Chapter 9

Demonstration of the comprehensive two-dimensional SFCxGC$_{ftp}$

9.1 Introduction

Comprehensive two-dimensional chromatography is a technique where two chromatographic separations are coupled together in such a way that the initial separation is maintained by subsequent steps. For the SFCxGC$_{ftp}$ presented here, an initial polar analysis was performed on a silica gel column. A stopped flow and pressure drop focussing arrangement was used to transfer cuts from the SFC to a fast temperature programmed GC.

Two different types of silica gel columns were applied to group separation:

- The packed column silica gel separation of chemical classes with SFC is a well-known application[1]. It is used to separate petrochemical liquids into aliphatic, aromatic and poly-aromatic compound classes. A packed silica column was used with the SFCxGC$_{ftp}$ as the first dimension to analyze petrol and diesel samples.

- The use of a porous layer open tubular (PLOT) column for the group analysis of oxygenated compound classes was also demonstrated in Chapter 6. Here, the PLOT column will be used with SFCxGC$_{ftp}$ for the separation of oxygenated compounds relevant to the petrochemical industry and to analyze a lemon essential oil.

Compounds in a chemical class have similar chemical functionality but differ widely in boiling point. In order to analyze each class for this boiling point distribution, temperature programmed GC is required. Each SFC peak has to be sampled many times to ensure that the peak information of the SFC separation is conserved. Thus, very fast second dimension separations are required. To this end, the fast resistive GC developed in Chapter 4 will be applied.

Transfer of each fraction between the SFC and GC was facilitated with a special interface called the stopped-flow-pressure-drop-focusing modulator. This interface periodically stops the flow in the SFC, providing time for the volatility analysis. Through this interface, CO_2 gas from the SFC exit was exchanged for hydrogen to facilitate a faster GC separation.

9.2 Experimental

9.2.1 The supercritical fluid chromatograph

As described in Chapter 6, a piston pump was used to deliver SFC grade CO_2 at 150 atm to a 2.1 mm x 250 mm column packed with silica gel. An electrically actuated internal loop injector with an internal volume of 0.2 µL was used for sample injection. The column was coupled through a low dead volume T-connector to two integral restrictors[2]. One restrictor was connected to the detector and the other to the split/splitless injector on a Varian 3300 gas chromatograph.

9.2.2 The modulator

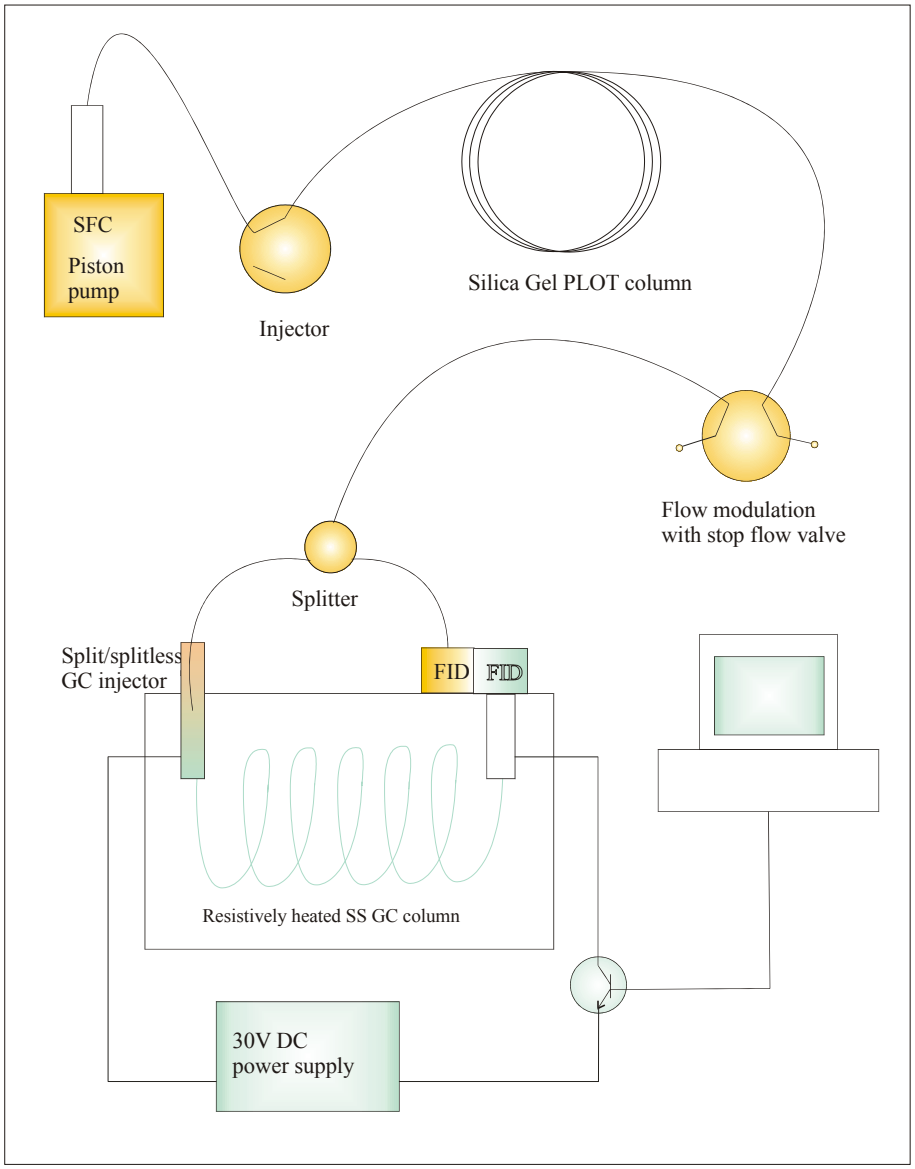
Flow modulation was achieved with an electrically actuated six-port valve with all but two adjacent ports closed off. Valve switching was controlled with a TTL pulse from a multipurpose input/output board. The valve was placed in between the splitter-restrictor assembly and the SFC column outlet. The eluent was transferred to the split/splitless injector on the Varian 3300 through one of the SFC restrictors. The injector splitter was opened and closed with a solenoid valve controlled from the PC by TTL pulse. A bypass switch was installed to allow the splitter valve to be opened and closed either from the computer or through the normal GC control panel.

