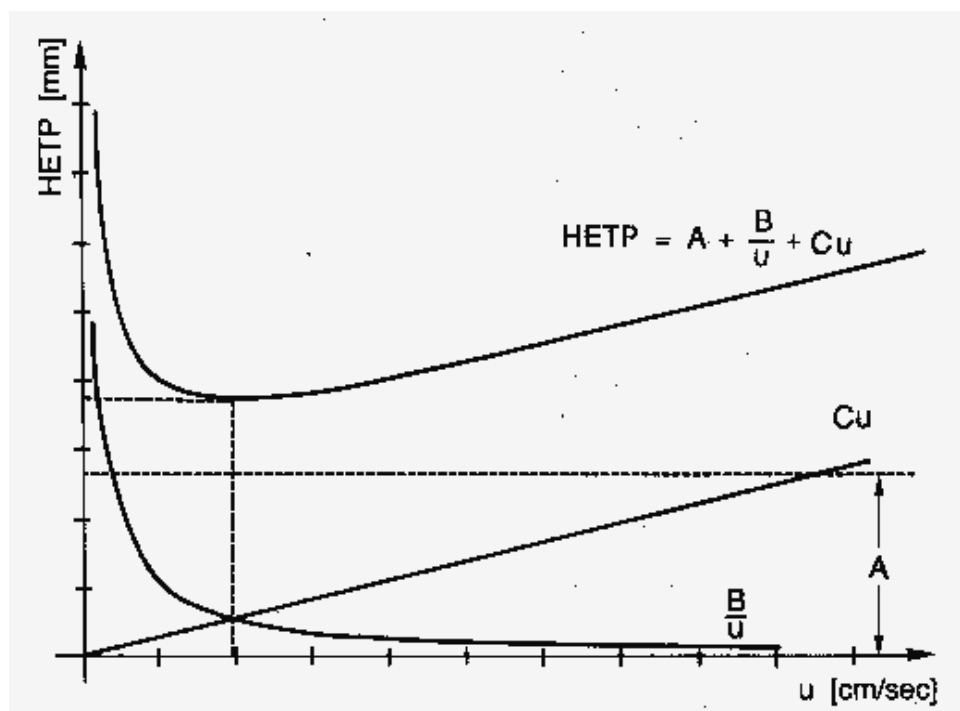
Fig. 9 Influence of Linear flow rate, u, on peak broadening during chromatographic separation (Efficiency) measured as HETP [27]

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

Cu =   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 (α). 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 (^{1}D) 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 (^{2}D). 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) ≈ 0, R ≈ 0).
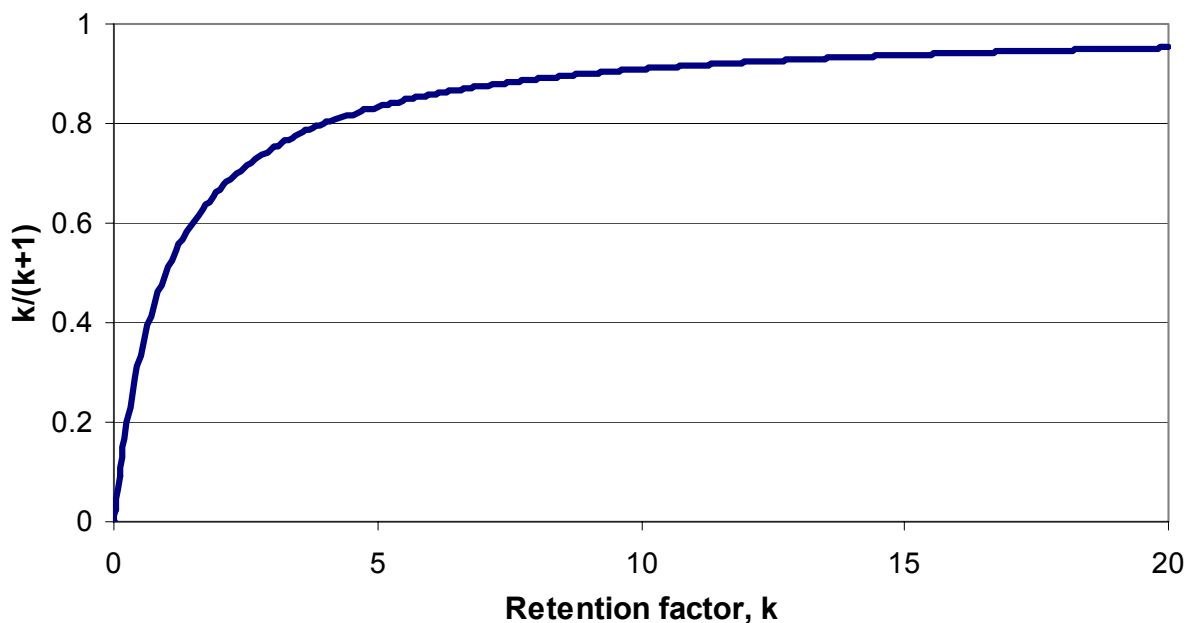
Fig. 10 The graph of k/(k+1) against k [23]

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_{opt}) with reduction in diameter [31].
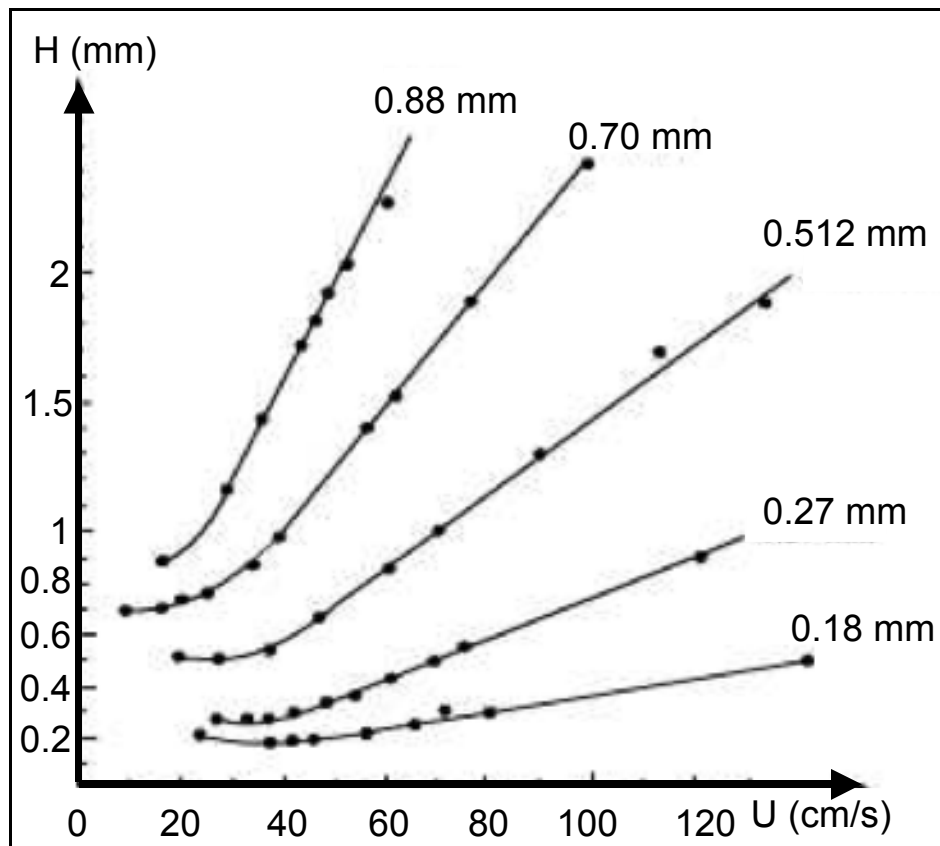
Fig. 11 The change in the Van Deemter curve with change in column inner diameter [31]

In terms of the Van Deemter equation, both changes are a result of the smaller Cu 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 serries, 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

Table of Contents

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 C9, 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_{44}). 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_{70}), but it was fast followed by Trestianu *et al.*[40] with their High-Temp *Simdist* method capable of eluting compounds up to C_{120} at $430°C$ and up to C_{140} in the final isothermal hold. These new developments eclipsed the use of normal distillation as, even under reduced vacuum conditions, alkanes above C_{60} 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 Emision-Detection (AED), are coupled to the system. The most important *criterium* 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.

Chapter 5

Optimisation of GC x GC parameters

Table of Contents

Chapter 5

Optimisation of GC x GC parameters

5.1   Introduction

As discussed in Chapter 3, the optimisation of any system is the most important part before the start of analysis. The fundamentals of optimising a two-dimensional gas chromatography system are exactly the same as in normal one-dimensional gas chromatography. The difficulty in optimising a two-dimensional system is that, instead of separately optimising only the parameters involved in one gas chromatographic system, two integrated gas chromatographic systems have to be optimised together.

For overall optimisation of a gas chromatographic system, it is necessary to follow a chronological path in order to keep track of changes and eventually optimise all the different parameters. Guidelines to optimise one-dimensional gas chromatography already exist, and it was also used as a guideline to optimise the two-dimensional system. In this dissertation the optimisation strategy included the:

- •   Linear flow rate in both columns
- •   Stationary phase selection of the second-dimension
- •   Temperature programming of the first- and second-dimension columns
- •   Optimisation of the modulator
- •   Atmospheric conditions influencing the modulator operation

This optimisation strategy represents a logical and sequential guide to optimise the different parameters involved in GC x GC. The coupling of two chromatographic columns gives rise to some more parameters to be optimised. These include the operation of the coupling interface (the modulator) to trap and inject first-dimension eluents into the second-dimension and to achieve this with minimal "wrap around" of second-dimension peaks. A wrap around occurs when the

modulator injects the next elution portion of the first-dimension column before the second-dimension separation of the previous portion is completed and overlap occurs between peaks of successive second-dimension chromatograms.

The following sections are divided into the general experimental setup in which the optimisation was done and a discussion of the different procedures to optimise the two-dimensional system.

5.2   General experimental setup

5.2.1 Instrumentation

The instrumental requirements for GC x GC are very similar to that of normal GC; in general a GC oven is modified to include a modulator (or coupling interface) between the two chromatographic columns. In addition to the modulator a second-dimension oven can be inserted into the main GC oven to have independent temperature control of the second dimension. The rest of the instrument is fundamentally the same, with an injector and a detector.

In our experiments an Agilent 6890A GC oven (Agilent Technologies, Wilmington, USA) was used, fitted with an Agilent split/split less injector and a fast flame ionisation detector (FID).   Hydrogen was chosen as carrier gas, because of its good separation properties. Hydrogen is ideal for fast gas chromatographic separations and where maximum separation is required in a fixed time. The injector was set to $250°$C with split mode, under constant pressure conditions. The FID operated at $300°$C with an airflow of 400 ml/min, hydrogen flow of 50 ml/min and nitrogen makeup of 50 ml/min. The signal obtained from the FID was recorded at 200 Hz.

The GC x GC modifications on this instrument were the following: A dual stage nitrogen cooled modulator (Zoex corp., Michigan, USA) was installed with the modulation interface between the two chromatographic columns. A second-dimension oven (Zoex corp., Michigan, USA) was installed for the second column which could be controlled by the main GC through its auxiliary

functions.   The column combinations used in this work was chosen in accordance with the most popular choice in the literature. The first-dimension, chosen to do boiling point separation and to operate under the same conditions of one-dimensional chromatography, was a HP-1, 30 m L (length), 0.25 mm ID (internal diameter), 0.25 µm df (film thickness)   column (Agilent Technologies,Wilmington, USA). Either of two columns was used as second-dimension column; a Polyethylene glycol (PEG) column Rtx-Wax, 1.05 m L, 0.1 mm ID, 0.1 µm df (Restek International, USA) and a 17% cyanopropylphenyl - 83% dimethyl polysiloxane column Rtx-1701, 1.05 m L, 0.1 mm ID, 0.1 µm df (Restek International, USA). The second-dimension columns were selected to do polarity separation under fast GC conditions.

5.2.2 Computer software

The control of the instrumentation and final analysis was done with a number of programs. The technique is still under development thus the software that was needed to run it was a combination of different modules. These modules include a program to control the modulator, a data acquisition program and then various programs to interpret and analyse the data.

5.2.2.1 Operating software

To operate the different parameters on the GC-oven, the built-in controls on the instrument were used. These parameters included the temperature programs of both dimensions, the column head pressure and the injector/detector settings. The modulator control, as stated previously, was controlled separately by means of a program specifically designed to switch the hot and cold jets to obtain the required modulation of the first-dimension eluents.

5.2.2.2 Data acquisition software

Quite different from normal one-dimensional chromatography, the data generated in GC x GC can be seen as numerous second-dimension chromatograms and not just a single long chromatogram.

The data can thus either be collected and stored as individual second dimension chromatograms, stored in one long continuous chromatogram, where every five or six seconds (depending on the modulation frequency) can be interpreted as a new second dimension chromatogram, or the individual second dimension chromatograms can be stored in a two-dimensional matrix format where the matrix rows represent the different second dimension chromatograms. The latter method of storage was preferred in this study, because of the data input required by the data visualisation software. For both data storage methods automatic data interpretation techniques are available, such as used in normal one-dimensional chromatography.   These interpretation techniques would typically include peak-find routines, peak-integration and even peak deconvolution. Some of these interpretation techniques have been used in this study and will be shown in later sections.

5.2.2.3 Data analysis and visualisation

Of great importance in a two-dimensional technique is to be able to have a visual interpretation of the acquired data. The data can be shown in a traditional two-dimensional plot (time, signal intensity - chromatogram) or, with the added dimension it can now be projected into a three-dimensional plot (first-dimension time, second-dimension time, signal intensity - chromatogram). This three dimensional plot provides a detailed view of the data, however, most of the data can be lost behind bigger chromatographic peaks. To eliminate this problem, the three-dimensional plot can be shown on a two-dimensional contour plot, with colours indicating the height (chromatographic intensity) of the individual peaks. Examples of these contour plots will be shown throughout this dissertation, because of its usefulness in easily interpreting GC x GC data.

Three programs used in the data analysis and visualisation were Matlab (Mathworks Inc.,USA), Excel (Microsoft, USA) and Transform (Research Systems, Noesys ver. 2.0, USA).   Matlab was used in some data manipulation (baseline subtraction, data shifting, *etc.*), extracting individual second-dimension chromatograms from the two-dimensional data matrix, reconstructing first-dimensional chromatograms and for the plotting of some of the chromatograms. Most of the Matlab programs had to be programmed in the laboratory and are therefore included in the *appendices*. The visualisation of the data in three-dimensional view and in contour plot was done on a mapping

software package from Noesys Research Systems called Transform. For the optimisation calculations and presentations, Excel was used.

5.2.3 Samples used

In order to optimise the system various samples or component mixtures were used. For the determination of linear flow rate, methane gas was used. Methane gas was the obvious choice since it is a very volatile gas that would move through the columns at unretained speeds and is also detectible by the FID.

For modulator optimisation a C_6 - C_{22} n-alkane mixture was used to monitor modulator peak shapes over this wide boiling point range. The n-alkane mixture was made up in the laboratory using 2.00 μg of each of the alkanes in a n-hexane solution. For total system optimisation (column 1, column 2, modulation) a diesel sample was used to provide closely eluting peaks, for determination of both first-dimension (^1D) and second-dimension (^2D) resolution.

5.3   Optimising the column parameters

In order to optimise the column parameters the following procedure was followed. Firstly the column dimensions were chosen, this was done based on popular column choices used in the literature. Secondly the column stationary phases were selected according to the test compounds and sample used (diesel in this case). Thirdly the temperature program in the first-dimension was selected so that there would be a sufficient number of second-dimension analyses per first-dimension peak. Fourthly the second-dimension temperature offset was selected so that a sample would be eluting in a maximum area of the separation plane. These steps of column optimisation are discussed again in a later section.

The steps mentioned above are used as point of departure for the resolution optimisation. To determine the optimum column flow conditions the resolution of two sets of compounds were

calculated and compared at different column head-pressure. To verify the method; first-dimension "dead times" were determined, linear velocities of both columns calculated, k- and α-values calculated for the test compounds, and plate heights for the second-dimension calculated.

The layout of this section is therefore chosen to give a chronological order of experiments and calculations done. In the conclusion section the simplified method is proposed for normal work that bypasses the more complicated experiments and calculations done here only to verify the method.

5.3.1 Linear flow rates

To determine the linear flow rate in the first column, methane gas was injected into the column at 25°C. The methane will move through the column unretained due to its high volatility and low affinity to the stationary phase. The time for the methane to move through the column ("dead time") will thus define the velocity of the carrier gas through the column. In a dual column system the gas has to move through both columns. The time the gas spends in the second column is, however, negligibly short compared to the time spent in the first column. The time the methane takes to move through the system thus serves to measure the "dead-time" of the first-dimension column.

Due to carrier gas compressibility the linear flow rate of the second column cannot be <u>calculated</u> directly from column geometry considerations and is a lot more difficult to determine. It is nearly impossible to <u>measure</u> this "dead-time" directly, thus it had to be <u>calculated</u> form the known parameters.

The first step in this calculation is to determine the head pressure of the second column. This was done by iterating with a software package from Hewlett Packard (GC Method Translation) that is used to translate one set of chromatographic parameters to a another chromatographic system with, for example, a different column or outlet pressure. To understand the logic behind this method, it has to be reminded that the exit of the first column is at the same pressure as the inlet of the second. The inlet pressure of the first column is that of the set pressure on the inlet gauge, while the exit of the second column is at atmospheric pressure (FID operates at atmospheric pressure). The "dead-time" of the first column is already determined, and thus the only unknown parameters remaining are the pressure in-between the two columns and the linear flow of the second column. With the Method Translation software and the indicated inlet pressure, the operator estimates and modifies the exit pressure of the first column until the correct "dead time" of the first column is calculated. By this iterative procedure, the intermediate pressure is determined.

The second step of the calculation uses the intermediate pressure, calculated in the first step, to calculate the linear flow rate in the second column, again by using the Method Translation software. Of course the program requires input as to the dimensions, type of gas, temperature and (atmospheric) exit pressure.

5.3.2 Optimisation of the flow rates for optimum resolution

As a point of departure, as mentioned before, a number of parameters were taken from well-established literature methods. These parameters included the length, diameter and stationary phase thickness of both the first- and second-dimension columns. A temperature gradient of 1 °C/min was used for the first-dimension separation, giving first-dimension peaks of about 30 seconds peak width. This peak width provides sufficient time for multiple sampling by the second dimension column. It is reminded that slower temperature gradients do not decrease the resolution, only increase the elution time (see influence of decreased temperature, increased k and k/(k+1) on the general resolution equation discussed in chapter 3). The temperature gradient of the second-dimension was the same as that for the first-dimension but at a raised temperature. The temperature difference between the two columns was adjusted according to the sample used and the stationary phase in the second dimension. The time allowed for second dimension separation (modulation period) was kept short enough to maintain a minimum of four cuts per first-dimension peak.

Given these predefined parameters, the study now focussed on adjusting the flow rates through the two columns for maximum resolution between compounds in both columns. As the two columns are linked to one another their linear flow rates cannot be independently adjusted without changing some of the column parameters such as diameter and length. The optimisation was thus done by changing the inlet head-pressure, with increments of 20 kPa (10 kPa increments in the optimum region), and calculating the resolution between two selected compounds for each separation dimension at the different head-pressures. (Finding optimum conditions for both columns at the

identical inlet pressure would indicate a good choice of matching column dimensions.)

The compounds used for the calculation of the first-dimension resolution were two C7 alkanes from a diesel sample that eluted close to 25°C. The two compounds chosen for the second-dimension were compounds that also eluted in the same temperature range, co-eluting in the first-dimension but separating in the second-dimension. The latter two compounds were an alkane and a cyclo-alkane, also of the C_7 group. Figure 12 gives a graphical representation of the optimisation strategy with GC x GC peaks eluting at about 25°C from a diesel sample.

In the case of the first-dimension optimisation, the experiment was refined by running the system in normal one-dimensional mode by shutting down the gas pulses of the modulator. The one-dimensional run was needed because of the low number of data points generated for defining first-dimension peaks in a two-dimensional chromatogram (generally only four data points per chromatographic peak). This procedure resulted in using different set of chromatographic peaks in the same elution temperature range for the calculation of the first-dimension resolution, due to co-elution with other peaks in the one-dimensional chromatogram.
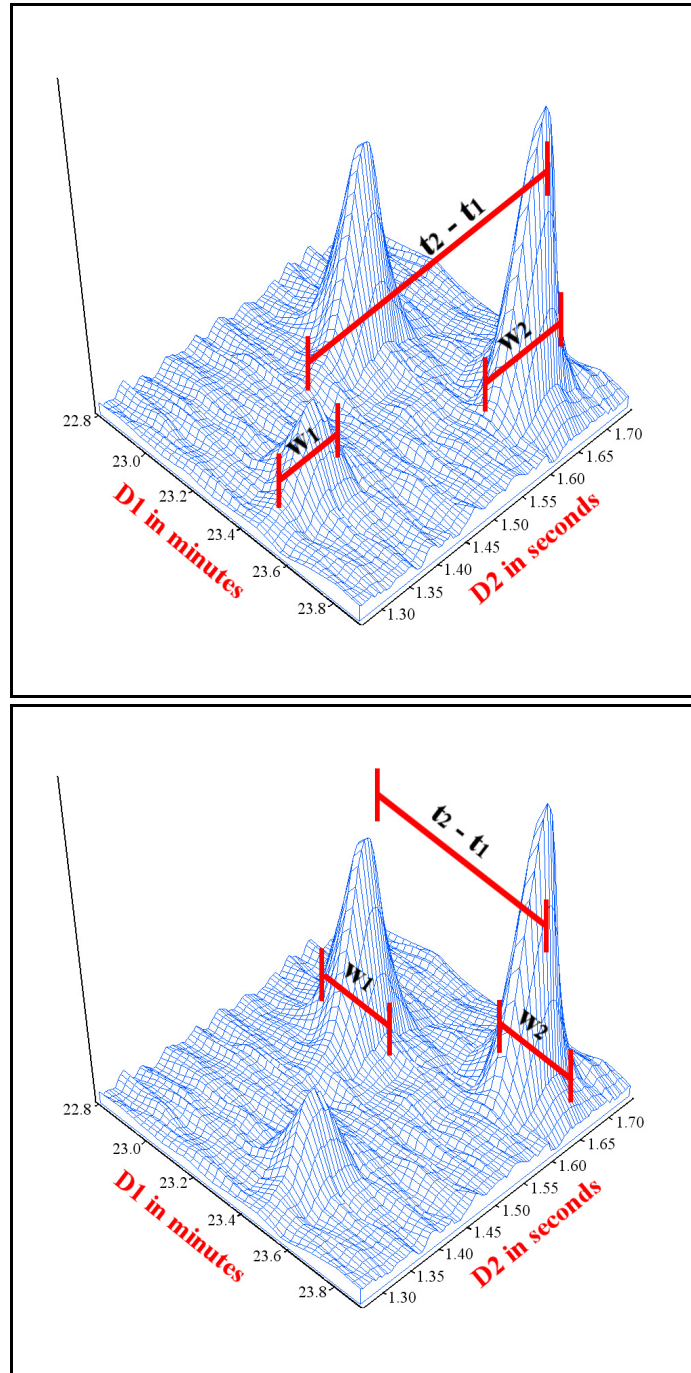
Fig. 12 Graphical representation of the optimisation strategy with GC x GC peaks eluting at

about 25 ℃ from a diesel sample.

The results obtained from the different inlet-pressures can be shown in figures 13, 14 and 15. For the raw data, see Appendix B. Table 5 gives the respective linear flow rates of both dimensions corresponding to the inlet head pressures as used in the figures.

Table 5 Inlet pressures corresponding to the average linear flow rates in the first- and second-dimension		
Inlet pressure (kPa)	Linear flow rate first-dimension (cm/s)	Average linear flow rate calculated for second-dimension (cm/s)
320	47.617	753
280	42.015	664
220	35.713	520
190	34.841	442
185	33.612	432
175	32.101	409
170	31.744	396
150	28.570	350
130	25.061	304
125	24.419	292
120	23.612	281
115	22.856	269
110	21.977	256
100	19.979	234
90	18.314	210
80	16.514	187
60	12.754	140

Resolution against linear flow rate first-dimension
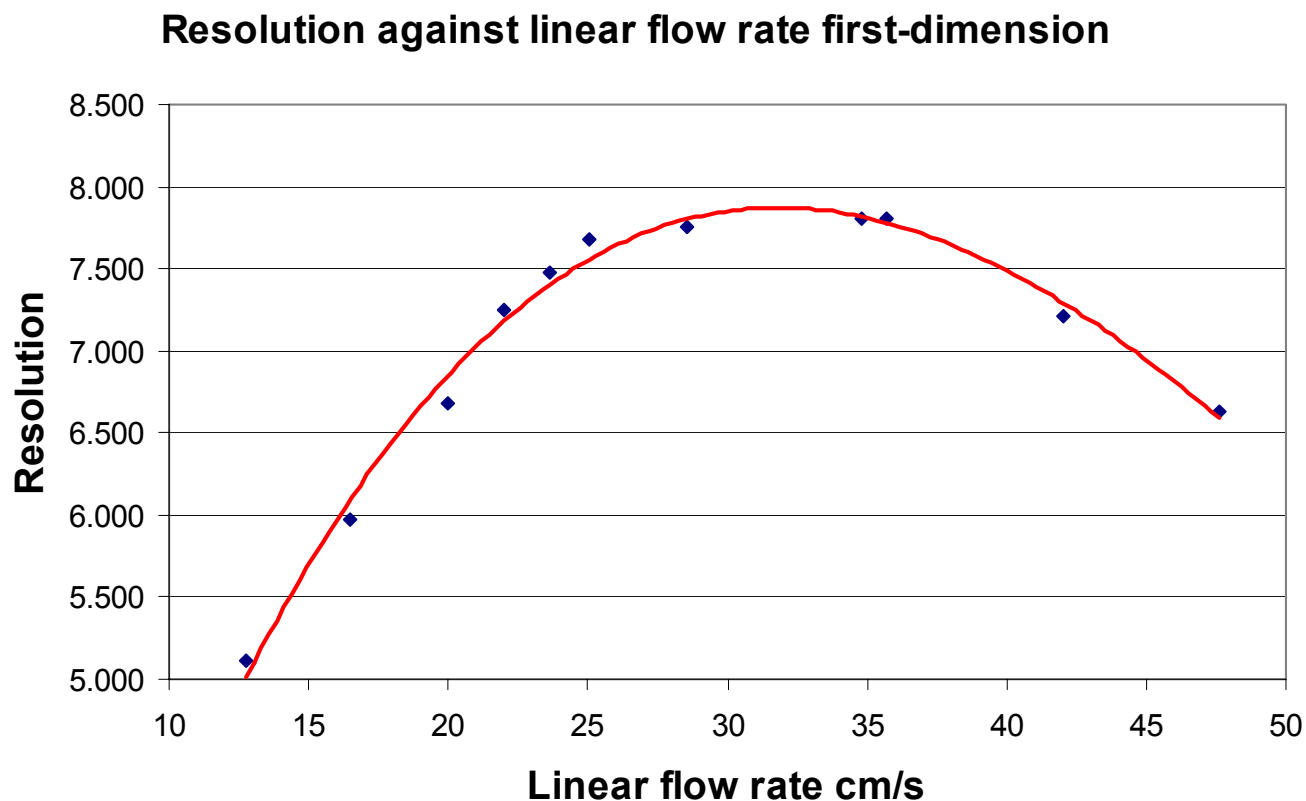
Fig. 13 Resolution plotted against the linear flow rate of the first-dimension at different inlet

pressures.

From these figures it can be seen that an optimum in resolution is not obtained at the same inlet-pressure. One way of fully optimising the system would be to adjust the length or diameters of the columns so that the optimum is reached at the same inlet-pressure, but this would be work for a future project. In this study a good compromise could be made between the resolutions of the two columns. The resolution of the first column changes very little at the top of the curve, while the resolution curve of the second column decreases rapidly towards the high flow side.

Resolution against linear flow second-dimension
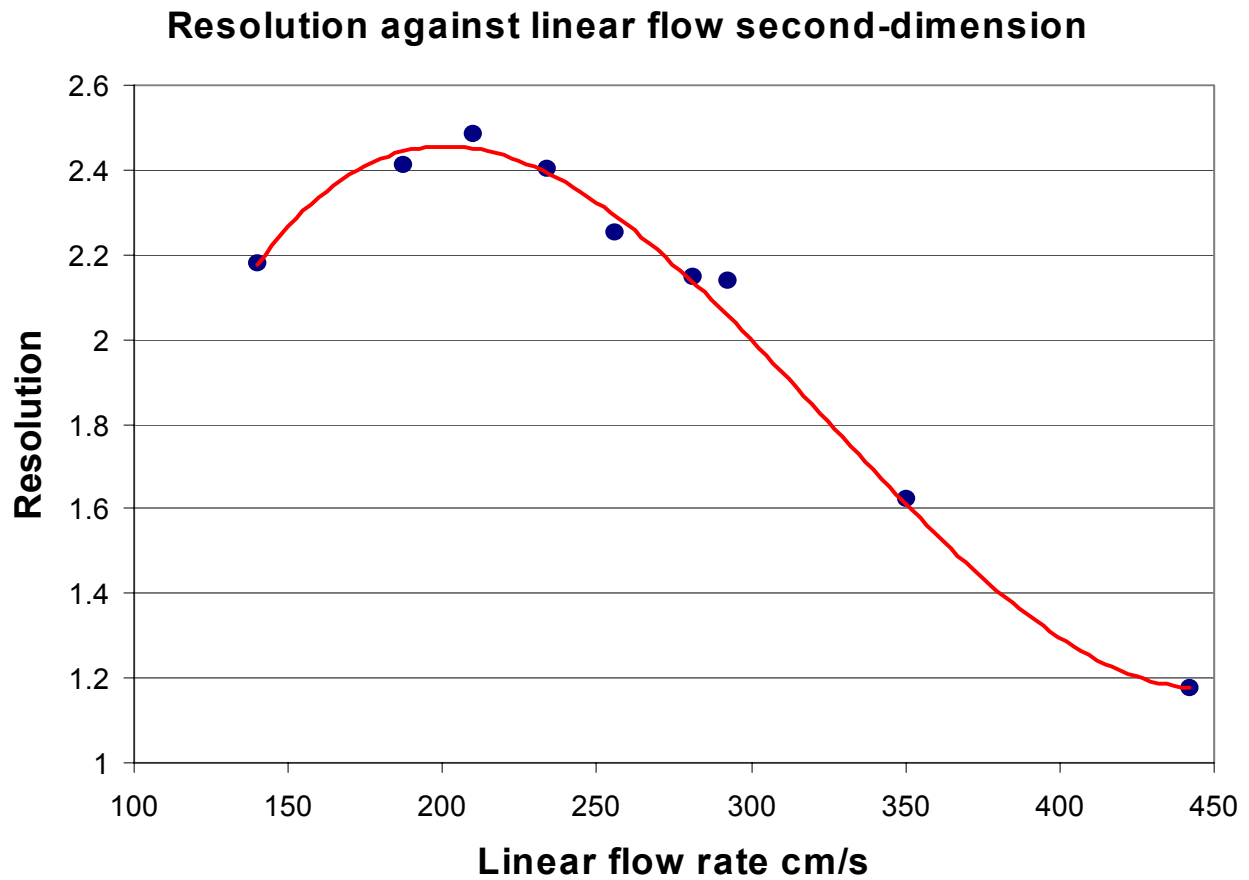
Fig. 14 Resolution plotted against the linear flow rate of the second-dimension at different inlet

pressures.

It was thus decided to run the first-dimension at a slightly slower and the second-dimension slightly

faster than their respective optimum flow rates. The inlet-pressure corresponding to this point was

110 kPa and provides 88% of the optimum resolution in the first-dimension and 90% of the optimum

resolution in the second-dimension.

Resolution against system inlet pressure
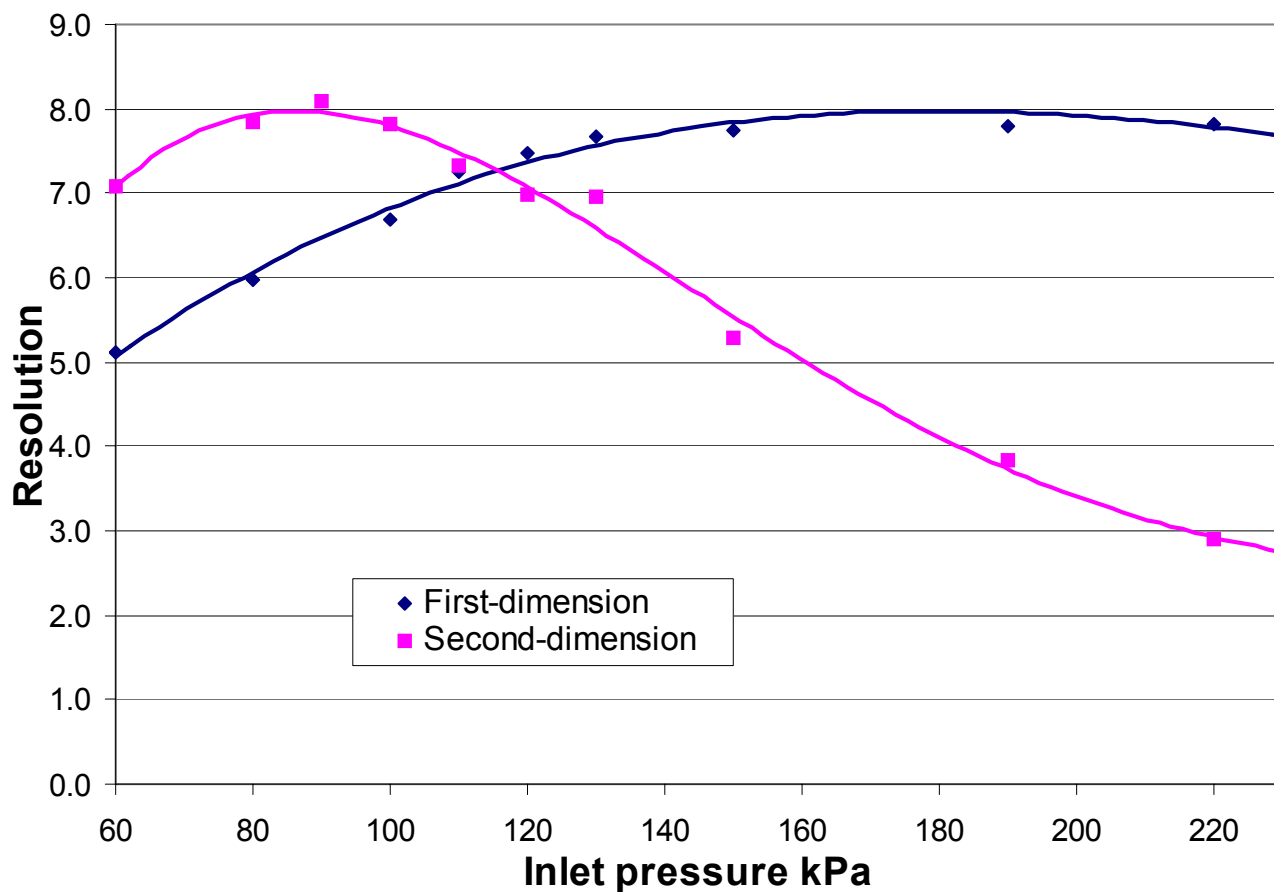
Fig. 15 Plot of resolution of the first- and second-dimension against the system inlet pressure (the absolute resolution values of the second-dimension are adjusted by a factor of 3.20 for better visual comparison).

The reason for using this resolution determination rather than the Van Deemter plot in the flow optimisation study should be highlighted at this point:

1) Although the Van Deemter plots would be the more conventional/ theoretical approach it would require isothermal operation of both columns (constant k-values required for N and H calculations)

which is difficult to achieve in practise.

2) Resolution determinations are just as valid under the more practical temperature programming conditions and do not require the determination of absolute retention times, which are not easily obtained in the GCxGC system used in this study.

3) Referring to the resolution equation (see Chapter 3): Provided the α- and k-values for the two peaks used do not change appreciably with flow rate, the resolution achieved will be directly related to the plate number and plate height. Flow optimisation of the resolution method under these circumstances would then be equivalent to plate height optimisation (Van Deemter plot)

4) In any event resolution optimisation as performed in this GCxGC study has more practical importance, as it incorporates both columns and modulator performance.

This resolution optimisation strategy should, however, not be done without investigating potential errors of interpretation. In this study a fixed temperature program in the first dimension was used. This would result in the compounds used for the calculations eluting at slightly different temperatures (and k-values) with the change in flow rate. The first-dimension elution temperature is also of concern for the retention behaviour of the second column since the analysed compounds are subsequently subjected to slightly different second-dimension column temperatures under different flow rates. This variation in temperatures would slightly affect the k-values and possibly the α-values with consequences on resolution as predicted by the resolution equation (Chapter 3), but unrelated to the flow rates. Compounds of similar chemical nature were therefore chosen for the calculations since these compounds do not produce significant changes in α-values with temperature. The changes in α- and k-values with flow would both introduce changes to the

resolution (R) values that are test compound related but not column performance (N) related, i.e. cannot be ascribed to changes in longitudinal diffusion or slow radial equilibria. The slow temperature program chosen in the first-dimension produces large elution k-values (the lowest k values for a particular compound occur at their highest temperature in the column, i.e. at elution) such that the k/(k+1) term approaches unity as depicted in Figure 10 (Chapter 3). Thus only slight changes in α are likely to contribute to changes in resolution with flow rate in the first-dimension - an effect suppressed by selecting two peaks that are chemically very similar, so that their relative k values do not change much with temperature.

This is not true for the second-dimension, as fast GC is often performed at very small k-values (k < 4). This more critical case is therefore dealt with in greater detail below:

In Tables 5 and 6 the k- and α-terms of the test compounds are calculated for the respective column head pressures used. This requires determination of absolute retention times and linear flow rate. In practise this is not done that easily as there is no trigger for the beginning of second-dimension chromatograms and the absolute retention times of peaks are thus uncertain. This was also one of the major considerations for using the more practical resolution approach since in this method only relative retention times are required. With the calculated "dead time" (section 5.3.1) the absolute retention times of the test compounds can be calculated. This was done by firstly adjusting the two-dimensional contour plot so that the unretained compounds from the second-dimension (low boiling, non polar compounds, i.e. the C3 and C4 alkanes) are on the zero position of the time axis. The "dead time" is then added to this zero position to give absolute retention times.

Table 6 The k - values and k - term used in the resolution equation				
Inlet Pressure	Peak 1		Peak 2	
	k - values	k / (k + 1)	k - values	k / (k + 1)
60	2.497	0.714	2.896	0.743
80	2.834	0.739	3.280	0.766
90	3.050	0.753	3.540	0.780
100	3.188	0.761	3.714	0.788
110	3.371	0.771	3.922	0.797
120	3.422	0.774	3.984	0.799
130	3.936	0.797	4.532	0.819
150	4.333	0.813	5.000	0.833
190	5.641	0.849	6.401	0.865

Table 7 The α - values and α - term used in the resolution equation		
Inlet Pressure	α - values	$(\alpha - 1) / \alpha$
60	1.160	0.138
80	1.157	0.136
90	1.161	0.138
100	1.165	0.142
110	1.164	0.141
120	1.164	0.141
130	1.151	0.131
150	1.154	0.133
190	1.135	0.119

As predicted the k-values (Table 6)are rather small and hence the k-term of the resolution equation could vary a lot, but at the region of resolution optimum (i.e. 90 - 120 kPa) the k-term deviated within a 2% (7% over the whole range measured). The α-terms (Table 7) have an even lower deviation of only 2% over the total head-pressure range measured.

Whereas the resolution values (R) are also affected by the retention factors (k) and α value for the test compounds, N values depend only the column performance as measured by $N = (t_r/\sigma)^2$.