The entire column was cooled down to the ramp starting temperature with CO_2, using the sub-ambient temperature control of the Varian 3300. External oven control was achieved through a small modification on the temperature control board of the GC. An override switch was installed to allow either the GC to operate normally or for the LabVIEW program to take command of the temperature control PCB that turns the oven, oven fan and cooling on or off.
After concentration of analytes on the column, a five second equilibration time was allowed for the pressure in the injector to normalize.

9.2.3 Resistively heated gas chromatograph

One meter of a 0.25 mm i.d. SE-30 stainless steel column (Restek Ultra alloy) was tightly coiled. The coil diameter was 1.5 cm. The column was connected to the split/splitless injector and FID detector on a Varian 3300. A 30V-power supply was connected to the heated column connectors on the injector and detector legs. Graphite ferrules ensured good electrical contact between the connectors and the metal column. The detector and injector legs were electrically isolated from the body of the GC .The current trough the column was controlled from the computer by adjusting the base voltage on a power transistor. A very small thermocouple was constructed from type K thermocouple wire. The wires had diameters of 25 micrometer (Goodfellow, Cambridge GB). The thermocouple was glued to the column with a tiny spot of polyimide resin (Alldrich). The temperature was controlled through PID feedback on the thermocouple signal. A program written in LabVIEW (Version5.1.1) with the LabVIEW PID control kit was used for temperature control (See LabVIEW program 4.a). A flexible heater tape was coiled around the detector and injector legs to ensure that they reached the upper temperature of the ramp.
The fast resistive GC is described in detail in Chapter 4.

Figure 9-1: Schematic of Instrumentation used for SFCxGC$_{ftp}$



The first rudimental embodiment of the technology is depicted in the photograph shown in Figure 9-2. Three computers, two GC ovens, a power supply, electronics box, SFC pump and cooler take up a good 3m^2 of laboratory space. Further development will see the reduction in components to one computer, one GC oven and a SFC pump with built in peltier element for cooling.

Figure 9-2: Photograph of the comprehensive two-dimensional supercritical fluid gas chromatograph.

9.2.4 Description of the operation of the SFCxGC$_{ftp}$

9.2.4.1 Control before a run

- The legs of the FID and split/splitless injector inside the GC oven are heated above the maximum temperature of the GC ramp.
- The FID on the fast GC is connected to an external fast amplifier. As described in Chapter 4 this allows for the observation of the very narrow peaks eluting from the fast GC.
- With the SFC pump filled with CO_2, it is pressurized to the operating pressure while the stop flow valve is open. Typically, a pressure of 150 atm is used.
- The modulation program (LabVIEW program 9.a) senses the position of the injector valve. This allows the program to know when an injection occurred and automatically starts modulation and data acquisition with the Chromperfect software. The valve should be in the loading position (blue) before the SFCxGC software is run. (LabVIEW program 9a).
- The column cooling is turned on automatically when the SFCxGC program (LabVIEW program 9a) is started and the operator waits for the ramp starting temperature to be reached before injection.

9.2.4.2 Control during a run

- After the computer senses an injection, the data acquisition software starts collecting data from the FID that is connected directly to the SFC via the split (see Figure 6-1)
- The stop flow valve is opened for 5 seconds or any other user defined interval. This value, as described in Chapter 7, is determined by the SFC peak widths. While the stop valve is open for sample to be collected, the pressure inside the injector increases to about 20 psi.
- After the 5 seconds collection period the stop flow valve is closed, the split on the injector is opened and a 5 seconds equilibration period allows for pressure in the injector to return to the operating pressure (typically 4 psi) for fast GC operation. The oven fan and cooling is also turned off at the beginning of this period.
- At the same time as the GC temperature program starts, the Chromperfect software starts collecting data from the FID connected to the GC.
- The maximum temperature of the ramp is reached typically in about 30 seconds, where after the oven fan and cooling is turned on to facilitate rapid cooling of the GC column. This phase takes about 20 seconds.
- Cooling is continued until the starting temperature of the ramp is reached and this temperature is maintained while the next sample is collected.
- This process is repeated until the end of the SFC run. The number of cycles can be predetermined from an unmodulated SFC run. However the operator normally terminates the analysis after the last expected peaks are observed.
- A typical GC cycle time of 1 minute is realized.

9.2.4.3 Data collection and handling

- Each GC run is stored in a separate file. Data files generated by Chromperfect have the format <name.##r>. The numbers are automatically generated by Chromperfect and increased form 00r to 99r where after the filename is automatically changed to <name1.##r> All the GC runs of a specific SFCxGC$_{ftp}$ analysis are stored together in the same directory.
- The data files collected by Chromperfect are converted to tab delimited text.

- These files are collated into a single text matrix. The collation program uses the file extension to arrange files in order in the matrix. This has the implication that only a hundred files can be collated at a time. (See LabVIEW program 5)
- A hundred text files are collated in a matrix at a time and the matrixes are then combined in Matlab to provide the entire SFCxGC$_{ftp}$ analysis as a single matrix.
- Transform software are used to produce surface and interpolated images for presentation and analysis.

9.3 Results and Discussion

9.3.1 Chromatograms obtained with the packed column

9.3.1.1 Analysis of a standard mixture

Figure 9-3 shows the analysis of a standard mixture containing the n-alkanes between decane (C_{10}) and tetracosane (C_{24}), the mono-aromatic compounds benzene, toluene, and a mixture of the xylenes and the di-aromatic compounds naphthalene and methyl-naphthalene. Here the high degree of order obtained with a SFCxGC$_{ftp}$ analysis can be seen.
To the left of the chromatogram the n-alkanes are found decreasing in volatility from top to bottom. The mono-aromatic compounds elute next and are separated according to boiling point to show the different members of this group. Finally the di-aromatic compounds elute from the SFC and are analyzed for volatility. Good separation is obtained between naphthalene and methyl-naphthalene with a difference of only one methyl group.