Resolution values can be converted to N values once the k and α values of the test substances are known. By rearrangement of the well known resolution equation (Chapter 3) we obtain:

$$\sqrt{N} = 4R\left(\frac{k_2 + 1}{k}\right)\left(\frac{\alpha}{\alpha - 1}\right)$$

The square root N values, thus calculated and presented in Figure 16, show that for our set of second-dimension peaks ($k \approx 3$), the optimisation in resolution and in column performance is found at much the same inlet pressure/ linear flow rate. (Note that the optimum N value is found at a marginally higher flow rate - justification for our theoretical concern over k and α values.) This means that flow optimisation of the R value for the selected pair of compounds does indeed optimise N values or column performance, obviating the need for the much more involved calculation of N values that require absolute retention times.

sqrt (N) against inlet pressure
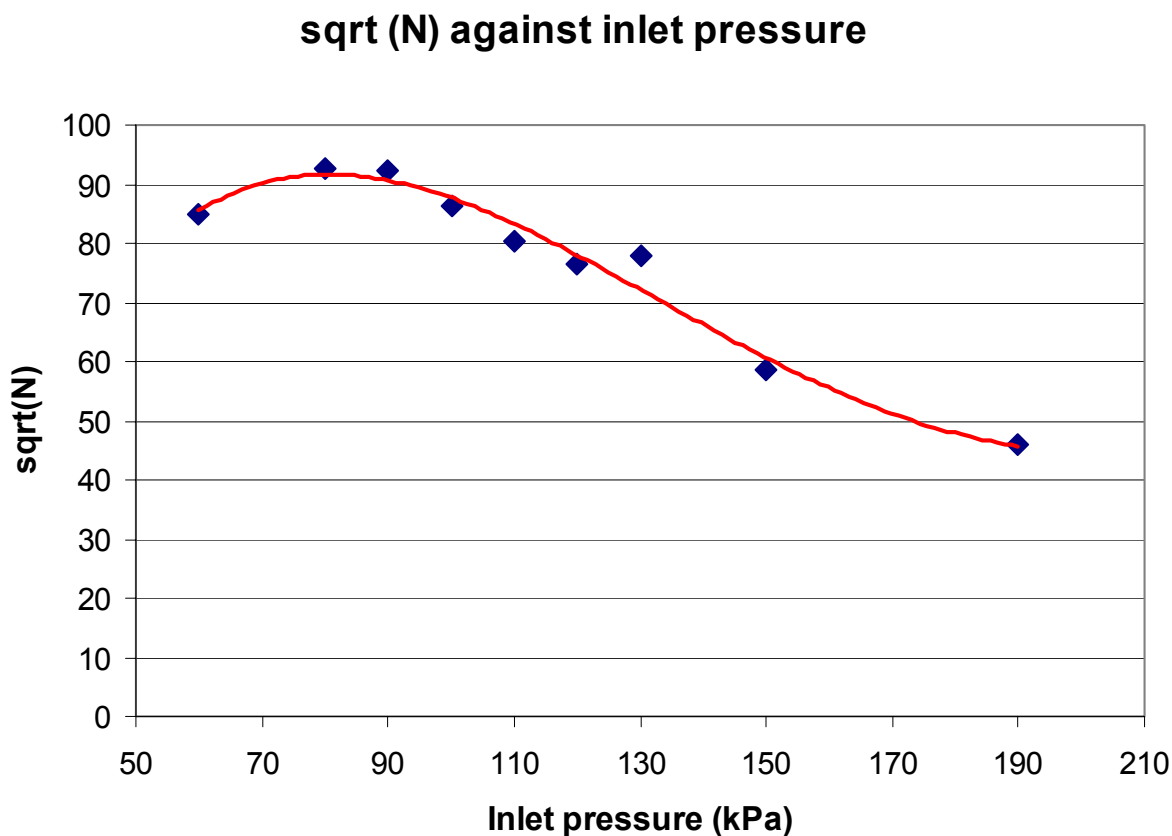
Fig. 16 The plot of the square root of the plate number (N) against the inlet head pressure for the

evaluating the k- and α-term dependency of the resolution optimisation method

With absolute retention times known a second method of verification, the traditional Van Deemter

plot, can be done in the case of the second-dimension. This would be the fundamentally correct way

of column optimisation. Plate heights can be calculated by the following equation:

In practise, the equation found in Appendix B, based on the peak width at half height, is used.

Van Deemter Plot of two second-dimension retention peaks
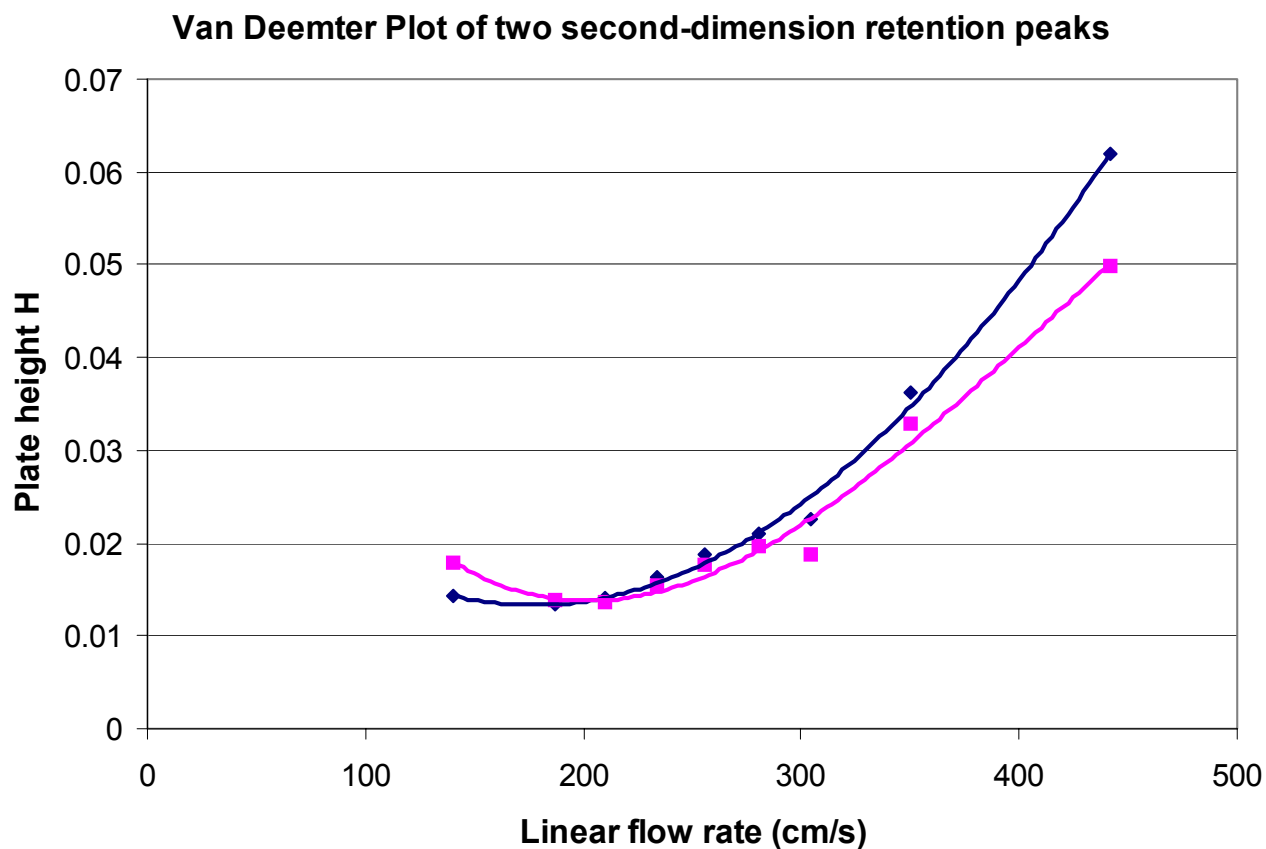
Fig. 17 The plate height for the two peaks used in the resolution calculations is calculated and plotted against the linear velocity of the second-dimension to give Van Deemter plots of the second-dimension.

From figure 17 it is clear that the optimum determined in the resolution approach is the same as the square root N and Van Deemter optimum in the graph, i.e. 200 cm/s. The minimum plate height obtained in this way is also a good indicator for evaluating the efficiency of the modulator injection.

The Van Deemter plot shows a much steeper gradient of the curve in the fast linear flow rate region than what is expected for a 100 μm column (see figure 11). Together with the minimum plate height that is about 35% higher than theoretically predicted for the 100 μm i.d. column, this indicates that the modulator injection into the second-dimension still has a negative contribution to overall system performance. This contribution (σ_i) has an enhanced effect at higher flow rates, where compounds elute with narrower peaks in time.

$$H = \frac{L}{N} = L\left(\frac{\sigma_t}{t_r}\right)^2 \qquad [22]$$

$$\sigma_t^2 = \sigma_{col}^2 + \sigma_{inj}^2 \qquad [16]$$

This would result in larger H values at higher flow rates (lower t_r) as σ_t/t_r will increase. The result is a steeper flow dependant curve than expected from pure column considerations. Indeed it can be speculated that σ_{inj} could even increase with flow rate due to cooling effect of the higher internal carrier gas flow.

The result of the flow optimisation study shows up the danger of blindly using literature Van Deemter [52,53] results or generally accepted optimum flow rates for a specific inner diameter column.

In figure 18 a comparison of using the generally accepted linear flow rate (40 cm/s) and the new recommended linear flow rate (22 cm/s). From the two diesel chromatograms in figure 18, it is

obvious that there is a much better separation in the resolution-optimised chromatogram and that there is even "baseline" separation of compounds previously overlapping (compounds circled in red).

To summarise: this study indicates that the resolution *vs* inlet pressure curves (Figure 15) for both dimensions are sufficient for GC x GC flow optimisation. It obviates the need to determine absolute retention times and linear flow rates. The more involved Van Deemter optimisation for the second-dimension does, however, give added information as to modulator performance.
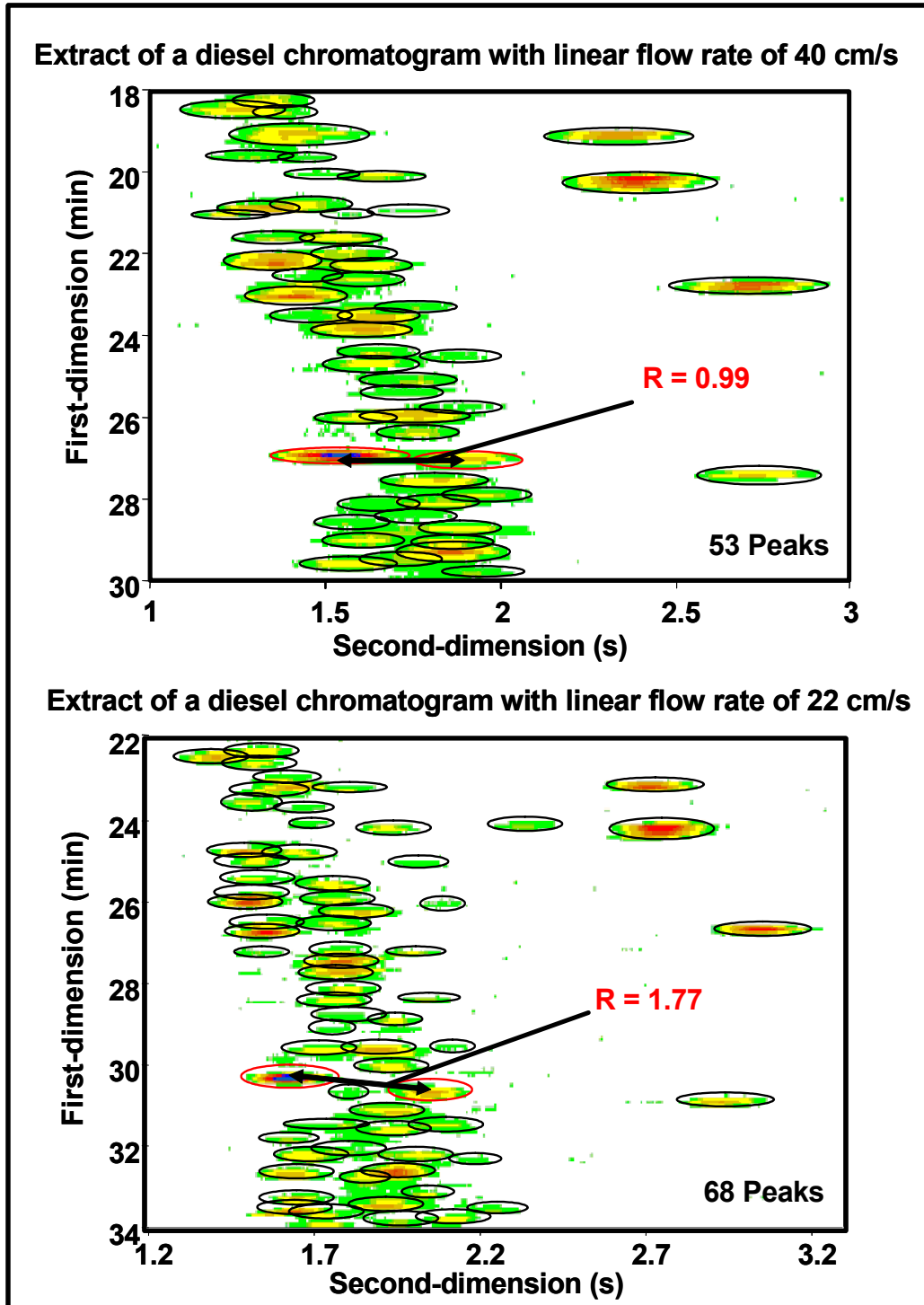
Fig. 18 A comparison between diesel chromatograms using the generally accepted linear flow

rate of 40 cm/s for the first-dimension and the optimised linear flow rate of 22 cm/s.

5.3.3 Choice of second dimension stationary phase

The choice of a stationary phase in chromatographic separations generally depends on the type of separations required and the type of sample to be analysed (see discussion on dimensionality Chapter 2). As discussed in Chapter 3 the two different stationary phases used in this study were a PEG column and 17% cyanopropylphenyl - 83% dimethyl polysiloxane column. The PEG column which is quite a polar column is in principle the best phase to provide "polarity" separation (see orthogonality consideration Chapter 2). The PEG column can separate compounds of different polarity with great efficiency, but the time required to separate these compounds becomes increasingly long as the polarity range of compounds in a sample increases. The second-dimension, however, has a limited time allowed for the compounds to elute. For the most polar compounds to elute within the fixed time frame (modulation period) the column is run at higher temperatures, which result in compounds eluting early in the chromatogram to be "squashed" together (lower R values at too low k values). This behaviour is referred to as the general elution problem (section 3.2.4). In principle, temperature programming in the second-dimension will solve this problem; a technology not yet available for the fast, repetitive second-dimension. Presently the PEG column is probably best reserved for samples containing compounds in a narrow polarity range.

The use of a less polar column like the 17% cyanopropylphenyl - 83% dimethyl polysiloxane column allows a lower temperature separation and decreased "squashing" of early eluting peaks. The separation of different polarity classes obtained with this column is less than that of the PEG column. The 17% cyanopropylphenyl - 83% dimethyl polysiloxane column can, however, separate

compounds eluting early (i.e. alkanes and cycloalkanes) in the chromatogram much more efficiently. The eventual aim of this study was to investigate potential applications for the analysis of diesel samples, which contain mostly compounds of low polarity alkanes and cyclic alkanes, as well as some medium polar (mono-aromatic, di-aromatic and tri-aromatic) compounds.. The 17% cyanopropylphenyl - 83% dimethyl polysiloxane column proved to be more robust in that it seemed to have a much longer life time than the PEG column. Both columns had an upper temperature limit of 270°C but the PEG column required operation at or above its recommended maximum temperature when diesel samples were analysed at higher temperatures (higher temperatures to get the compounds to elude in the defined modulation period). The 17% cyanopropylphenyl - 83% dimethyl polysiloxane column was, therefore, the column of choice in this optimisation study.

In figure 19 a comparison between the two different columns can be seen. From these figures some of the above concerns for not using the PEG becomes more apparent. In the accompanying extraction sections of the figures it can be clearly seen that the PEG column is able to separate compounds with great efficiency, but can do so only in specific narrow polarity ranges (in the case presented here it was best suited for separating the aromatic bands and thus the alkane and cyclo-alkane bands is "squashed" together, small k-values), the cyanopropylphenyl column on the other hand provides a more even separation of all the compound groups analysed (k-values bigger than three).
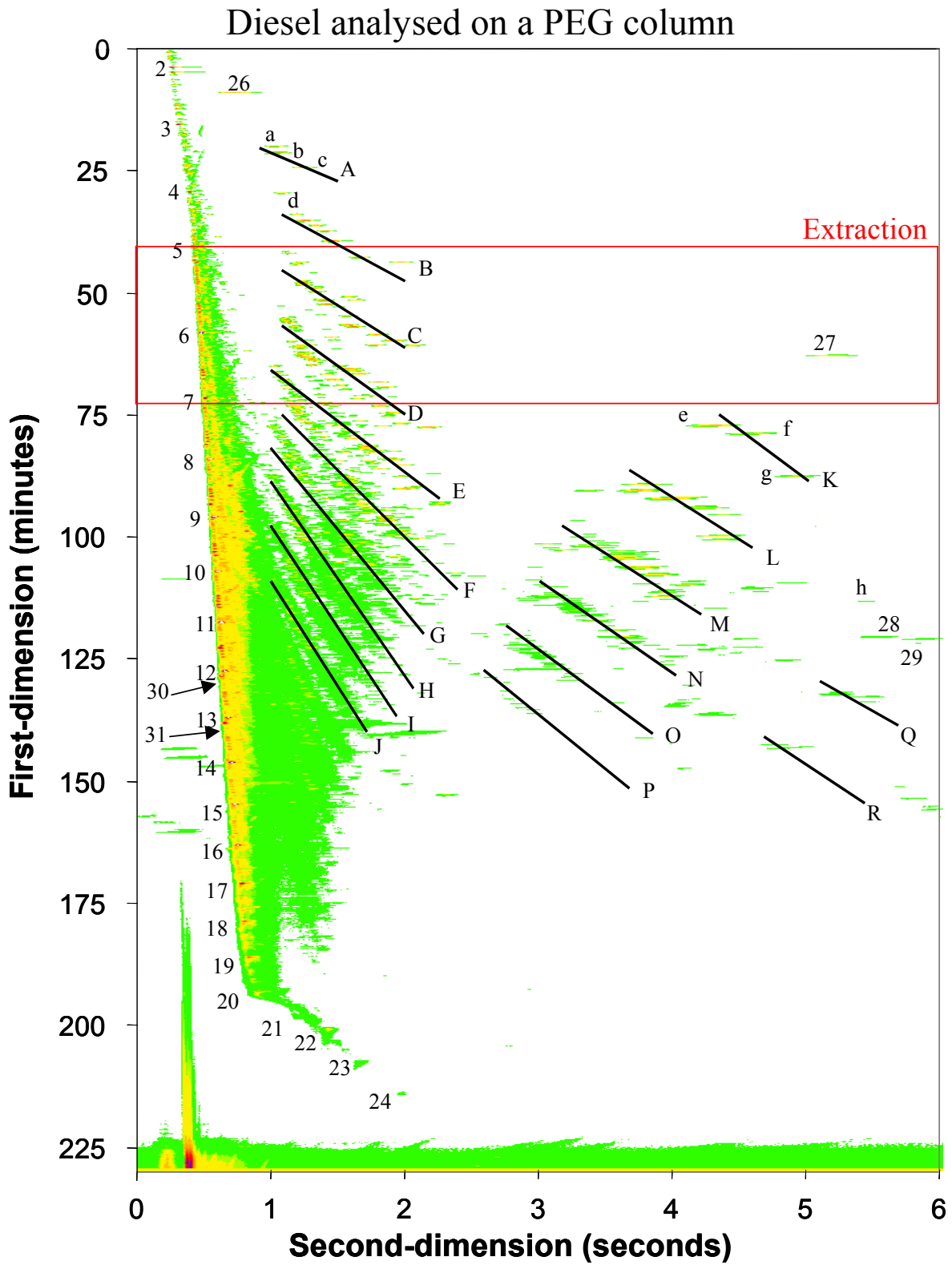
Fig. 19a The chromatogram of a diesel sample analysed on a PEG second-dimension column

Diesel analysed on a PEG column (2D extraction of Figure 19a)

Diesel analysed on a cyanopropylphenyl column
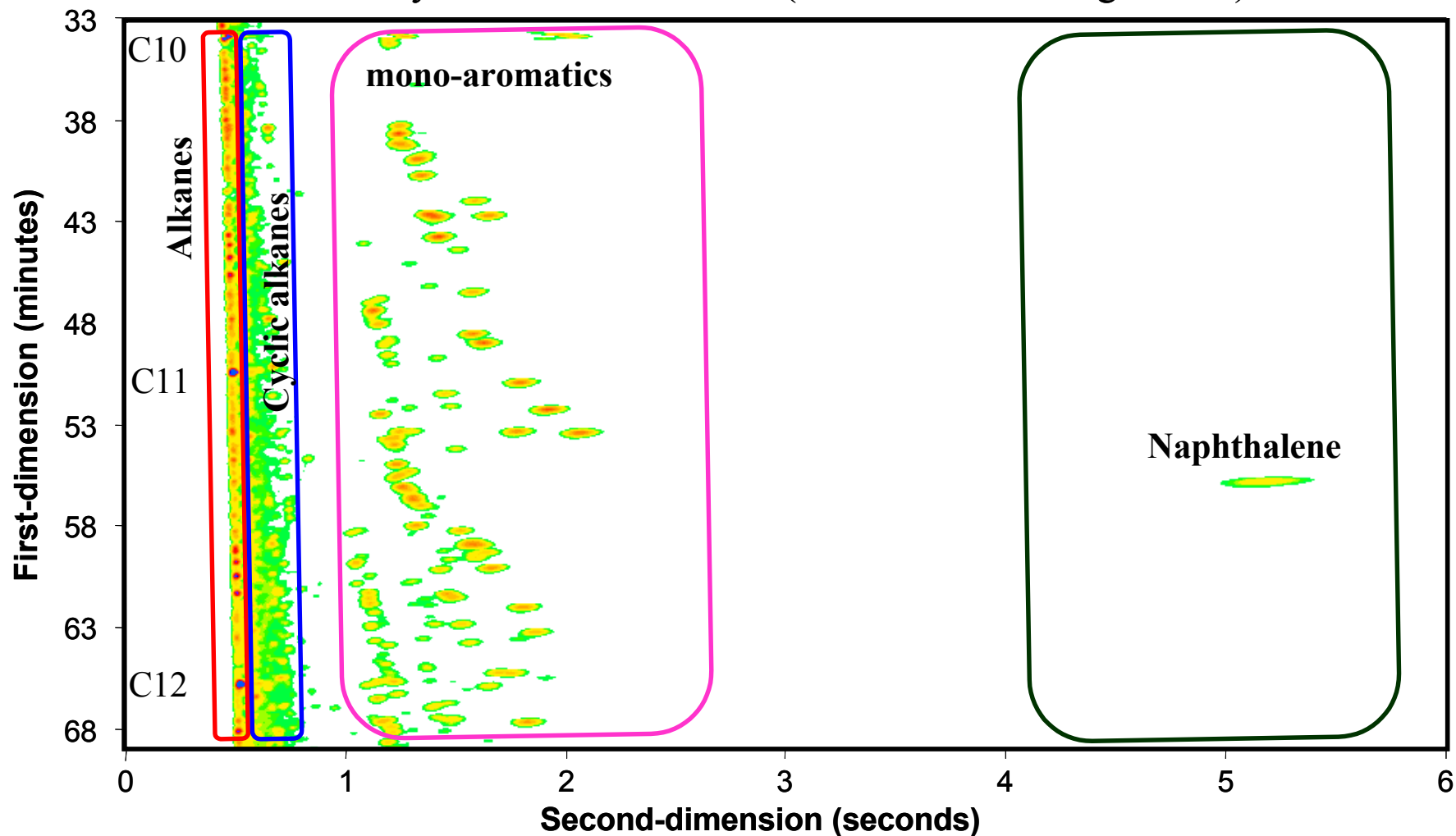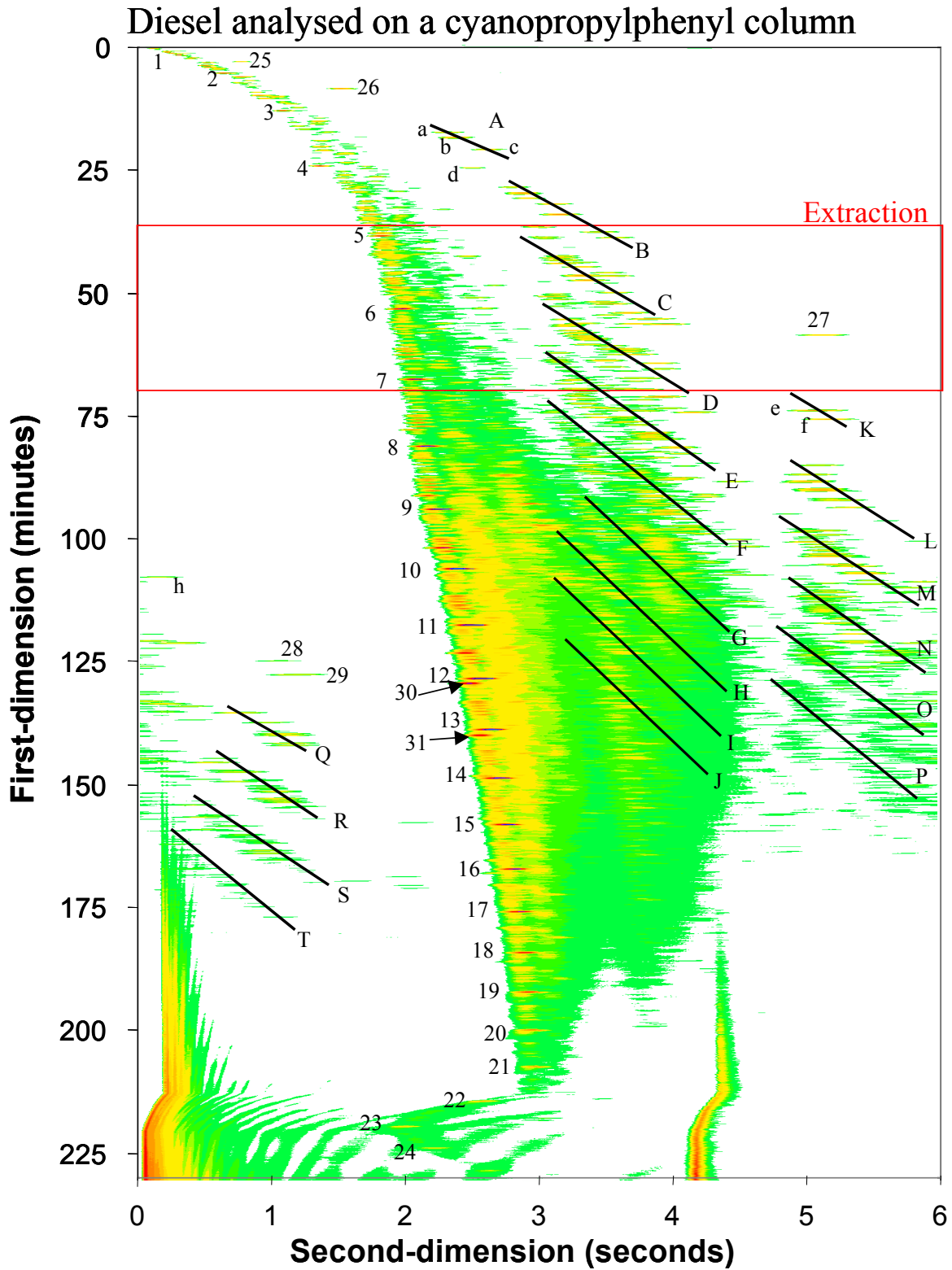
Fig. 19b The chromatogram of a diesel sample analysed on a cyanopropylphenyl second-dimension column

Diesel analysed on a cyanopropylphenyl column (2D extraction of Figure 19b)
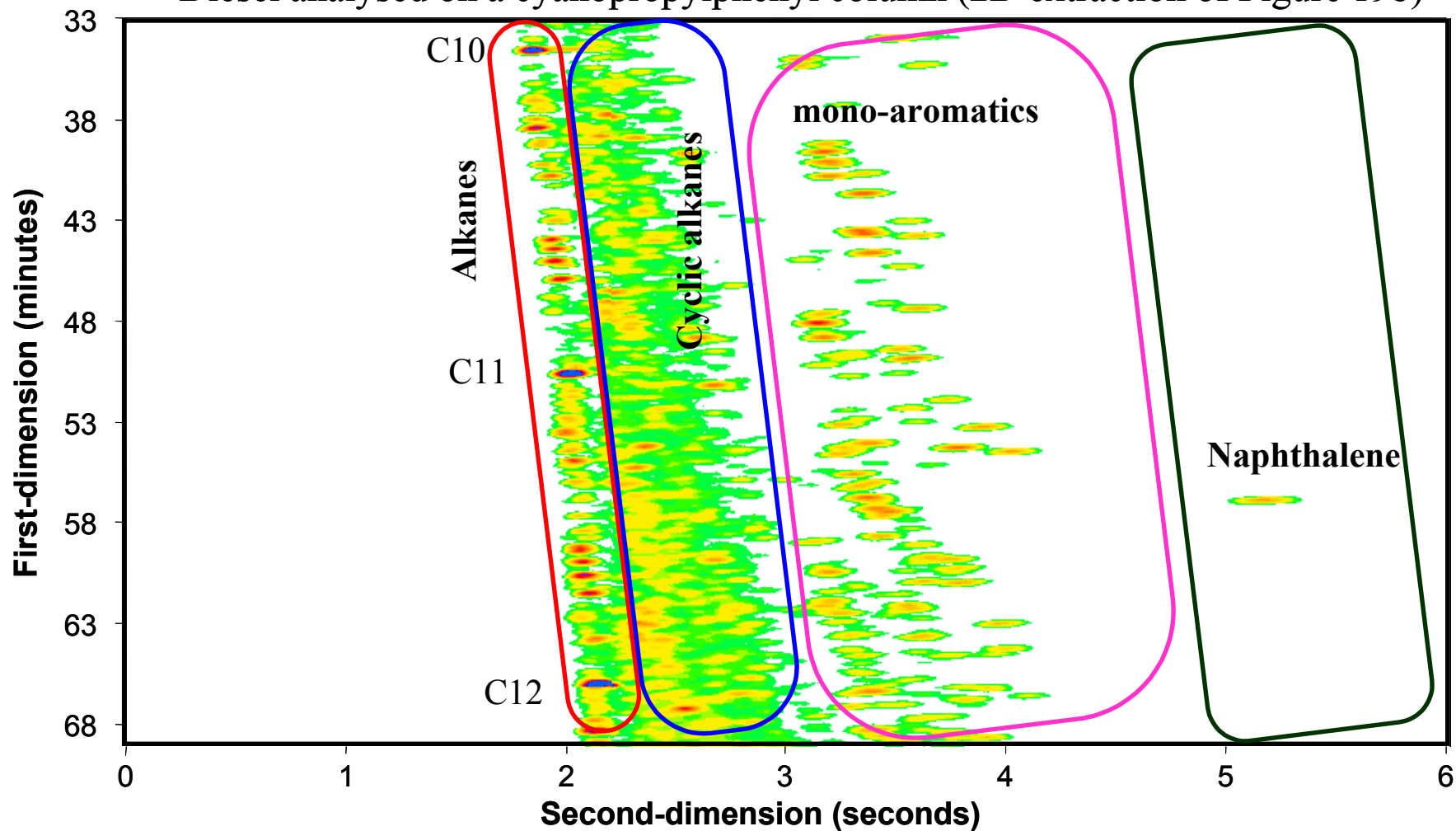
Table 8 Names of peaks used in the diesel and paraffin chromatograms [17,55]			
Individual compounds		**Some more identified compounds**	
1	n-C6	a	ethylbenzene
2	n-C7	b	meta + para-xylene
3	n-C8	c	ortho-xylene
4	n-C9	d	isopropylbenzene
5	n-C10	e	2-methylnaphthalene
6	n-C11	f	1-methylnaphthalene
7	n-C12	g	bi-phenyl
8	n-C13	h	fluorene
9	n-C14		
10	n-C15		
11	n-C16		
12	n-C17		
13	n-C18	**Grouped compounds**	
14	n-C19	A	$(CH_x)_2$-benzene
15	n-C20	B	$(CH_x)_3$-benzene
16	n-C21	C	$(CH_x)_4$-benzene
17	n-C22	D	$(CH_x)_5$-benzene
18	n-C23	E	$(CH_x)_6$-benzene
19	n-C24	F	$(CH_x)_7$-benzene
20	n-C25	G	$(CH_x)_8$-benzene
21	n-C26	H	$(CH_x)_9$-benzene
22	n-C27	I	$(CH_x)_{10}$-benzene
23	n-C28	J	$(CH_x)_{11}$-benzene
24	n-C29	K	$(CH_x)_1$-naphthalene
25	Benzene	L	$(CH_x)_2$-naphthalene
26	Toluene	M	$(CH_x)_3$-naphthalene
27	Naphthalene	N	$(CH_x)_4$-naphthalene
28	Phenanthrene	O	$(CH_x)_5$-naphthalene
29	Anthracene	P	$(CH_x)_6$-naphthalene
30	Pristane	Q	$(CH_x)_1$-anthracene or $(CH_x)_1$-phenanthrene
31	Phytane	R	$(CH_x)_2$-anthracene or $(CH_x)_2$-phenanthrene
		S	$(CH_x)_3$-anthracene or $(CH_x)_3$-phenanthrene
		T	$(CH_x)_4$-anthracene or $(CH_x)_4$-phenanthrene

5.3.4 Temperature difference between the columns

From section 5.3.3 it is obvious that there are specific temperatures required for fast second-dimension separation. The second-dimension is operated at a constant temperature difference with the first-dimension temperature. The temperature difference depends on the sample used, the dimensions and the stationary-phase of the second-dimension column. In our case we never changed the column dimensions. The optimisation of the temperature difference has to take the following into consideration: The modulation period (time allowed for each second-dimension chromatogram) is predefined by the temperature program of the first-dimension as this will dictate the time-width of the peaks eluting from the first-dimension. This first-dimension peak has to be analysed several times by the second-dimension column, a generally accepted number of cuts being four over each first-dimension peak. For a temperature program of 1°C/min, providing *ca.* 30 s first-dimension peak widths, the preferred second-dimension time is five to six seconds.

The temperature of the second-dimension column now needs to be adjusted in order to fully utilise this predefined time. The temperature thus needs to be low enough to allow the compounds to separate, but high enough (large enough k value, see Figure 10) not to stay in the column too long to co-elute with the next second-dimension portion ("wrap-around", figure 20). The temperature should be selected for the peaks to elute over the full separation time. For the PEG column the optimum temperature difference when analysing diesel was observed to be 30°C and that of the 17% cyanopropylphenyl - 83% dimethyl polysiloxane column to be 20°C higher than the first-dimension temperature.
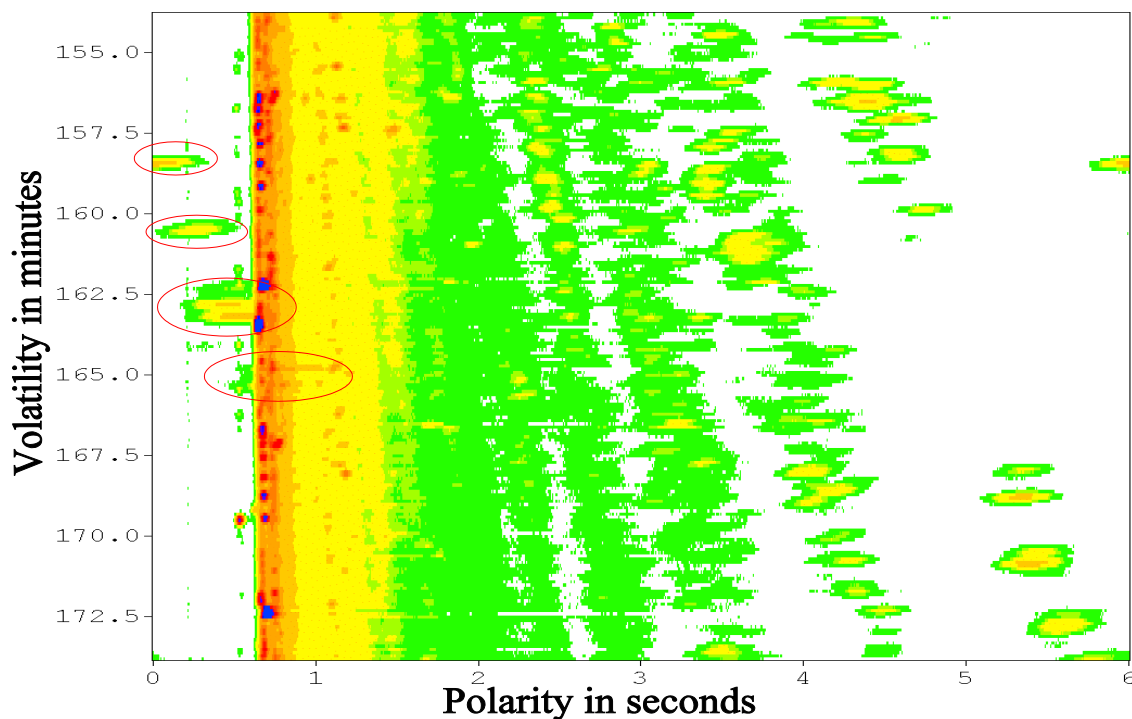
Fig. 20 The peaks circled in red on the left of the picture represent second-dimension eluents that overlap with those of the next second-dimension run. This phenomenon is called "wrap-around"

5.4   Modulator optimisation

The modulator used in this study is a prototype and great care has to be taken in the operation and maintenance thereof. The most critical factor in operating a GC x GC is for the second-dimension injections to be very sharp and reproducible. It was observed that the peak sharpness can be increased by using shorter heat pulse times, but this needs to be monitored very closely as too short

heat pulses can cause inefficient heating of the cold spots. In figure 21 the result of too short or too long heat pulses can be seen.
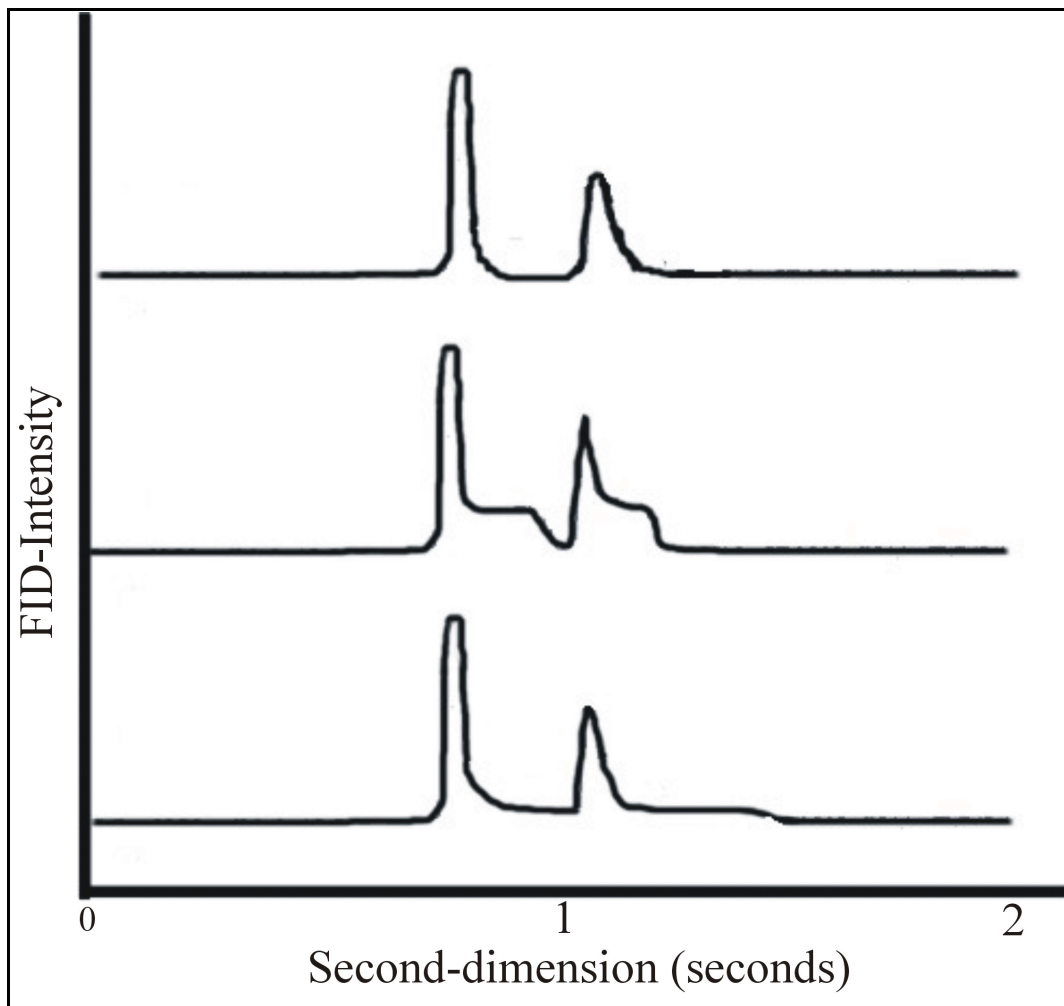
Fig. 21 Actual peak profiles obtained with adjustment of the heat pulses, top figure indicating a good peak shape (60-180 ms heat pulse), the middle one a too short heat pulse (smaller than 60 ms) and the last one a too long heat pulse (longer than 180 ms).

The step that is formed in the middle chromatogram (behind the short heat pulse peak) indicates that not all of the eluent trapped on the cold spot is injected at once and the rest is injected in a delayed step. The sharp decline at the end of the step indicates that the cold jet has been reactivated and is cooling the spot down, trapping the remainder of the eluent that should have been injected if the pulse was of sufficient heat. The too long heat pulse has a similar effect but now unmodulated eluents from the first-dimension column are starting to move through the trap due to the trap being too warm for the cold jets to trap this eluent. The sharpest peak shapes were found when using a heat pulse of between 60 ms and 180 ms. The longer pulse was chosen due to some weather related effects discussed later.

The effect of using an increased gas pulse pressure was also examined. The increase of the gas pressure in the hot jets worked effectively at oven temperatures below 150°C but at higher oven temperatures the results were negatively affected. The negative effect was due to a too short heating section in the modulator for the larger volume hot pulses and thus resulting in pulsing cooler gas onto the cold spot than required to remobilise the trapped compounds. The last portion of the hot pulse volume comes from outside the GC oven and indeed cools the trapped area too below the oven temperature.   As a modification to the modulator it would therefore be advised to use a longer heating section (e.g. an additional 0.5 m of a metal tube being inside the oven) so that a larger amount of hot gas can be blown onto the cold spot in a shorter time-frame.