Over the range of compounds analyzed, very good orthogonality is observed. Compound classes are clearly separated. With this presentation different groups are arranged in straight vertical bands. The wavy appearance of the spheres is an unfortunate result of retention time irreproducibility of the fast GC.

Figure 9-3: SFCxGC$_{ftp}$ of a Petrochemical standard using a silica gel packed column

SFC: Pressure =150 atm, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 250°C at 450°C/min. Flow rate = 1m/sec H_2

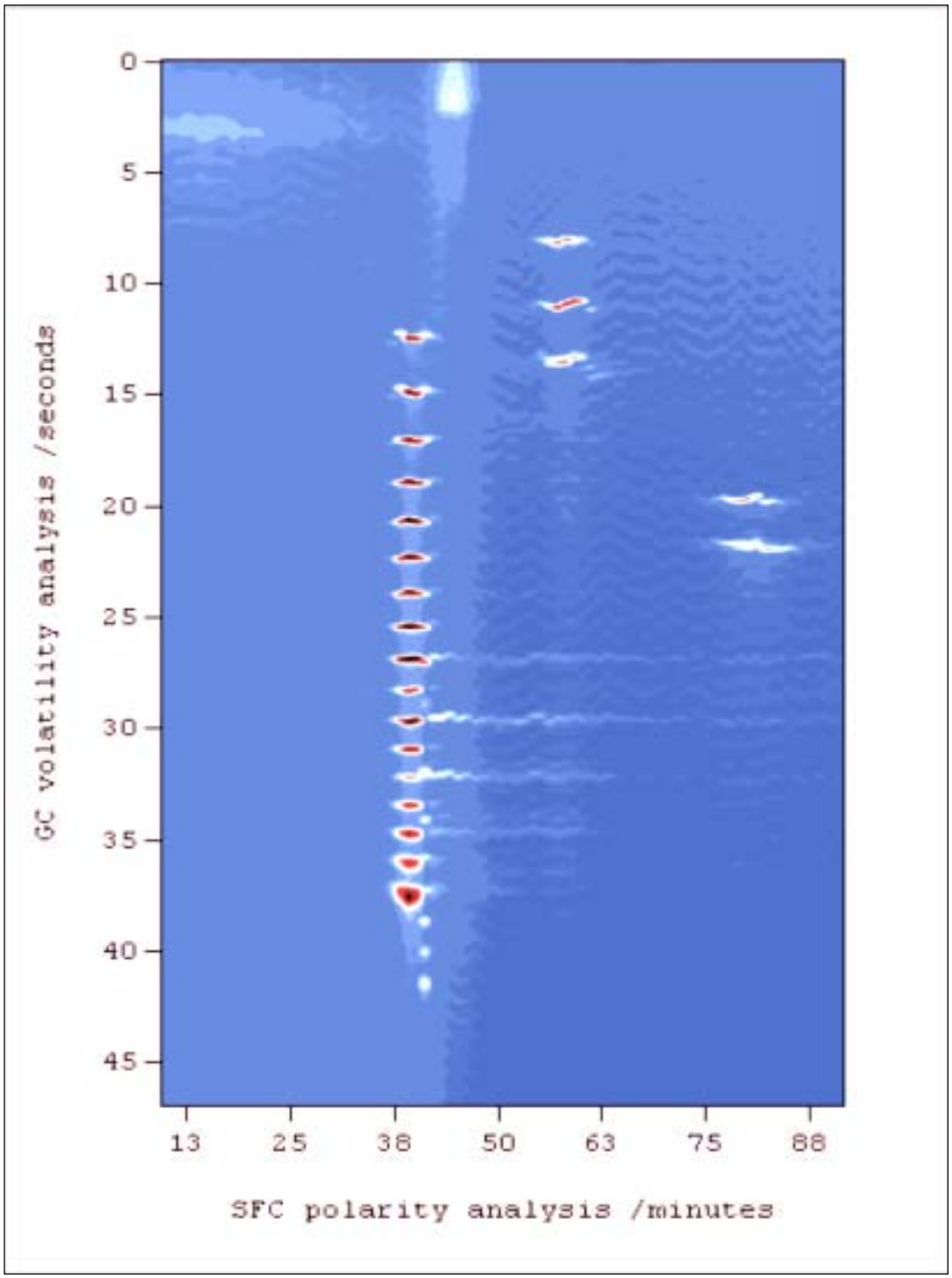
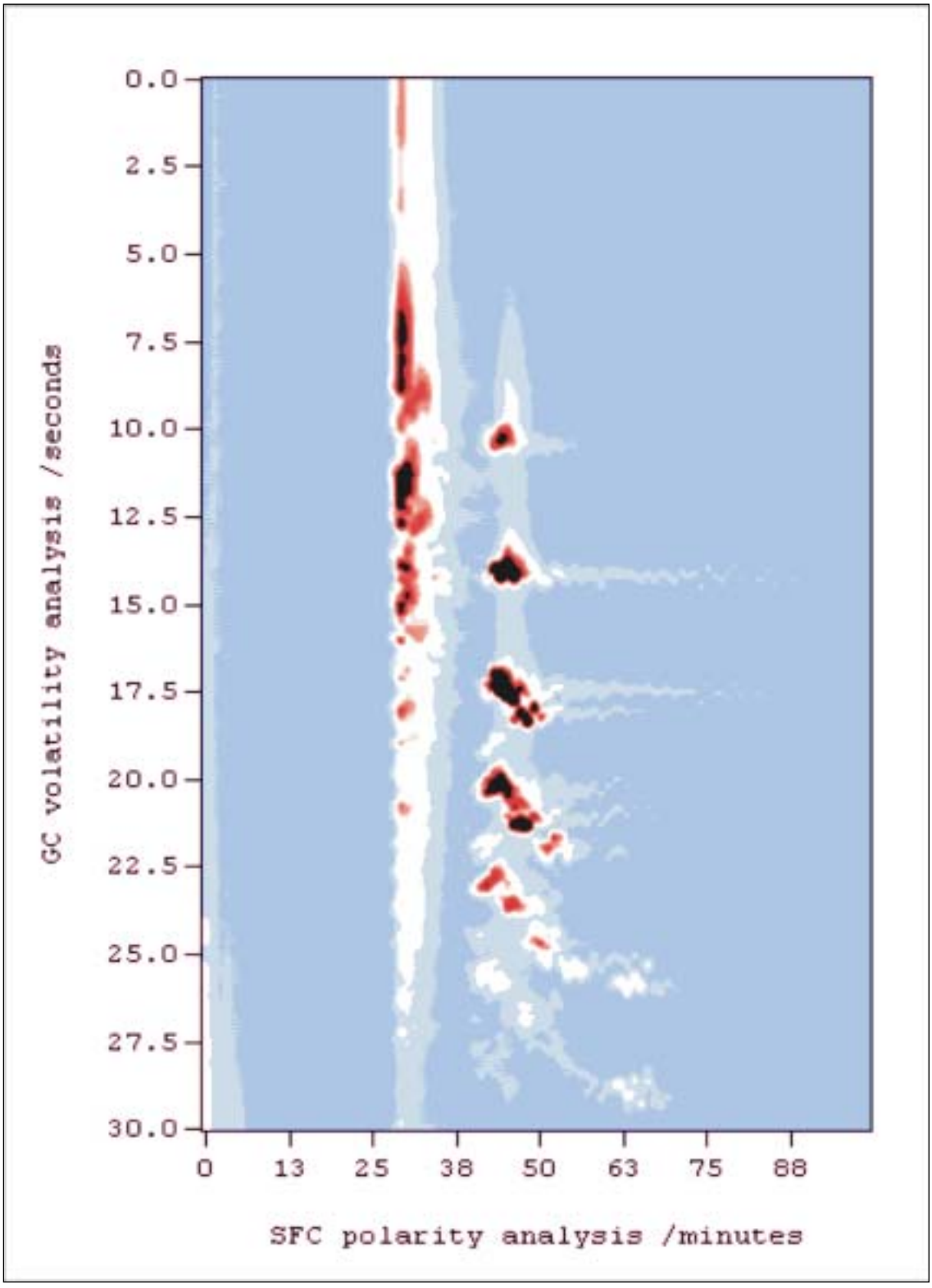