During a period of high humidity in the laboratory it was noticed that the cold spots would condense water from the atmosphere and cause ice formation. The ice collecting on the column has a much higher thermal capacity, resulting in the heat pulses being inefficient in remobilising the eluents

trapped in the cold zones. This inefficiency ranged from increased peak widths to permanent trapping of eluents.   In a first attempt to reduce this effect, a lower cold flow pressure was used to just trap any first column eluents. This reduction in cold flow rates worked quite well at higher oven temperatures, but at lower temperatures it was ineffective in trapping the more volatile eluents. To utilise these reduced cold flow rates, different flow stages thus had to be defined for the cold flow. At temperatures below 10°C a cold flow of above 40 ml/min is needed, between 10°C and 40°C a flow of 30 ml/min was effective and above 40°C the cold flow can be reduced to 20 or 15 ml/min. The reduced cold flow rates helped a lot in decreasing ice formation above 40°C but at lower temperatures other precautions had to be taken: The oven was cryogenically cooled to 10°C and lower with liquid nitrogen, to reduce the water content of the oven atmosphere. As the liquid nitrogen evaporates it displaces the humid atmospheric air from the GC oven. In normal cool down mode the oven vents open for faster cooling with laboratory air, also during the initial stages of the GC run when the cold jets of the modulator start to operate and freeze out laboratory moisture on the cold spots of the modulator.   As a further precaution silica gel was placed inside the oven and the oven vents were sealed with aluminium foil to keep the moisture levels down during instrument-down periods. These precaution measures have shown great improvement in modulator operation, in that the cold spots did not freeze up under these conditions, and that even lower flows (about 10 ml/min, compared to 20 ml/min without the precautions) of cold nitrogen air were required to efficiently trap the compounds eluting from the first-dimension column. From a cost point of view, this is of course, a great improvement.

Chapter 6

Analysis of diesel petroleum fractions

Table of Contents

Chapter 6

Analysis of diesel petroleum fractions

6.1   Introduction

The analysis of petroleum products is already well established and done routinely in many laboratories. The different techniques involved in the analysis of petroleum fractions are discussed in Chapter 4. In Chapter 4 it is also mentioned that GC x GC can be used as a valuable tool in the analysis of these samples. This section of the dissertation investigates the fingerprinting of different diesel samples. The eventual use of this fingerprinting technique would be to accurately identify diesel in pollution and arson studies.

A few interesting samples of specialist diesels were also examined for their differences in lubricating efficiencies. These samples were obtained from the Mechanical Engineering Department of the University of Pretoria and contained different diesel-paraffin mixtures used in the South African mining industry.

At the time of this study, no two-dimensional automated peak integration software for GC x GC was available, therefore, the fingerprinting and sample comparisons were done on a visual interpretation

of the three-dimensional data sets.   These comparisons included overlaying the different chromatograms and trying to spot differences in composition and even some colour differences in the colour coded plots that would indicate a difference in intensity (amount).   In short, the preliminary studies were done to see whether the technique could be applied to future studies in fingerprinting and quality control.

6.2   Experimental setup

Four different diesel samples were obtained from service stations in Pretoria. All the diesel samples in this section were analysed under the same gas chromatographic conditions. A temperature program of $1°C$/min from $10°C$ to $270°C$ was used for the first-dimension, while the second-dimension was kept at a constant temperature difference of $20°C$ above that of the first. The optimum flow conditions determined in chapter 5 were used by selecting 110 kPa inlet pressure. A sample of $0.2 \mu l$ was injected under a split of 1:65 at an inlet temperature of $250°C$. The FID was run at $300°C$ with air to hydrogen to nitrogen ratio of 400:50:50. A modulation period of six seconds was selected, based on the optimisation studies. The column set used was a HP-1 column for the first dimension and a RTX-1701 column for the second, the dimensions were the same as in the optimisation studies.

6.3   The analysis of four local diesels

Four local diesel samples were analysed to detect any compositional differences. Three of these diesels have their origin form crude oil and the fourth is a diesel synthesised by the Fischer-Tropsch process from coal. These four diesels show clear differences, even in their one-dimensional analysis (reconstructed from GC x GC data, see section 5.2.2.3 Data analysis and visualisation), as can be seen in the figure 22.
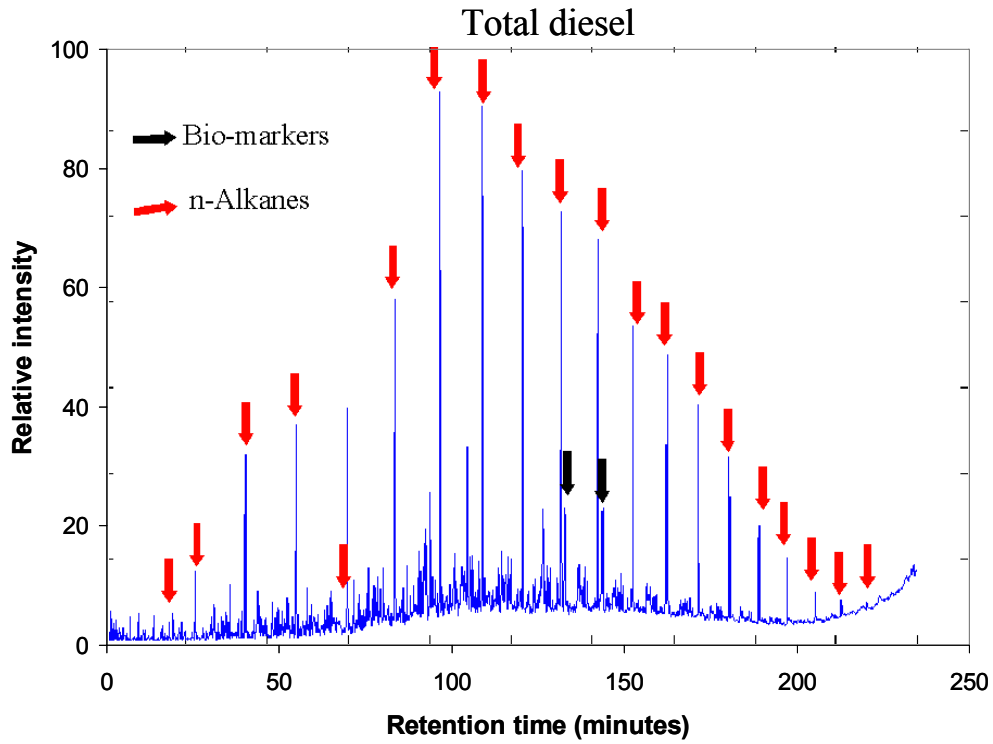
Fig. 22a A one-dimensional chromatogram of a Total diesel sample
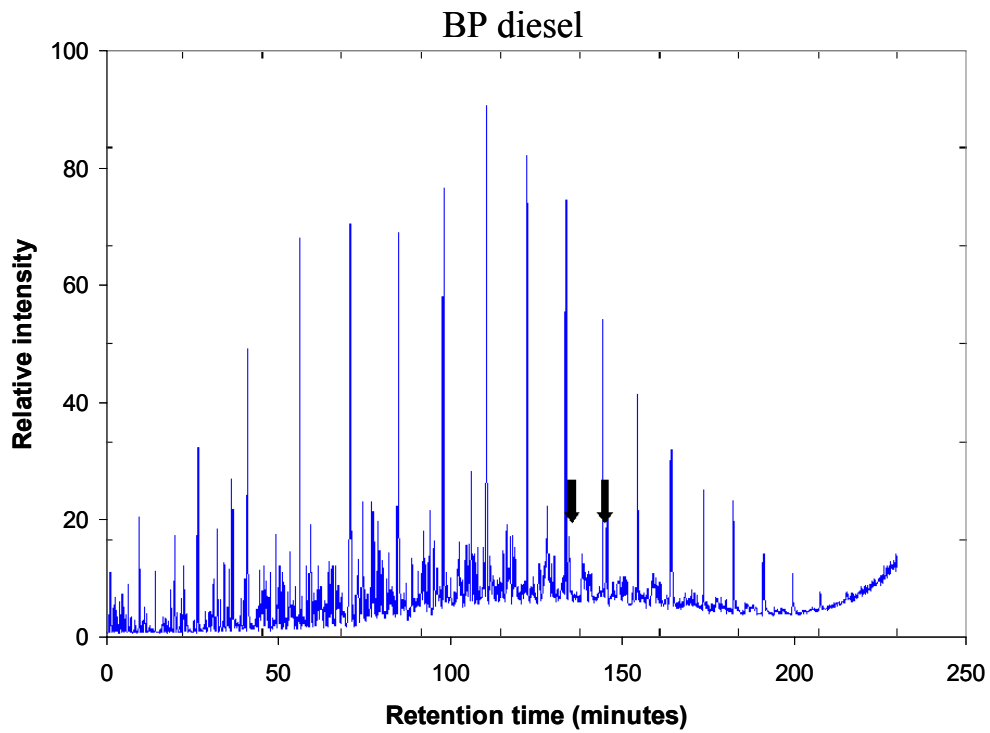
Fig. 22b A one-dimensional chromatogram of a BP diesel Sample
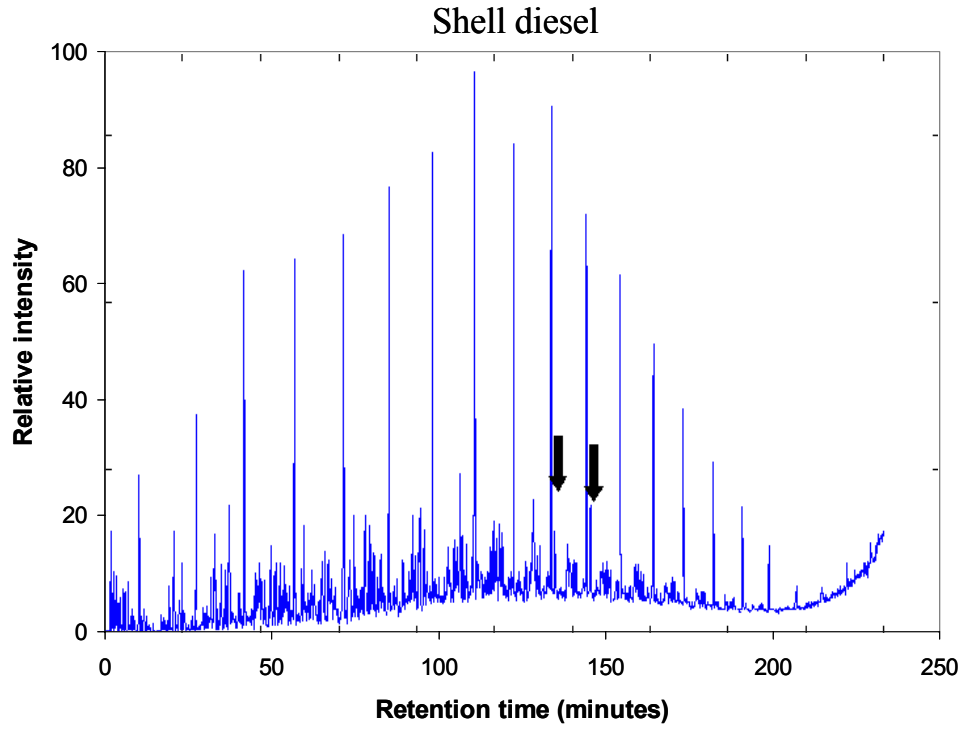
Fig. 22c A one-dimensional chromatogram of a Shell diesel sample
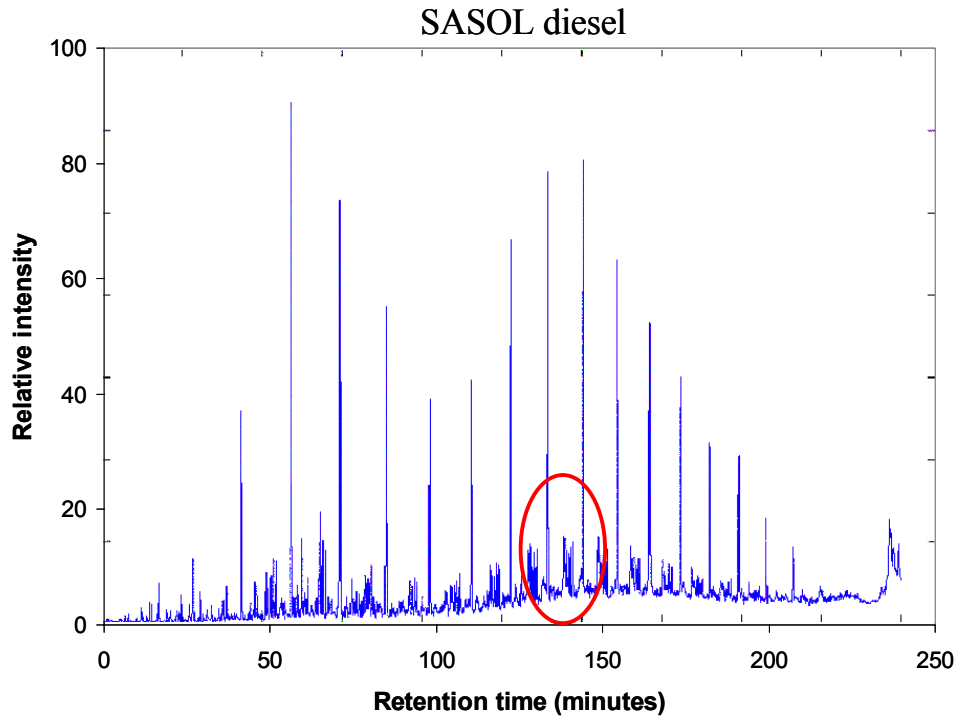
Fig. 22d A one-dimensional chromatogram of a SASOL diesel sample

The differences in these chromatograms are quite obvious: The n-alkane pattern (the peaks standing out from the rest, marked in figure 22a with red arrows). The biomarkers (marked in black arrows, figure 22a-d) can only be observed in the oil refined diesels but not in the diesel from the Fischer Tropsch process (area circled with red). There are also some differences in the smaller compounds seen between the n-alkanes, but no detailed information can be extracted due to peak overlap.

When analysing the data in two dimensions these differences are much more visible. Peaks invisible in the one-dimensional GC due to overlap can now often be allocated to specific compound groups or, in some cases, uniquely identified. In figures 23a - 23d the differences in the composition can be seen more clearly. See also Table 8 for compound identification.
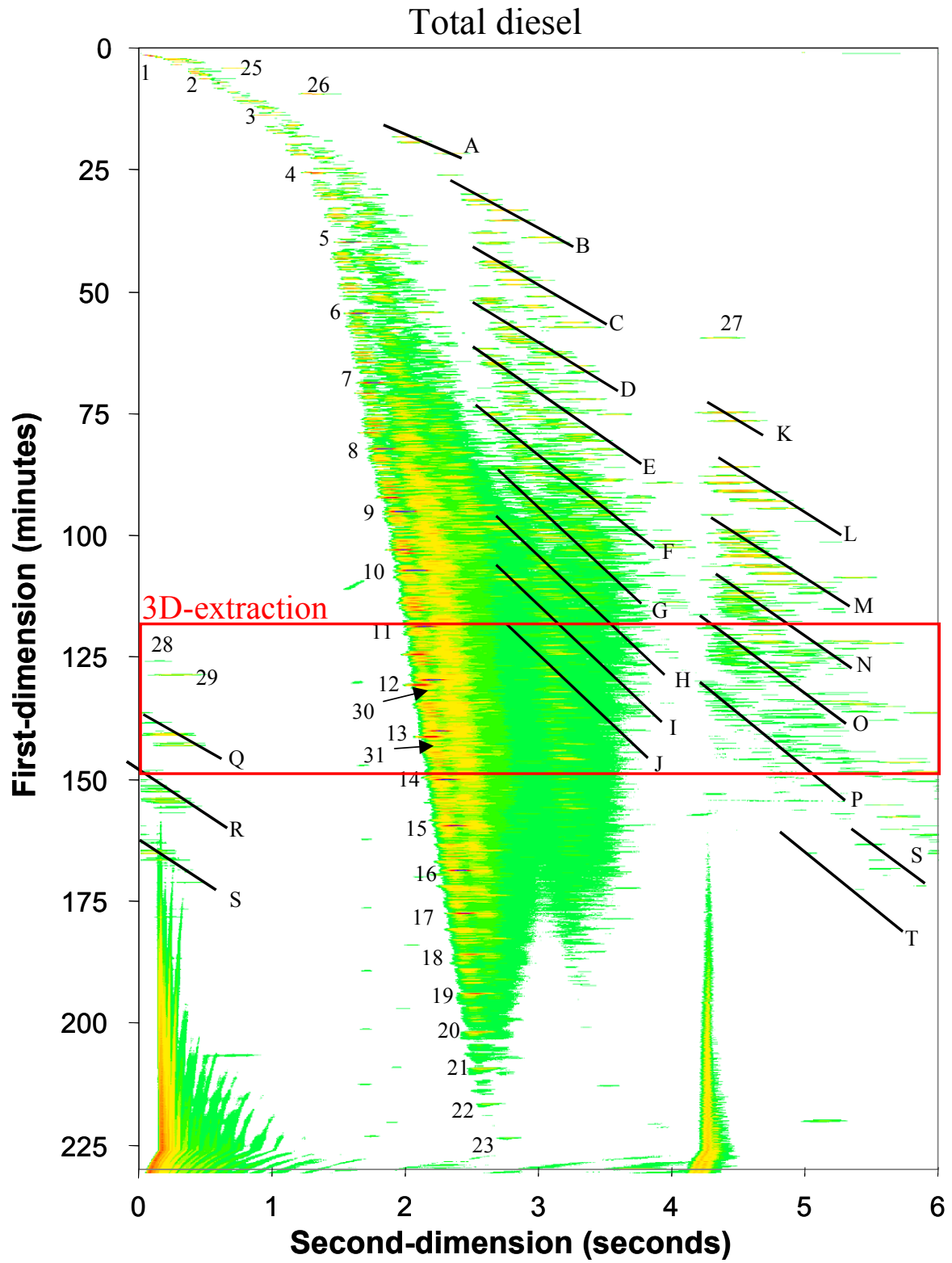
Fig. 23a A two-dimensional chromatogram of a Total diesel sample

Total diesel (3D extraction of Figure 23a)
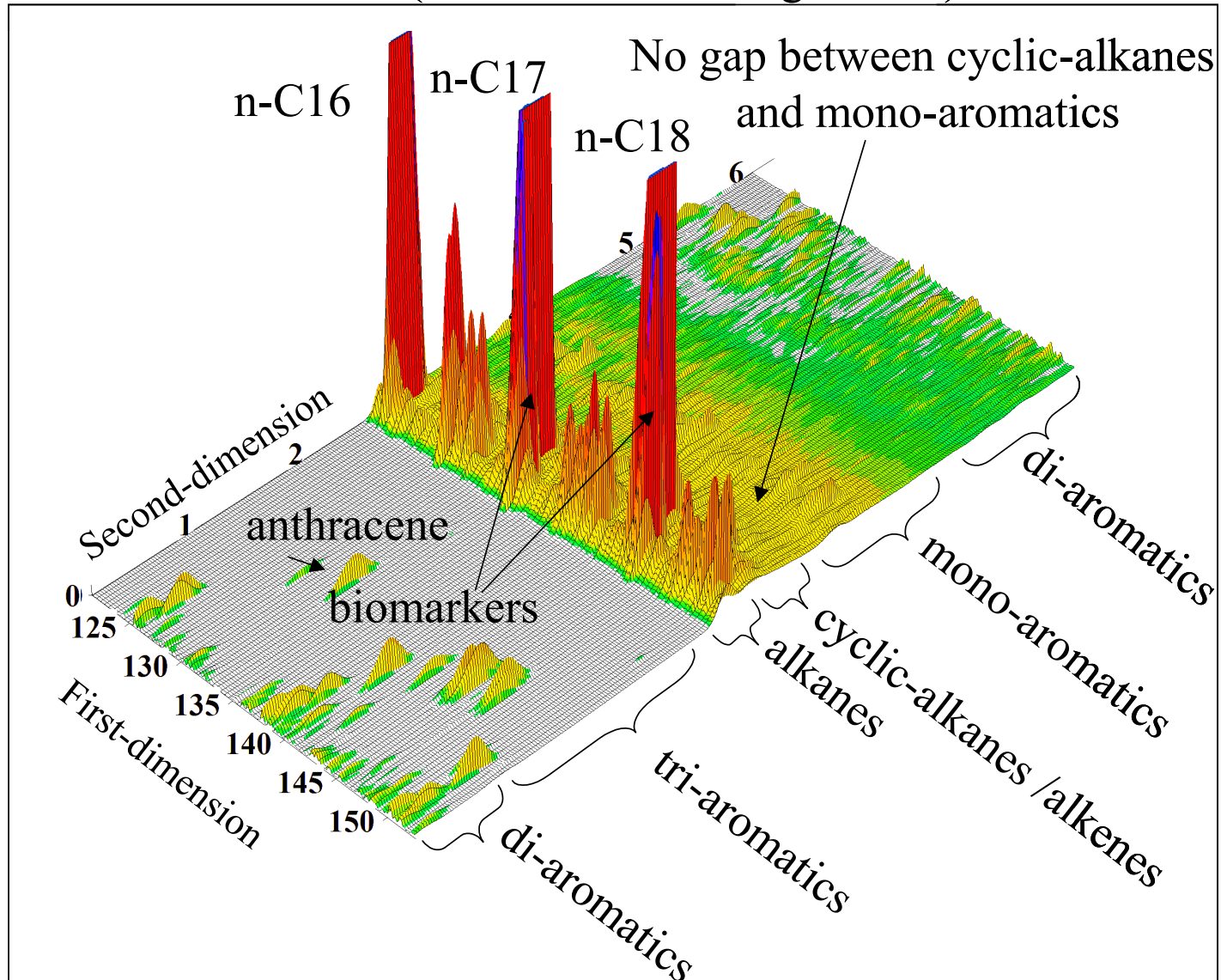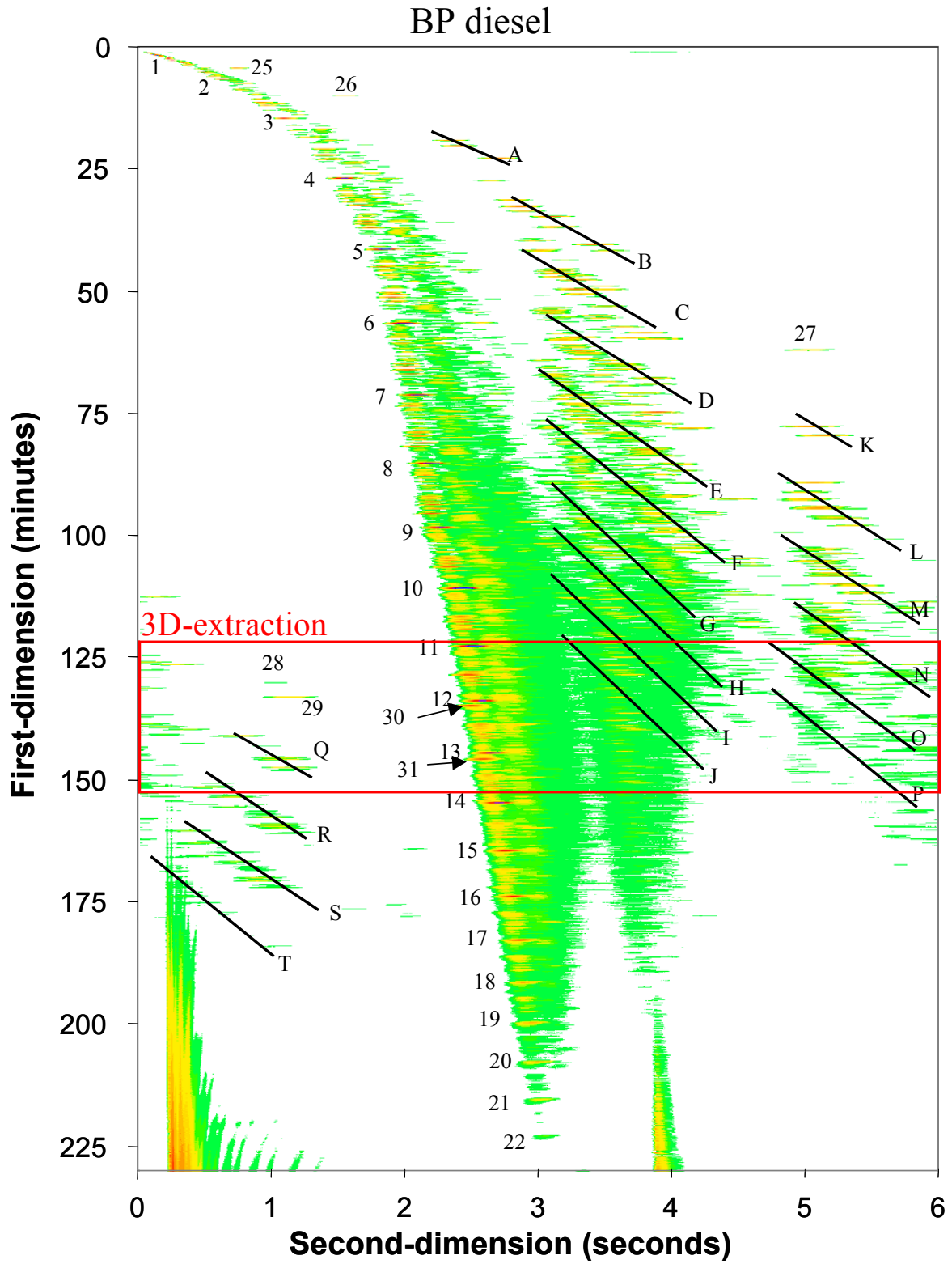
Fig. 23b A two-dimensional chromatogram of a BP diesel sample

BP diesel (3D extraction of Figure 23b)
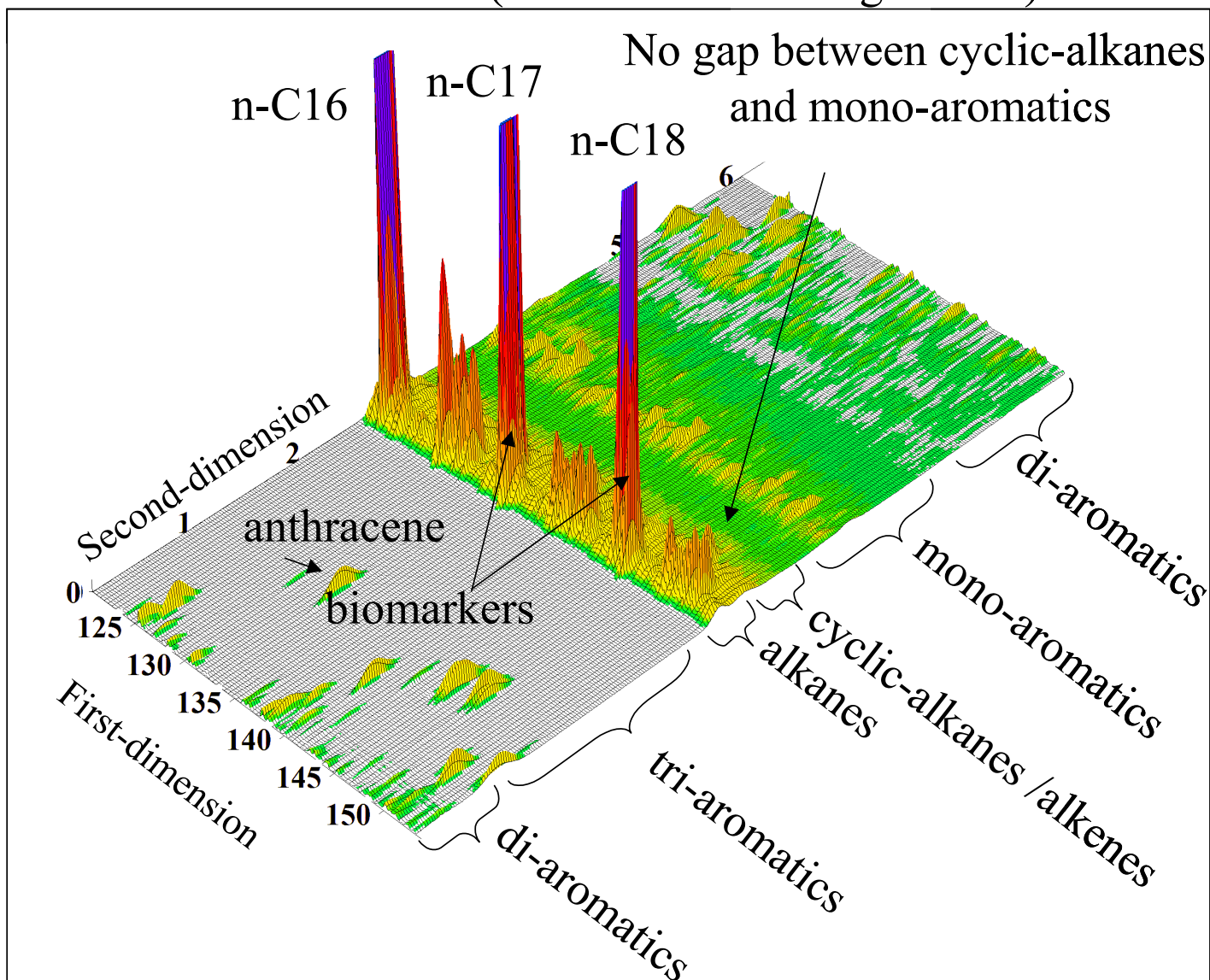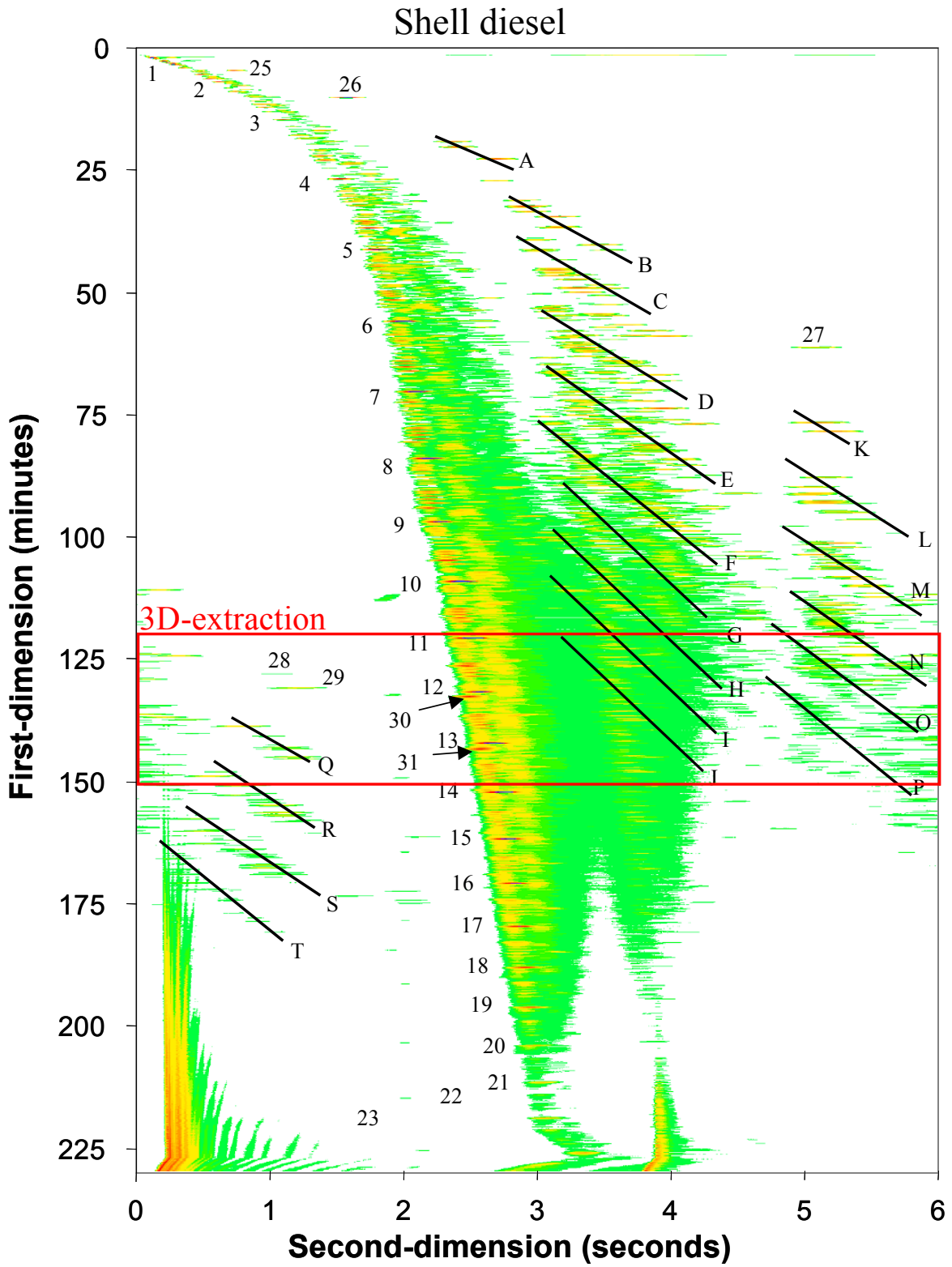
Fig. 23c A two-dimensional chromatogram of a Shell diesel sample

Shell diesel (3D extraction of Figure 23c)
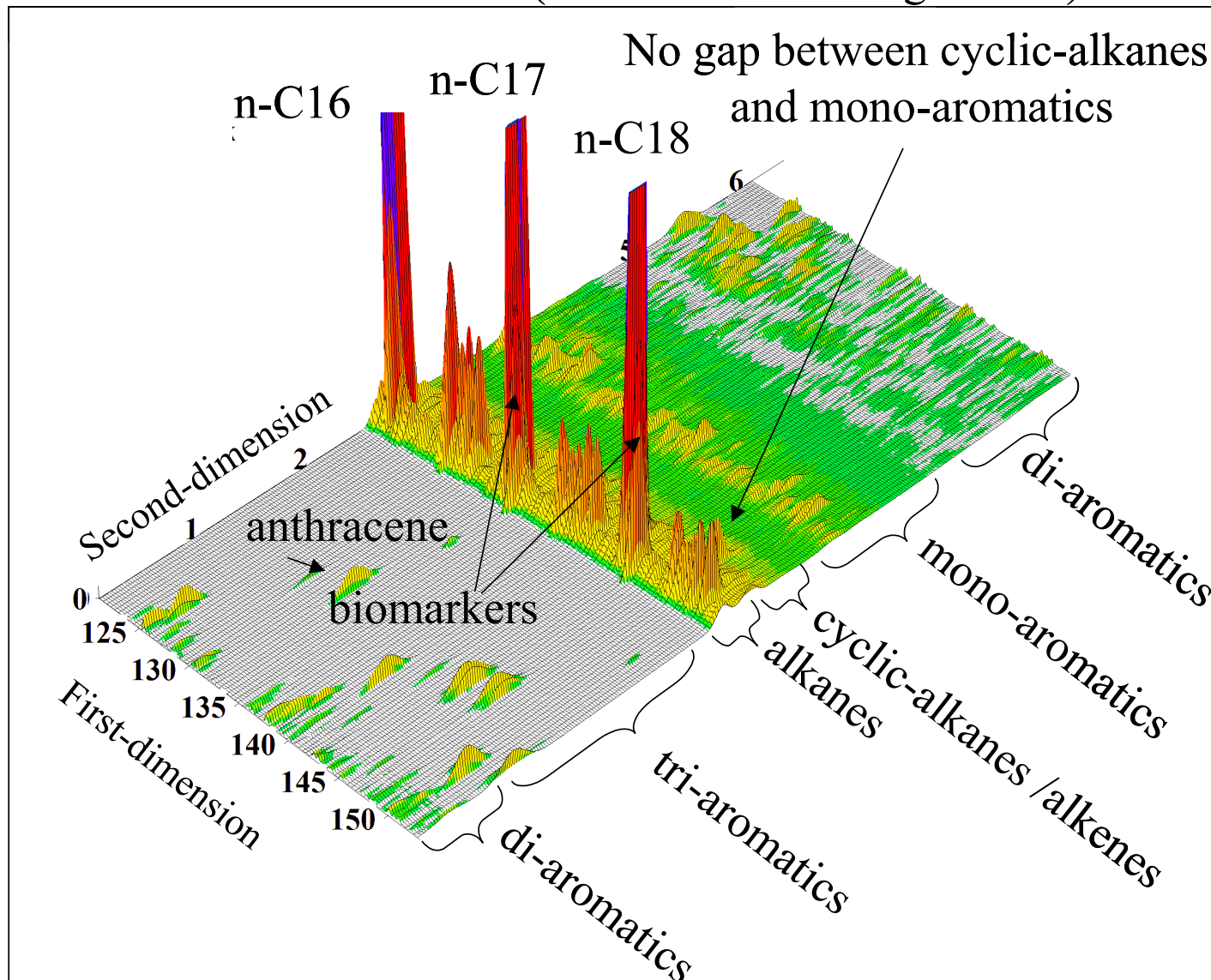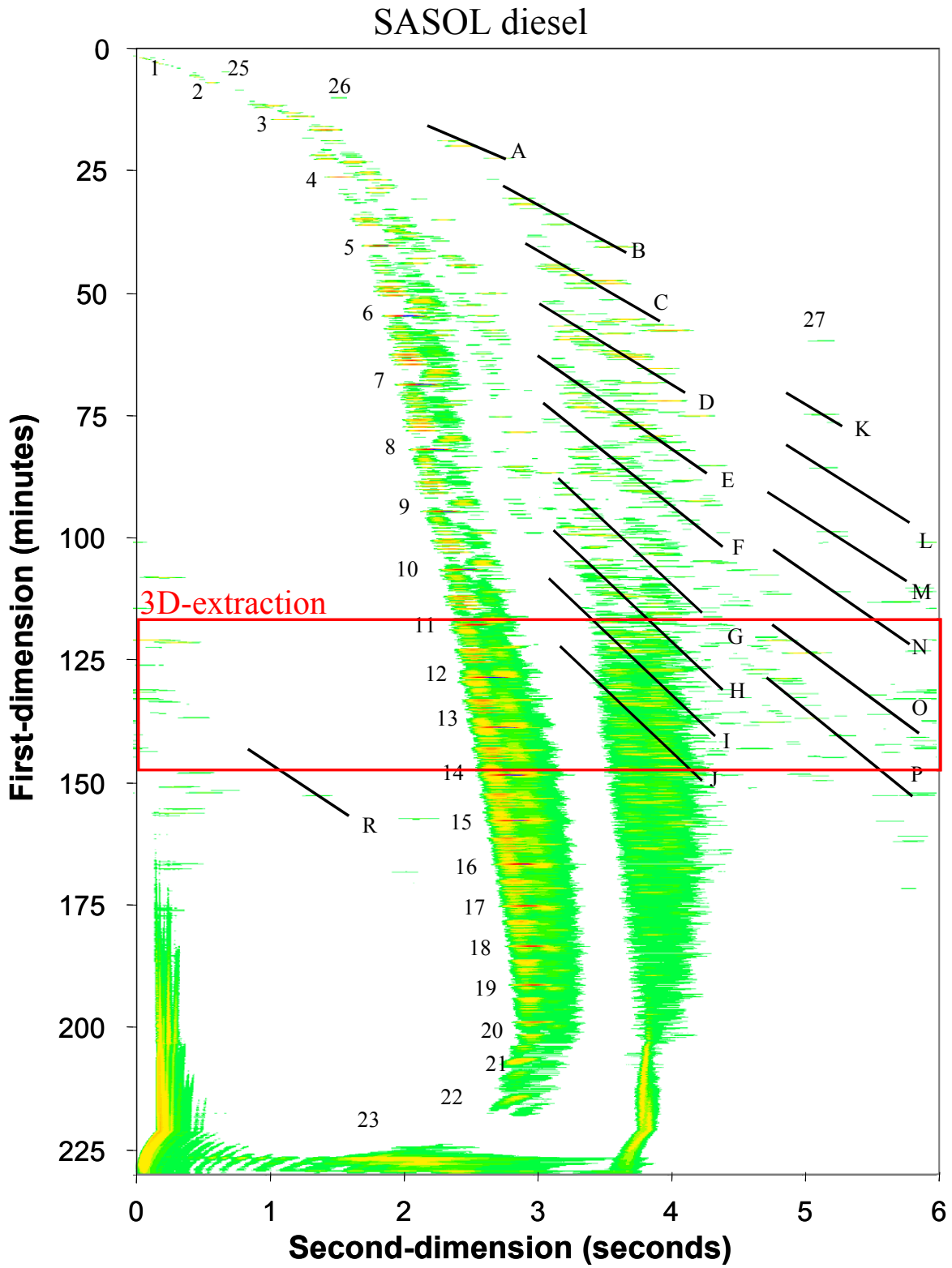
Fig. 23d A two-dimensional chromatogram of a SASOL diesel sample