Figure 9-4: SFCxGC$_{ftp}$ analysis of a petrol sample.
SFC: Pressure =150 atm, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 250°C at 450°C/min. Flow rate = 1m/sec H_2



9.3.1.2 Analysis of a commercial petrol sample

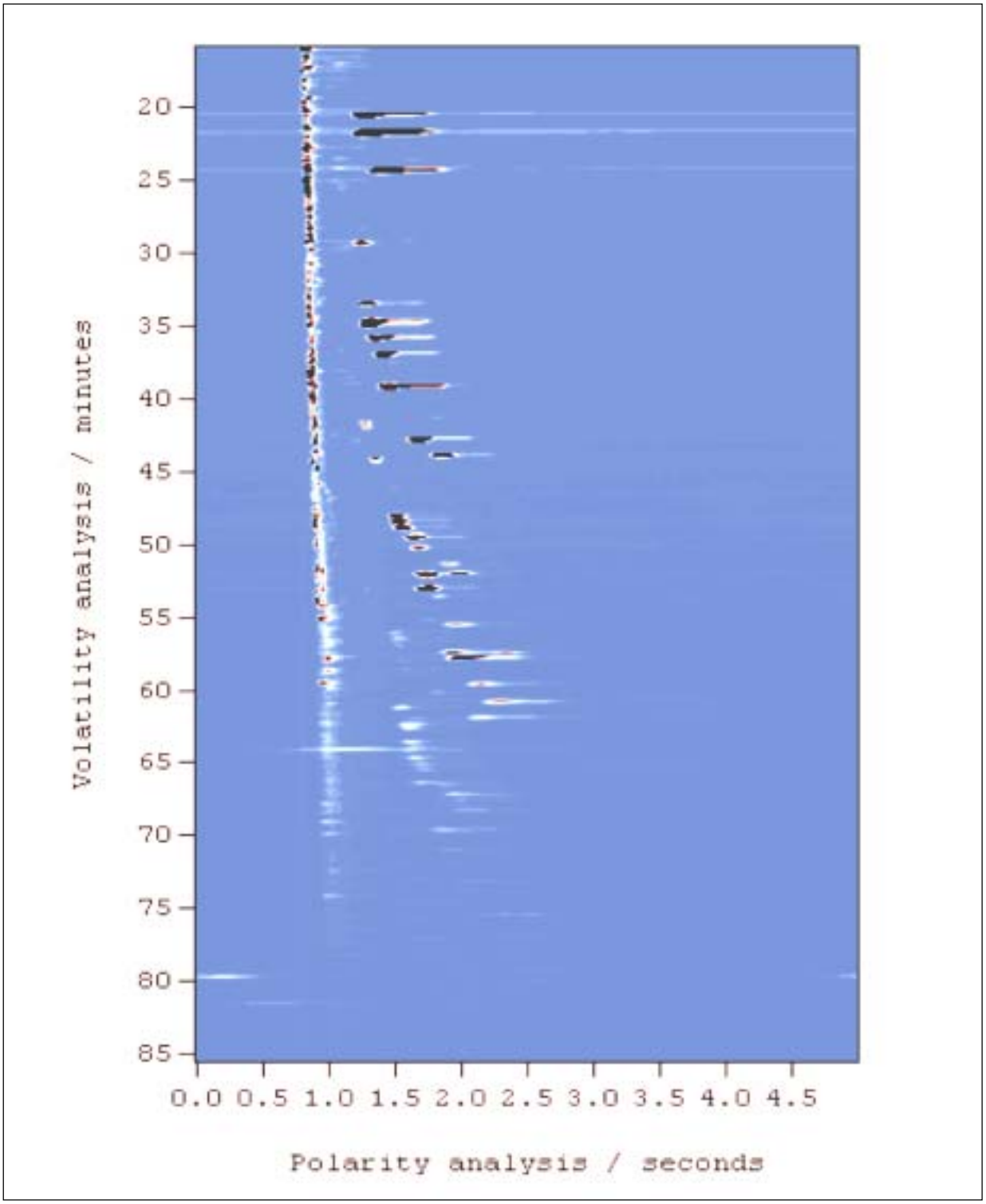
The SFCxGC$_{ftp}$ analysis of a petrol sample (Figure 9-4) correlates well with the chromatogram of the standard solution displayed in Figure 9-3. The aliphatic compounds on the left, decrease in volatility from the top to bottom. A set of spots that are not clearly separated from the alkane band is observed to the right of the alkane band. These spots probably belong to olefinic compounds. The mono-aromatic compounds are well resolved with respect to the increase in number of methyl groups present. Structural isomers form a diagonal band that indicates a second sample dimensionality present on the SFC column. The diagonal appearance of this band suggests correlation between the volatility and polarity axis for this sample dimensionality.
No di-aromatic compounds were observed in this petrol sample.