SASOL diesel (3D extraction of Figure 23d)
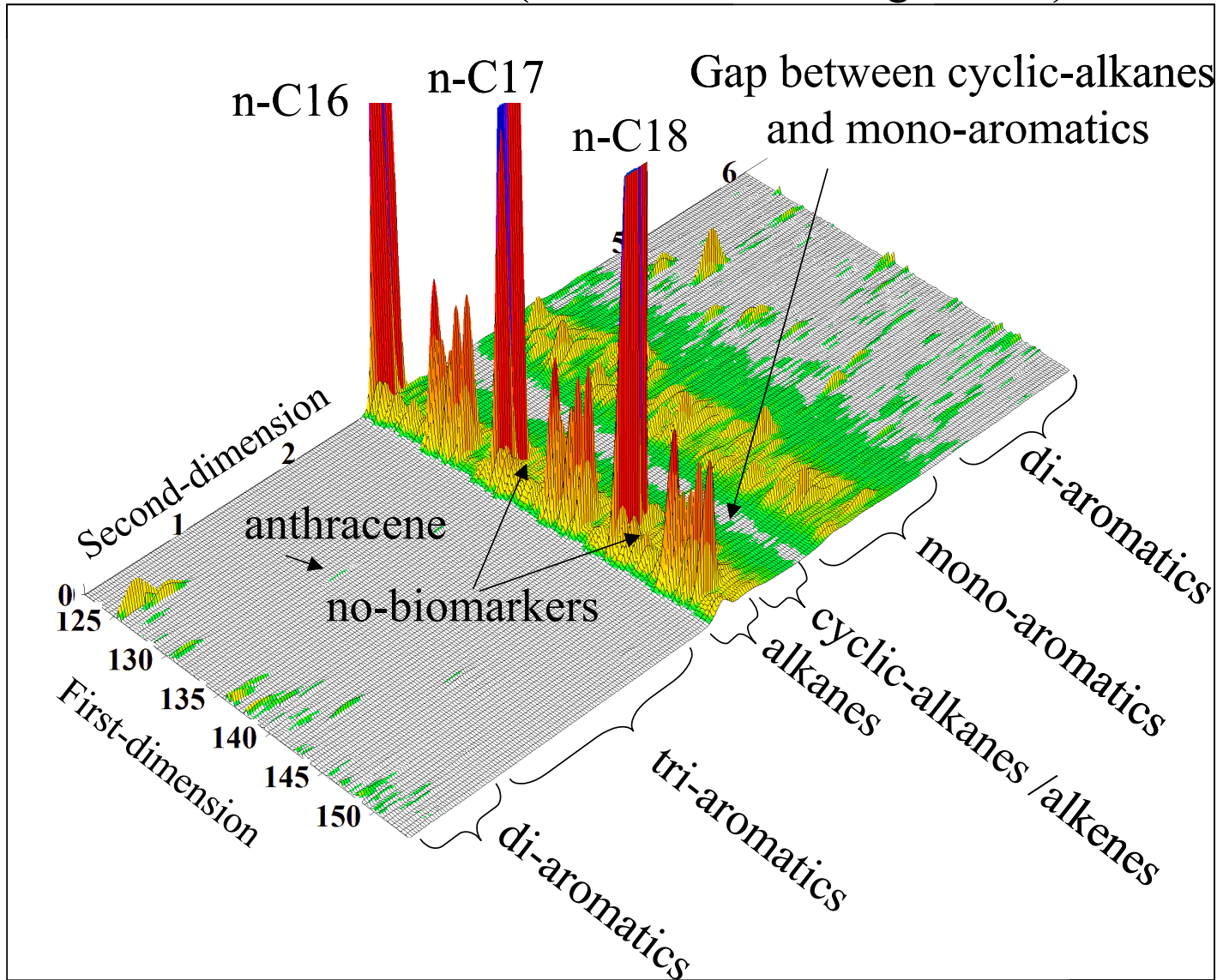
The two-dimensional chromatograms of the four diesel samples can readily be differentiated. The most obvious of these differences is in the group separations (aliphatic, mono-aromatic, di-aromatic and tri-aromatic), the Total and Shell diesel shows almost no separation between the aliphatic and mono-aromatic components while in the BP diesel there is a slight separation and in the SASOL diesel baseline separation is observed. This is due to the relative concentrations of the cyclic-alkanes (or alkenes) and the mono-aromatic compounds.   Differences in case of individual compounds are also found, this can be seen as peaks differing in colour in the respective chromatograms. Another obvious difference is that the tri-aromatics (groups Q,R,S and T) and biomarkers (peaks 30 and 31) in the SASOL diesel are almost absent. To be more specific on the concentration variances software for quantitative analysis (peak integration) is required.

6.4   Analysis of diesel-paraffin mixtures

The use of these mixtures in the mining industry arises from their much cleaner exhaust emissions. This is essential in a closed environment where the air needs to be circulated and remain safe for human activity. These specially blended fuels for the mining vehicles perform exactly the same as the diesels used on the surface but because of their lower aromatic content, their lubricating properties are greatly reduced. In diesel engines the lubrication of the fuel is a very important factor and can have a big influence on the lifespan and functionality of the engine.

As was seen in the previous section, a huge portion of the diesel constitutes aromatic compounds.

In the Fischer-Tropsch diesel it was mentioned that there is a clear gap between the cyclic-alkanes and the mono-aromatic part of the chromatogram, due to a lower concentration of the latter group. The Fischer Tropsch process is known to produce diesel fractions of lower aromatic content. Low aromatic content diesels provide less lubrication and are known to produce cleaner exhaust emissions.

The same applies to the paraffin in that it has a high cetane number but almost no aromatics to improve the lubrication. A further advantage of the paraffin is improved combustion of its shorter alkane chain lengths, hence the cleaner exhaust gasses. To improve the lubricating properties of the paraffin, aromatic compounds or a well lubricating diesel can be added. The study undertaken by the Department of Mechanical Engineering at the University of Pretoria was focussed at determining the fraction of diesel to give the optimal lubricating properties [56].

As the project of determining the best diesel-paraffin mixture was still in progress at the end of this study, we can only speculate on the significance of our analytical results. Figure 24 shows the different diesel-paraffin combinations evaluated. In the respective chromatograms, changes can be seen in the alkane boiling point range (with the addition of diesel to the paraffin there are more alkanes of higher mass). Also, as expected, the polar fraction of the paraffin increases with the addition of diesel, to such an extend that, in the 66% diesel sample, even some tri-aromatics (group Q the $(CH_x)_1$-anthracene and peak 29 anthracene) are observed. The mono-aromatics and di-aromatics also increase with diesel concentration. In the pure paraffin sample, only the mono-aromatics up to group G and di-aromatics up to group L can be observed. In the 66% paraffin

mixture, group M of the di-aromatics appears and group I of the mono-aromatics appears in the 50%
paraffin sample. The 66% diesel mixture almost contains all the groups also observed in the pure
diesel sample apart from groups J and N. Interesting phenomena, like why the 66% paraffin mixture
has better lubricating properties than pure diesel, [56] cannot be explained at this stage.
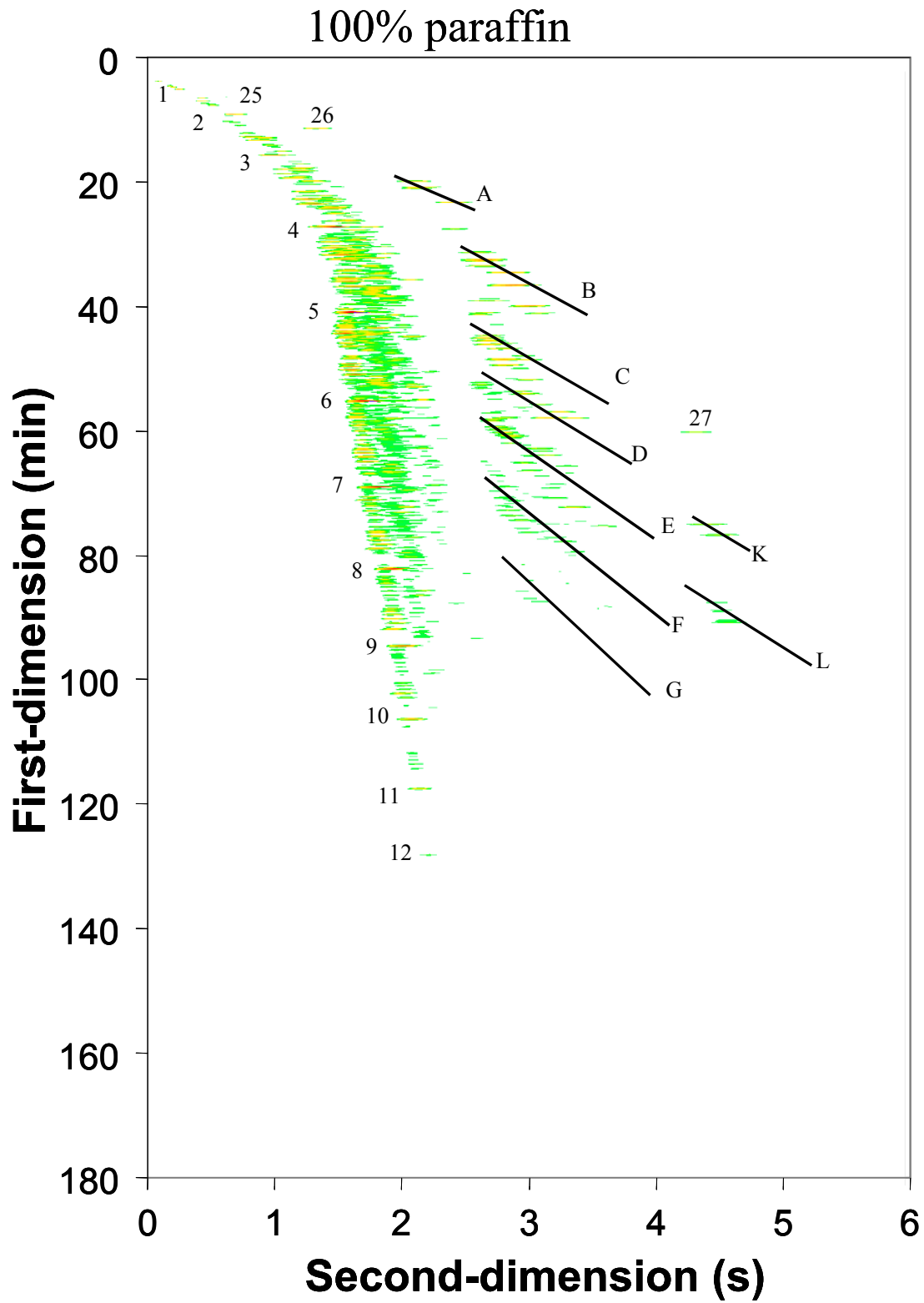
Fig. 24a A two-dimensional chromatogram of a 100% paraffin sample
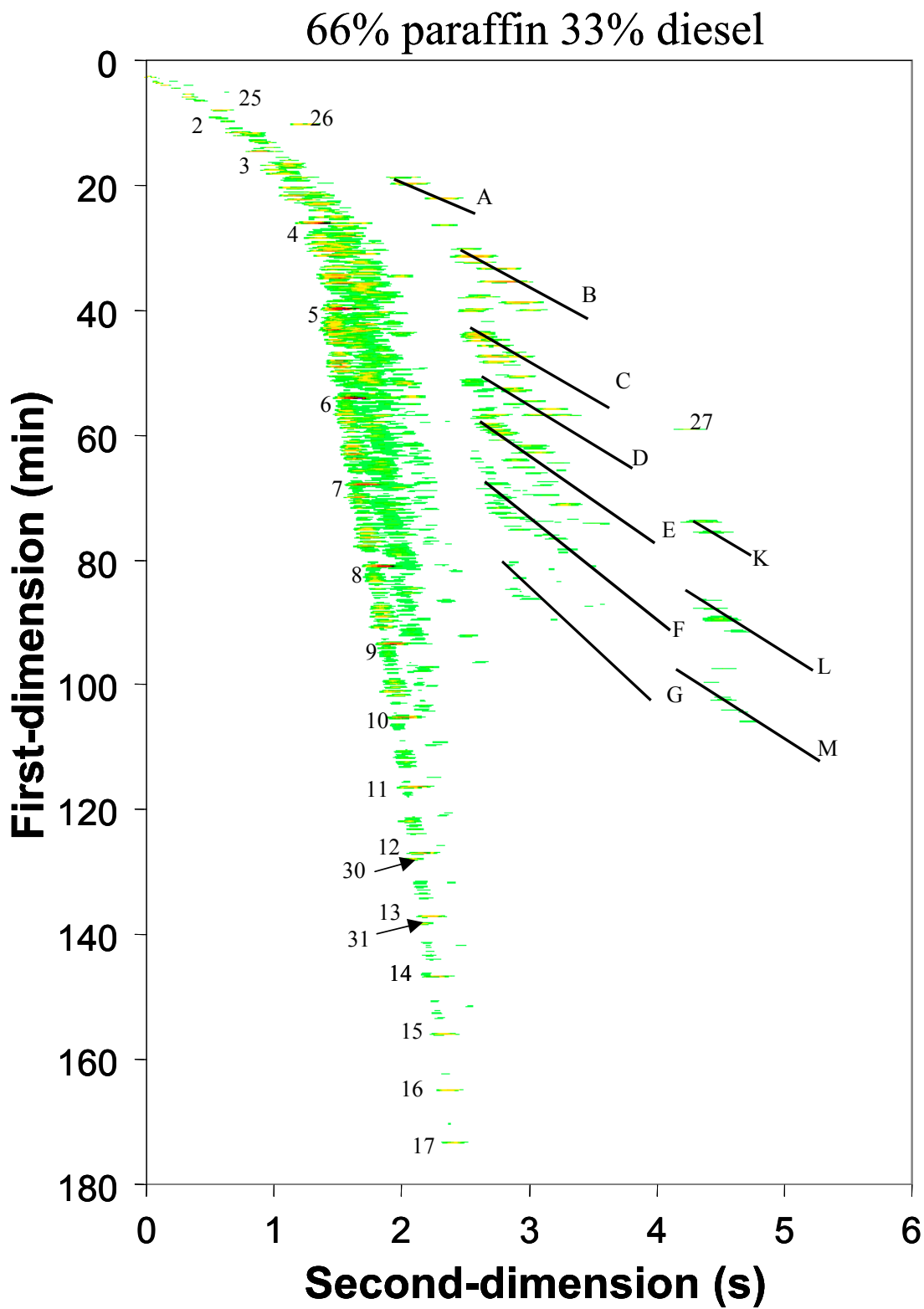
Fig. 24b A two-dimensional chromatogram of a 33% diesel 66% paraffin mixture
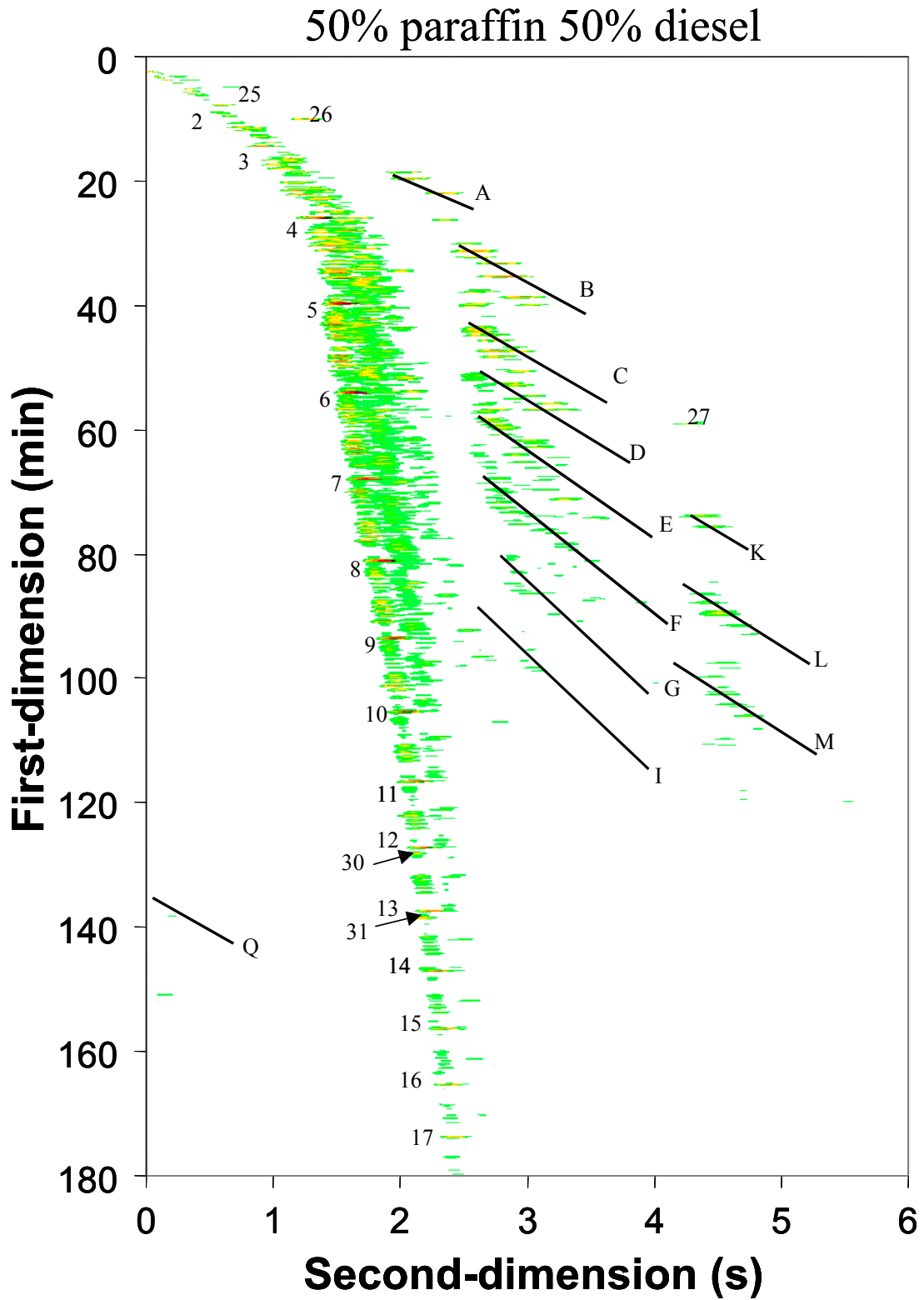
Fig. 24c A two-dimensional chromatogram of a 1:1 diesel : paraffin mixture
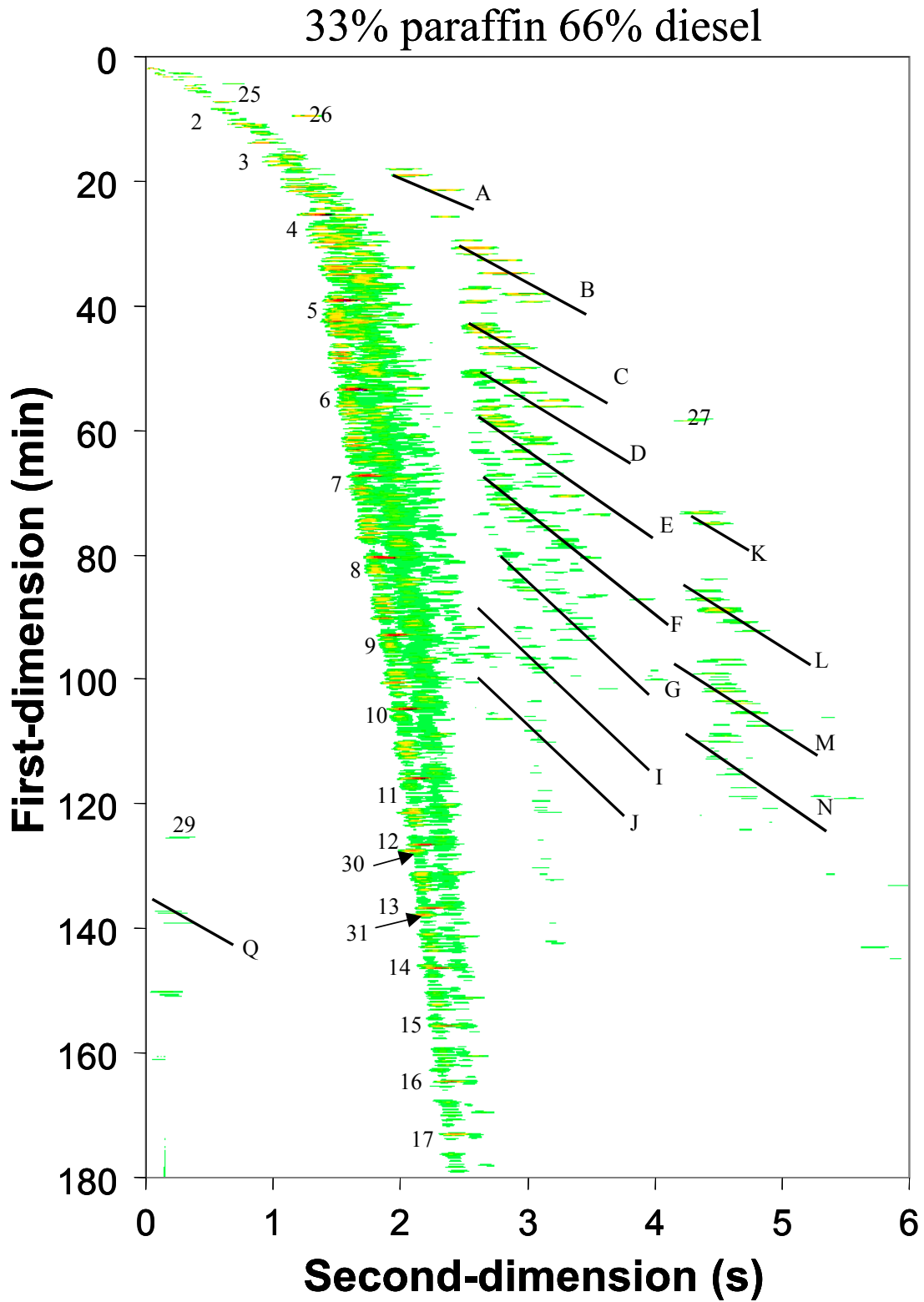
Fig. 24d A two-dimensional chromatogram of a 66% diesel 33% paraffin mixture
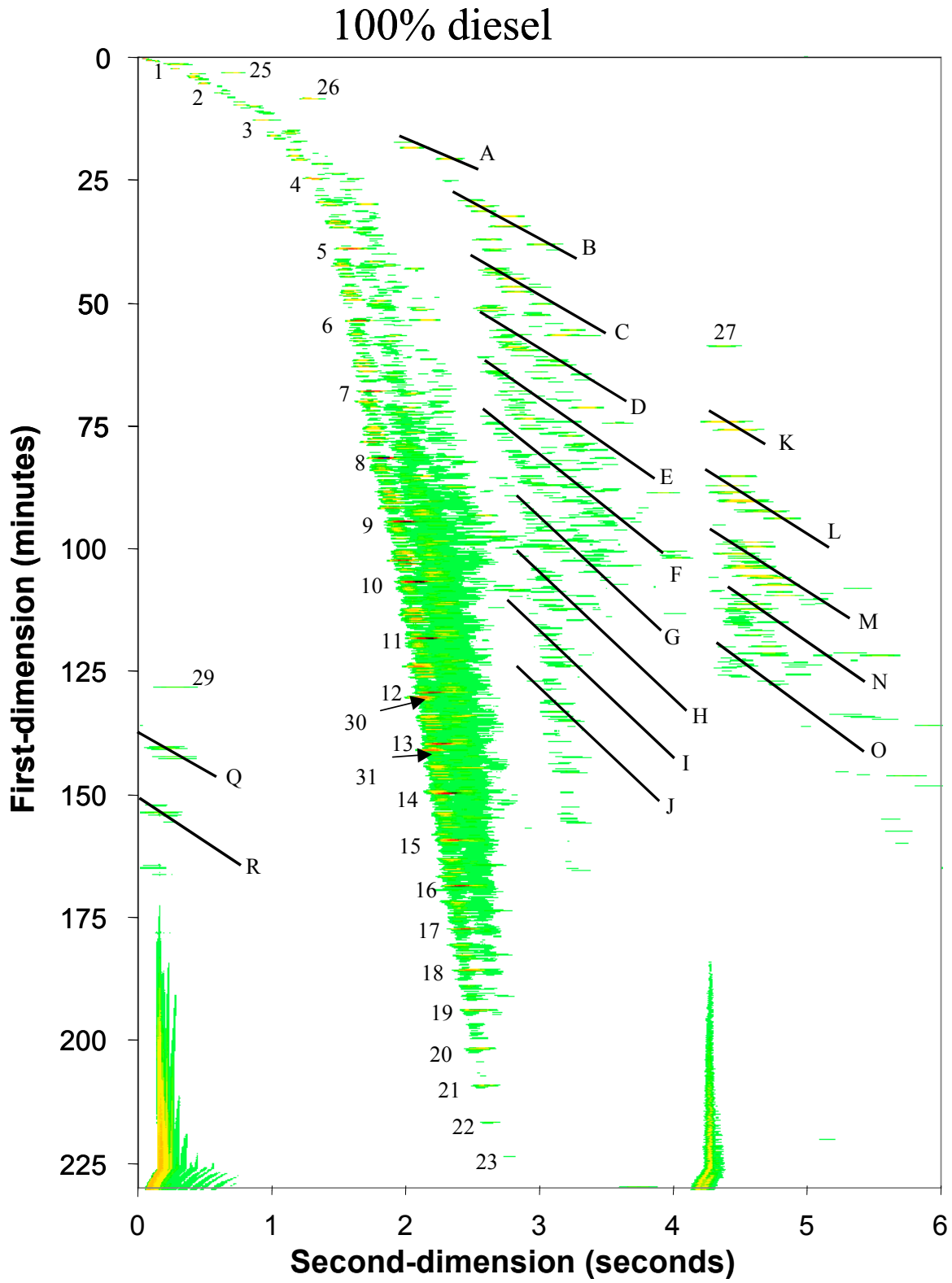
Fig. 24e A two-dimensional chromatogram of a 100% diesel sample