The analyzed sample originated from the Fischer-Tropsch process. It is lead free petrol that contains a transition metal complex as anti-knocking agent and lubricant and is marketed by Sasol as 'Dual Fuel™'. The manufacturer claims that it can be used in all car engines, whether they are designed for lead-free petrol or not. The amount of aromatic compounds in this sample is quite high. Aromatic compounds tend to increase the octane level of unleaded petrol.

The same sample was analyzed with a commercial GCxGC instrument (Figure 9-5) that used a modulator with alternating pulses of hot and cold gas, as described in Chapter 7. Earlier compounds including benzene and toluene were not shown in this extraction of the GCxGC chromatogram, as they were not successfully trapped at the analysis temperature.
The effect of the slow ramp rate and long column length on the volatility axis was clearly beneficial. The result is a much larger peak capacity on the volatility axis. The high peak capacity on the first dimension also implies an increase in the number of cuts transferred to the second column. Because the transferred cuts are simple mixtures that typically contain less than ten compounds they can easily be separated in a short time with limited peak capacity. This is an advantage over SFCxGC$_{ftp}$ where relatively complex mixtures are transferred to the second column for a fast analysis. As the number of chemical classes is limited and the group selectivity of the SFC separation is very high, the SFC can generate only a few peaks. Compounds can be spread out over the first axis by reducing this selectivity. However in this process correlation

between the two axes is increased. While more of the available separation space will be used, less separation space will be available due to the effect of correlation (See Figure 2-1).

Figure 9-5: GCxGC analysis of a petrol sample



In more complex samples, where much functionality may be present on a single compound, group boundaries will be less clearly defined and more of the available separation space may be utilized.

The overall run time for the GCxGC and SFCxGC$_{ftp}$ analyses are more or less the same, however, the GCxGC produces a much higher peak capacity per unit time. For the SFCxGC$_{ftp}$ with hydrogen used as carrier gas and the ramp rate optimized to give the highest possible peak capacity per unit time, the only way to increase peak capacity and keep the GC analysis time short is by decreasing the diameter of the capillary column. However, this was not possible with the current instrumentation, as smaller diameter stainless steel columns are not currently commercially available.
The polarity of the GCxGC column used for the polarity separation in this experiment was not high enough to separate the olefinic compounds from the bulk aliphatic group. This higher selectivity of SFC for polar compounds is an obvious advantage over GCxGC, especially for compounds of high molecular weight since selectivity that entails polar or chiral interactions tends to decrease at higher temperatures.

9.3.1.3 Analysis of a diesel sample

In Figure 9-6 the n-alkane peaks are clearly visible with a smaller grouping of compounds in between each alkane peak. The mono-aromatics were well separated from the aliphatic group. For the less substituted mono-aromatics, individual compounds could be observed. Due to the multitude of possible structural isomers and the limited available separation space, the highly substituted mono-aromatic compounds were not resolved. However, a good visual impression of the volatility distribution of a sample can easily be noted. Low levels of di- and tri aromatic compounds were also observed in this sample. Structural isomers were again arranged in diagonal bands. These bands were also observed in the GCxGC analysis of similar samples (See Figure 9-7).

Figure 9-6: Analysis of a diesel sample with SFCxGC$_{ftp}$
SFC: Pressure =150 atm, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 300°C at 450°C/min. Flow rate = 1m/sec H_2

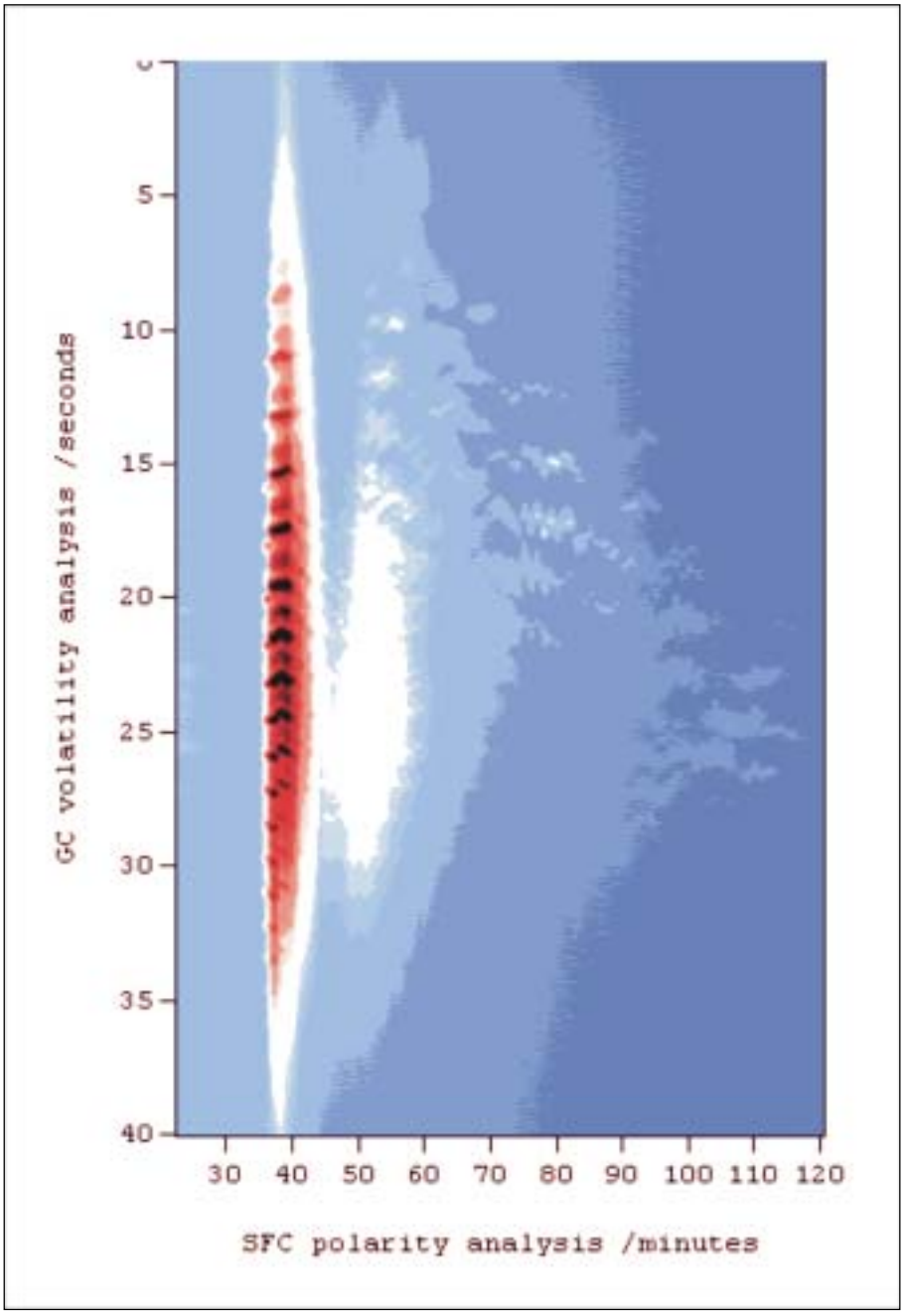
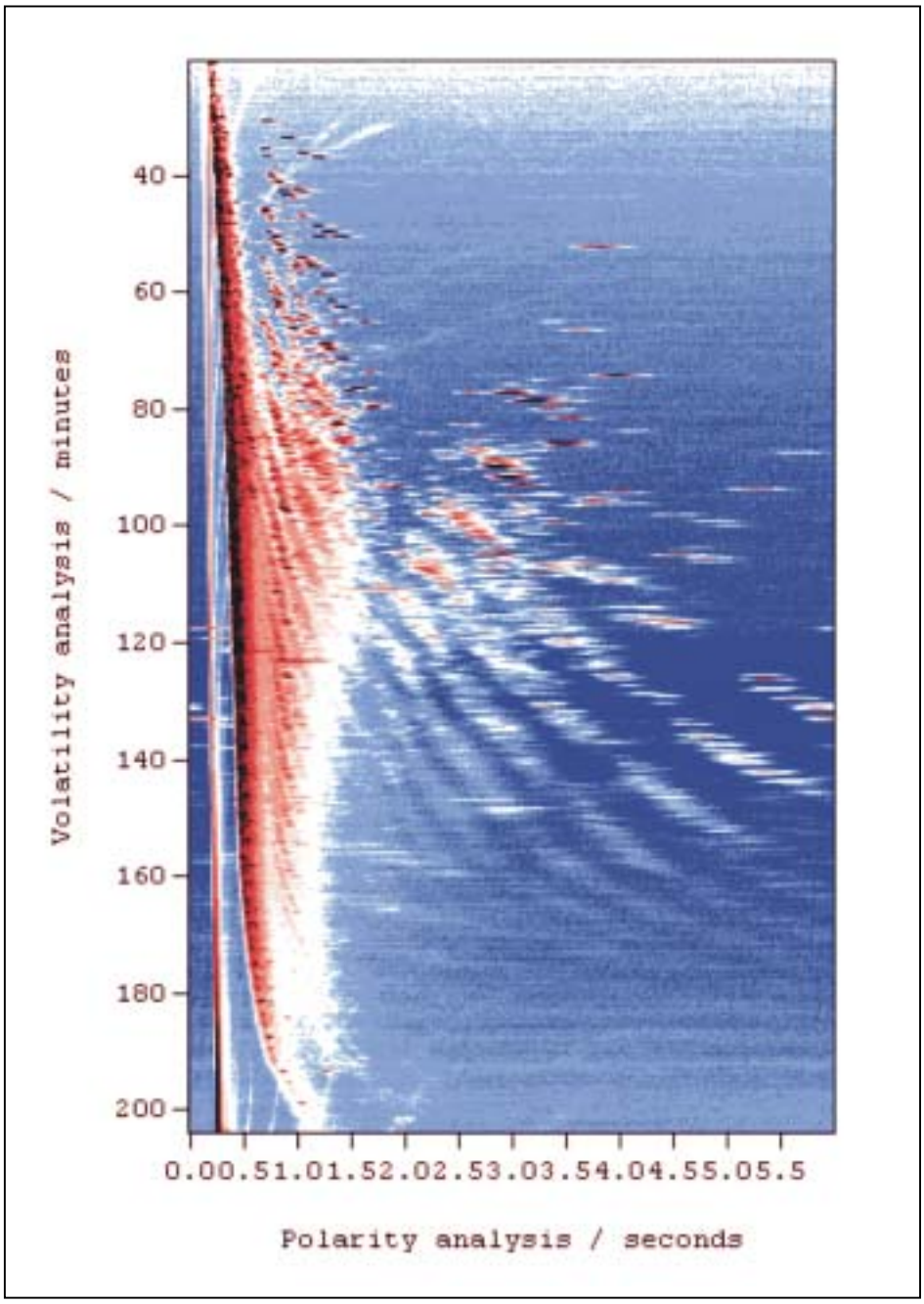


Figure 9-7: Analysis of a diesel sample with GCxGC



This figure demonstrates the immense peak capacity that can be produced with GCxGC instrumentation. However a more distinct separation is obtained between the aliphatic and aromatic compounds in SFCxGC$_{ftp}$ than for GCxGC. This is due to the very high selectivity that can be obtained with the low temperature group separation realizable with the SFC separation.