Chapter 7

Conclusion

Table of Contents

Chapter 7

Conclusion

In this dissertation the goal was to provide a guideline for the optimisation of GC x GC, as such a process is a lot more complex than in normal gas chromatography.   The path taken to achieve this optimisation led to some interesting and previously ignored parameters that play an integral role in GC x GC performance.   These included the careless use of "rules of thumb" to optimise linear flow rates, and the blind use of calculations done by the instrumentation without knowing the margin of error or even the formulas used!

It was also shown that GC x GC can be applied very successfully to the analysis of complex mixtures such as diesel petroleum samples. The applications on the diesel samples range from fingerprinting to quantitative component analysis.

7.1   Optimisation of GC x GC

The optimisation of a comprehensively coupled system proved to be a very complex procedure. Frequently used "rules of thumb" for the optimisation of one-dimensional systems involve approximations that are not obvious without careful understanding of the original optimisation studies. The one common error is to use the generally specified linear flow rate for a given column

inner diameter and the type of carrier gas. The optimum linear flow rate specified for columns with different diameters is good under conventional, atmospheric pressure conditions but is based on carrier gas diffusitivities at atmospheric pressures, that do not apply directly to high pressure exit conditions. In GC x GC such high pressures exist since the second dimension acts as a restrictor at the end of the first column and diffusion in the carrier gas slows down appreciably. This change in the diffusion can be clearly seen in the calculations done to determine the optimum linear flow rates. The van Deemter optimum flow rate for hydrogen in a 0.25 mm internal diameter column is in the order of 40 to 50 cm/s, but we determined that our system has a optimum linear flow rate in its first dimension of 30 to 35 cm/s which corresponds almost to the optimum linear flow rate of helium under normal pressure conditions in a similar column. As a synergistic effect, the lower values of the first-dimension linear flow rate also result in much improved second-dimension resolution due to the lower linear flow rates and a yet unknown modulator effect.

When the second-dimension Van Deemter plot was drawn, some interesting results were obtained. It has to be reminded that a Van Deemter plot of this dimension is reasonable since it is run under isothermal conditions. The last part of the curve usually dictated by the Cu-term was a lot steeper than plots of similar column dimensions. This is believed to be the result of a less than perfect modulator that gives a measurable contribution to the second-dimension bandwidth.

The simplified flow optimisation method is justified and is recommended for any similar optimisation studies where different columns need to be coupled in GC x GC (verified for test compounds with second-dimension retention factors larger than three (k > 3)). The final suggested

inlet pressure (110 kPa) which is slightly too low for the first-dimensions and sightly too high for the second gives extremely improved results to the chromatogram (Figure 18) where a "rule of thumb" linear flow rate (of 50 cm/s) is used. The use of a wide boiling point range test sample such as diesel seems justified, as the modulator optimisation is strongly dependent on column oven temperatures and therefore needs to be checked over a wide temperature range.

In summary we propose an overall GC x GC optimisation guideline that involves the following steps:

1   Column selection (first- and second-dimension column length, internal diameter and film thickness) and carrier gas selection.

2   Stationary phase selection (based on dimensionality considerations, the polarity range of the compounds within the sample and thermal stability of the column phases)

3   Temperature programming of the first-dimension and modulation period chosen to maintain the recommended four second-dimension analyses per first-dimension peak. These parameters would depend on the type of analysis and consideration of the speed *vs* resolution trade-off.

4   Versification of modulator performance over the full column temperature range anticipated for the sample.

5   Flow optimisation (resolution calculations of selected test compounds at different system inlet pressures). This is required once a new set of column dimensions is selected, that has not previously been optimised with the selected carrier gas. Test compounds should be well retained in both dimensions (k >3). A new set of column dimensions should be selected, if

the first- and the second-dimension linear flow optima are not compatible.

5   Second-dimension offset temperature should finally be adjusted so that the polarity range of the sample utilizes the full space of the two-dimensional separation plane without any "wrap-around"

7.2   The analysis of diesel petrochemical samples

This study confirmed the recent literature reports that GC x GC is a powerful technique for the analysis of petrochemical samples. In this method development study, different diesel samples were analysed and in their respective one-dimensional plots, clear differences can be seen regarding, for example, n-alkane patterns. These differences, however, are not clear enough to speculate on the differences in the total composition of the samples with different lubrication and combustion properties . In one-dimensional analysis, too many compounds co-elute and many are thus not identifiable.   When involving two-dimensional separations, these smaller differences can be investigated. Under visual inspection differences can be easily spotted, which is a clear indication that this method can be used for fingerprinting purposes.

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Appendix A

Software created for data handling (Matlab R12)

1   Background subtraction

Program to subtract a five-point average (five lowest chromatographic values) from each second dimension chromatogram value. This baseline subtraction was done to ensure a same level baseline for the two-dimensional chromatogram.

---

function matrikstemp = backgroundsubtractionp(matriks)

% Program to subtract 5 low point average from 2nd dimension

% chromatograms to get stable baseline in two dimensions

% To use tipe in variable = backgroundsubtractionp(matrix)

[vertikaal,horisontaal] = size(matriks);

tic

versoek = 0;

h = timebar('Background Subtraction','Progress')

```
horsoek = 0;

maksimum = 0;

matrikstemp = matriks;


while versoek < vertikaal;                              % while 1

    versoek = (versoek + 1);

    timebar(h,versoek/vertikaal)

    horsoek = 0;

    horsoektemp = 0;

    minimum1 = 9999999999999999;

    minimum2 = 9999999999999999;

    minimum3 = 9999999999999999;

    minimum4 = 9999999999999999;

    minimum5 = 9999999999999999;

    while horsoek < horisontaal;                        % while 2

        horsoek = (horsoek + 1);

        if matriks(versoek,horsoek) < minimum1;         % if 1

            minimum1 = matriks(versoek,horsoek);

            horsoektemp = horsoek;

        end                                             % end if 1

    end
```

```
horsoek = 0;

    while horsoek < horisontaal;

        horsoek = (horsoek + 1);

        if ((matriks(versoek,horsoek) < minimum2) & (matriks(versoek,horsoek) > minimum1))
                                                              % if 2

                                                              % if 3

            minimum2 = matriks(versoek,horsoek);

                                                      % end if 3

        end                                 % end if 2

    end

    horsoek = 0;




while horsoek < horisontaal;

    horsoek = (horsoek + 1);

    if matriks(versoek,horsoek) < minimum3;             % if 4

      if matriks(versoek,horsoek) > minimum1;           % if 5

        if matriks(versoek,horsoek) > minimum2;         % if 6

          minimum3 = matriks(versoek,horsoek);

        end                                 % end if 6

      end                                   % end if 5

    end                                     % end if 4
```

```
end

horsoek = 0;

while horsoek < horisontaal;

    horsoek = (horsoek + 1);

    if matriks(versoek,horsoek) < minimum4;                 % if 7

        if matriks(versoek,horsoek) > minimum1;             % if 8

            if matriks(versoek,horsoek) > minimum2;         % if 9

                if matriks(versoek,horsoek) > minimum3;     % if 10

                    minimum4 = matriks(versoek,horsoek);

                end                                         % end if 10

            end                                             % end if 9

        end                                                 % end if 8

    end                                                     % end if 7

end

horsoek = 0;

while horsoek < horisontaal;

    horsoek = (horsoek + 1);

    if matriks(versoek,horsoek) < minimum5;                 % If 11

        if matriks(versoek,horsoek) > minimum1              % if 12

            if matriks(versoek,horsoek) > minimum2          % if 13

                if matriks(versoek,horsoek) > minimum3      % if 14

                    if matriks(versoek,horsoek) > minimum4  % if 15
```

```
                    minimum5 = matriks(versoek,horsoek);

            end                                         % end if 15

          end                                           % end if 14

        end                                             % end if 13

      end                                               % end if 12

    end                                                 % end if 11

  end

                                                        % end while 2

  t = [minimum1 minimum2 minimum3 minimum4 minimum5];

  gemideld = ((minimum1 + minimum2 + minimum3 + minimum4 + minimum5) / 5);

  count1 = 0;

  for count1 = 1 : horisontaal

        matrikstemp(versoek,count1) = matrikstemp(versoek,count1) - gemideld;

  end

end                                                     % end while 1

close(h)

matrikstemp;

matriks;

toc
```

2 Program to reconstruct the one-dimensional chromatogram

Program for the reconstructing of a one dimensional chromatogram from a two-dimensional array by adding up all the values of any given second-dimension chromatogram, analogous to the reconstructed total ion chromatogram from GC-MS raw data

---

function onedimensionchrom(matriks)

% drawing one dimensional chromatogram from a dwo dimensional data set

```
[vertikaal,horisontaal] = size(matriks);

matrikstemp = matriks';

teller =   1:vertikaal;

i = 99999999;

while i > 99999

   i = input('What is the modulation period of the chromatogram in seconds ?: ');

   if i > 99999

      iii = input('The modulation period must be an interger between 0 and 99999 seconds !!! ');

   end

end

naam = input('What is the name of your chromatogram ?','s');

tottyd = (vertikaal*i)/60;

tydinkrement = tottyd/10;
```

```
onechrom=sum(matrikstemp(1:end,teller));

plot(onechrom)

inkrement = vertikaal/10;

set(gca,'XTick',inkrement:inkrement:vertikaal)

set(gca,'XTickLabel',{tydinkrement:tydinkrement:tottyd}),xlabel('minutes')

ylabel('intensity')

title(['1 Dimensional Plot of ',naam])
```

3   Program to extract individual second-dimension chromatograms

This program was designed to extract individual second-dimension chromatograms from the two-dimensional data array and plot it in a one-dimensional chromatogram.

```
function matrikstwee = twodimensionchrom(matriks)
% drawing two dimensional chromatogram from a one dimensional data set


[vertikaal,horisontaal] = size(matriks);

matrikstemp = matriks';

teller =   1:(vertikaal-1);

i = 99999999;

t = 99999999;

col = 99999999;

while i > 99999

   i = input('What is the modulation period of the chromatogram in seconds ?: ');

   if i > 99999

      iii = input('The modulation period must be an interger between 0 and 99999 seconds !!! ');

   end

end

while t > 99999

   t = input('What is the sampling rate in Hz ?: ');
```

```
if t > 99999

   ttt = input('The sampling rate must be between 0 and 99999 Hz !!! ');

end

end

while col > 99999

   col = input('What is the column you whish to plot -- amu = column + 1 + minmass exported ---:

');

   if col > 99999

      colll = input('The column must be between 0 and 99999  !!! ');

   end

end

inkrement = i * t

aantalchroms = vertikaal/inkrement

herstel = round(aantalchroms)


tydelik = matriks([1:inkrement], col);


teller = 0;

while teller < (herstel - 1);

   teller = (teller + 1);

   volg = inkrement;

   volg = (volg * teller);
```

```
    h = (volg + 1);

    n = (volg + inkrement);

    tydelik2 = matriks([h:n], col);

    tydelik = [tydelik tydelik2];

end

matrikstwee = tydelik';
```

Appendix B

Calculations

Equations used

a)   Linear flow rate of the first-dimension

u = (column length) / (dead time measured)

b)   Resolution

$$R = \frac{2(t_{R2} - t_{R1})}{4\sigma_1 + 4\sigma_2}$$

t_{R1} and t_{R2} are respectively the retention times of the two chromatographic peaks

$$\sigma_1 = \frac{hw_1}{2.355}$$

$$\sigma_2 = \frac{hw_2}{2.355}$$

Where hw the width at half height for the respective peaks represent.

c)   Plate number

$$N_1 = 5.54\left(\frac{t_{R1}}{hw_1}\right)^2$$

d.)   Plate height, H

$$H = \frac{L}{N}$$

where L is the column length and N is the plate number

Calculations of the respective linear flow rates of the two dimensions

Inlet pressure (kPa)	Dead Time First-dimension (s)	Linear flow rate first-dimension (cm/s)	Iterated pressure between the two dimensions (kPa)	Dead time calculated for seond-dimension (ms)	Linear velocity at second column exit (cm/s)	Average linear velocity calculated for second-dimension (cm/s)
320	60	47.617	343.00	139	2093	753
280	68	42.015	310.20	157	1686	664
220	80	35.713	258.50	201	1120	520
190	82	34.841	230.31	237	865	442
185	85	33.612	226.85	242	835	432
175	89	32.101	218.80	256	767	409
170	90	31.744	214.30	264	730	396
150	100	28.570	198.60	300	607	350
130	114	25.061	183.20	344	495	304
125	117	24.419	179.10	358	467	292
120	121	23.612	175.15	374	440	281
115	125	22.856	171.15	391	414	269
110	130	21.977	167.00	410	387	256
100	143	19.979	160.00	447	344	234
90	156	18.314	152.00	500	296	210
80	173	16.514	144.59	561	255	187
60	224	12.754	129.58	751	177	140

The calculation for the second-dimension resolution and plate numbers										
Inlet pressure (kPa)	Width at half height hw_1 (ms)	Width at half height hw_2 (ms)	Second-dimension column "dead time" (ms)	Peak 1 retention time t_{R1} (ms)	Peak 2 retention time t_{R2} (ms)	Plate number for peak 1 N2	Plate number for peak 2 N2	Peak 1 width at baseline $4\sigma_1$ (ms)	Peak 2 width at baseline $4\sigma_2$ (ms)	Resolution of peaks R
60	72	90	751	2626	2926	7369.44	5855.63	122.3	152.9	2.18
80	57	65	561	2151	2401	7889.34	7559.05	96.8	110.4	2.41
90	55	61	500	2025	2270	7509.91	7671.88	93.4	103.6	2.49
100	55	60	447	1872	2107	6417.95	6831.82	93.4	101.9	2.41
110	55	60	399	1744	1964	5570.28	5935.95	93.4	101.9	2.25
120	55	60	374	1654	1864	5010.20	5346.86	93.4	101.9	2.15
130	55	60	344	1594	1803	4653.30	5002.63	93.4	101.9	2.14
150	70	75	300	1600	1800	2894.37	3191.04	118.9	127.4	1.62
190	90	90	237	1574	1754	1694.47	2104.19	152.9	152.9	1.18

The calculations for the resolution in the first-dimension							
Inlet pressure (kPa)	Peak 1 retention time t_{R1} (s)	Peak 2 retention time t_{R2} (s)	Width at half height of peak 1 hw_1 (s)	Width at half height of peak 2 hw_2 (s)	Width at baseline Peak 1 $4\sigma_1$ (s)	Width at baseline Peak 2 $4\sigma_2$ (s)	Resolution between peaks 1 and 2
60	74686	81582	704	727	1328	1369	5.674382
80	59889	65575	483	496	905	999	6.838882
100	48408	53114	365	383	680	730	7.408175
110	45955	50441	320	358	585	653	7.790951
120	42281	46433	291	308	530	580	8.161903
130	39972	43920	283	296	498	530	8.028964
150	35254	38763	228	249	470	435	8.662154
190	28783	31675	185	203	379	362	8.776624
220	25365	27922	160	174	320	335	9.014573
280	20502	22582	140	150	286	291	8.445517
320	18065	19889	125	138	250	300	8.166388

Calculations of the square root N value				
P	k term	α term	R	sqrt N
60	0.7433	0.1379	2.181	85.0707
80	0.7663	0.1359	2.413	92.6943
90	0.7797	0.1384	2.487	92.1698
100	0.7879	0.1416	2.406	86.2954
110	0.7968	0.1406	2.253	80.4386
120	0.7994	0.1409	2.150	76.343
130	0.8192	0.1315	2.099	77.9403
150	0.8333	0.1333	1.624	58.469
190	0.8649	0.1187	1.178	45.8963