9.3.2 Chromatograms obtained with the PLOT column

The packed column was exchanged with a silica gel porous layer open tubular (PLOT) column. This allowed highly polar compound classes to elute as separated groups. The column facilitated the analysis of oxygenates in petroleum samples and the analysis of essential oils by SFC$_{PLOT}$xGC$_{ftp}$.

9.3.2.1 Analysis of a standard mixture of oxygenates

The SFC analysis of the oxygenated petrochemical standard described in Chapter 6 shows the useful separation that can be obtained by the PLOT column alone. Such a separation is not easily obtainable with other instrumentation. Even GCxGC^3 was unsuccessful. While GCxGC could separate alcohols, co-elution occurred between non-polar compounds and many of the important ethers, like DIPE, TAME and ETBE. When a similar standard is analyzed with SFC$_{PLOT}$xGC$_{ftp}$, the separation power of the technique becomes evident: TAME, MTBE and iso-propylether are well separated. The methyl and symmetrical ethers are divided into two separate groups. The volatility analysis of the second dimension separates these two groups further into their individual compounds. While ETBE was not available for testing the strong polar selectivity and the difference in volatility should also ensure good separation between ETBE and the other ethers.

The effective polarity of longer alcohols is reduced due to the effect of the long aliphatic chain. Thus, it is observed that dodecanol elutes slightly earlier from the SFC column than do the shorter alcohols like ethanol. This ‘molecular weight’ effect causes correlation between the two separation dimensions of polarity and volatility.

Figure 9-8: SFC$_{PLOT}$xGC$_{ftp}$ analysis of an petrochemical standard containing alkanes, ethers and alcohols.
SFC: Pressure =150 atm, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 250°C at 450°C/min. Flow rate = 1m/sec H_2

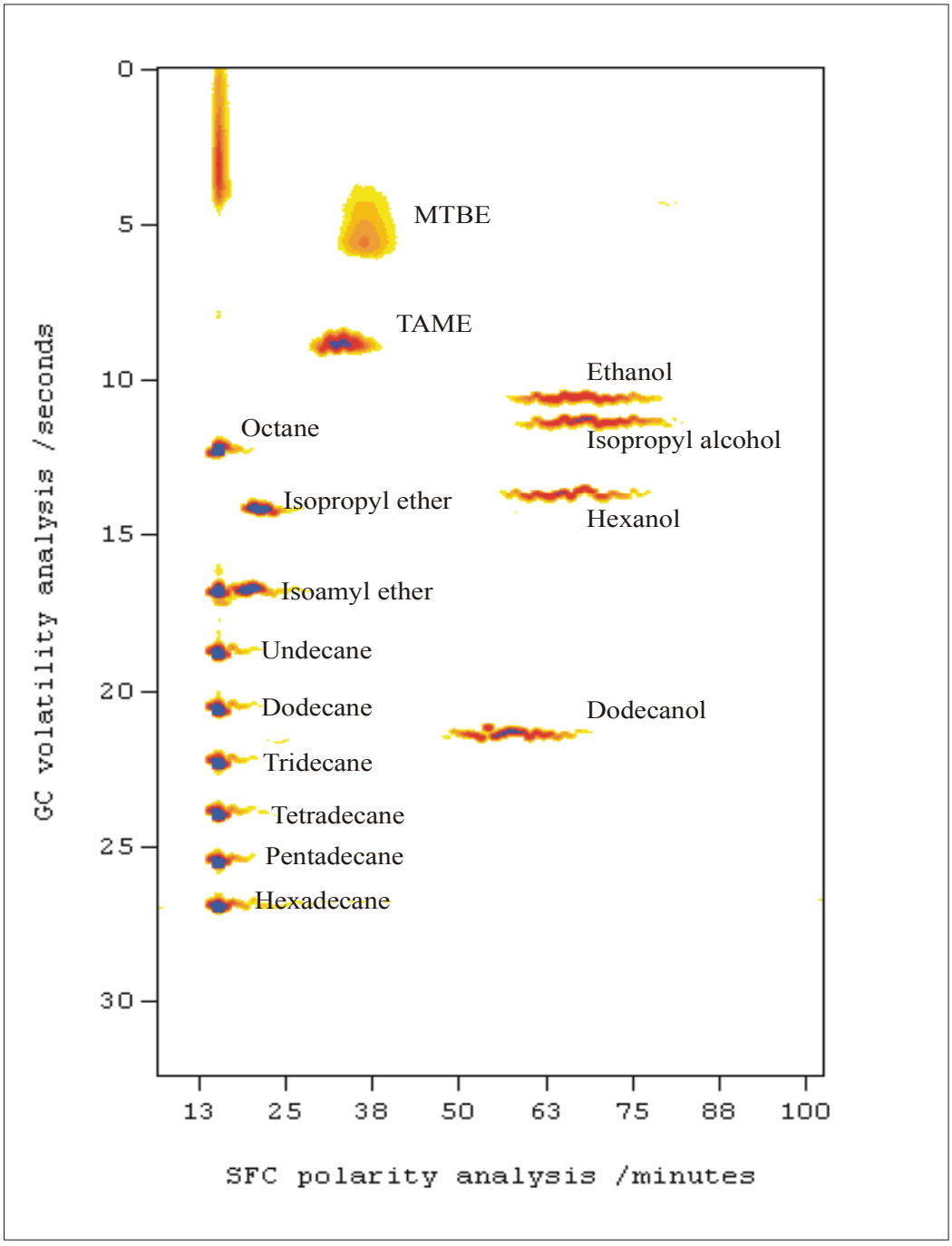
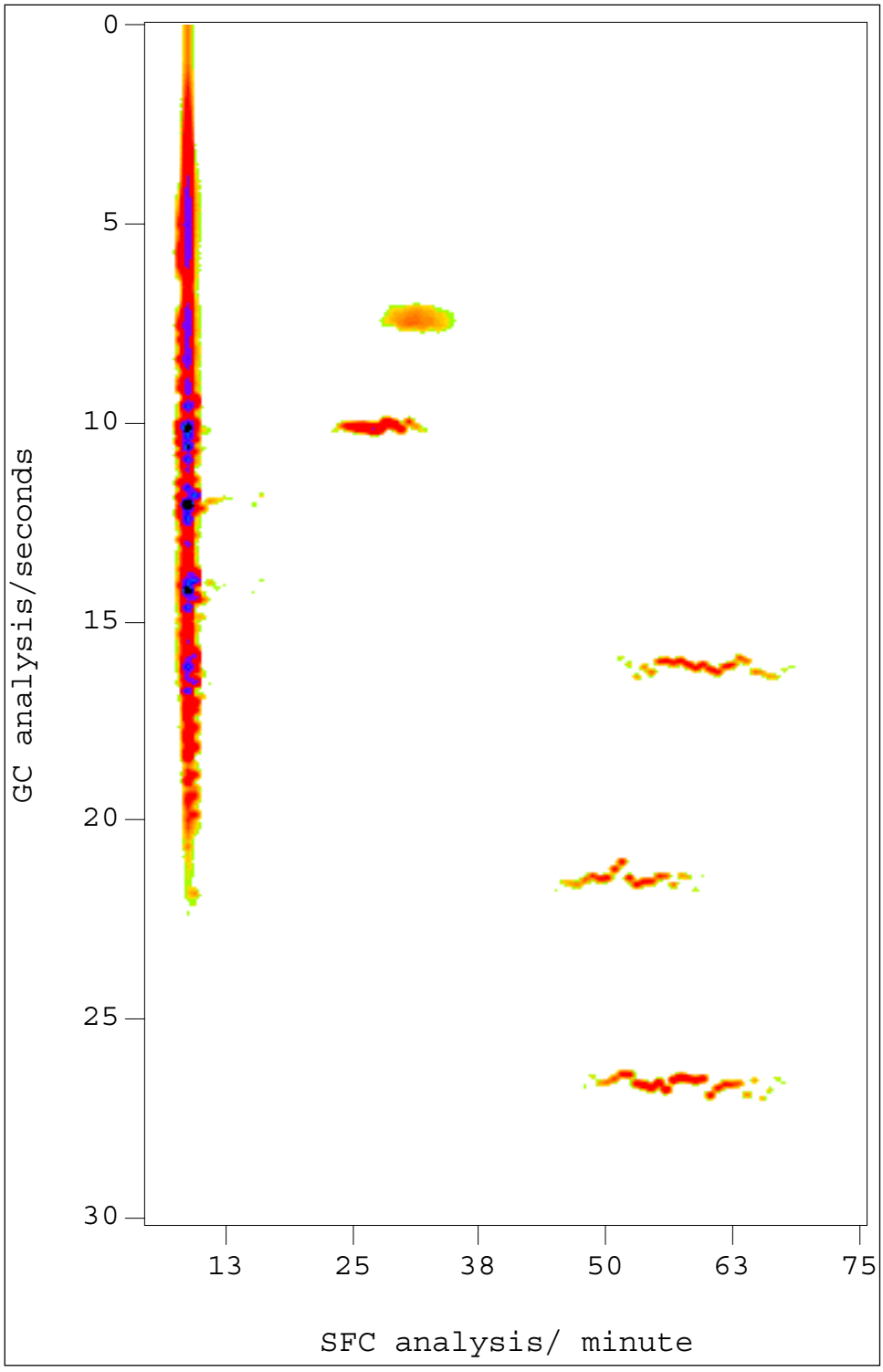


Figure 9-9: SFC$_{PLOT}$xGC$_{ftp}$ analysis of a commercial lead free petrol sample spiked with ethers and alcohols.
SFC: Pressure =150 atm, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 250°C at 450°C/min. Flow rate = 1m/sec H_2



9.3.2.2 Analysis of an unleaded petrol sample

The oxygenated standards added to the sample were all clearly separated form the non-polar aliphatic and the aromatic sample components. The standards added were isopropyl ether (IPE), t-amyl methyl ether (TAME), methyl t-butyl ether(MTBE) and iso-propanol, heptanol and dodecanol.

The petrol sample was also analyzed on its own without any oxgenates added. This is presented in Figure 9-10. The presence of a methyl ether, probably TAME by volatility axis displacement, is confirmed for the unleaded petrol sample with this chromatogram.

9.3.2.3 Analysis of a diesel sample(Natref LCO)

The Natref LCO sample analyzed by packed column SFC showed no clear distinction between the different groups. It was also noted that the sample contained some oxygenated compounds recovered through back-flushing of the column. With SFC$_{PLOT}$xGC$_{ftp}$ analysis (Figure 9-11) it can be seen that, especially at high volatilities, the non-polar peak is smeared towards increased polarity. This could be the result of high molecular weight poly-aromatic hydrocarbons as anthracene was just separated from an alkane in Figure 6-3, or possibly from high molecular weight ethers with two long side chains.

A range of compounds is also seen to elute with retention times that correspond to the retention of methyl ethers at ≈ 17 minutes on the polarity axis. These would be the compounds responsible for the peak on backflushing of the column in the previous packed column SFC analysis (Table 6-1).

Figure 9-10: SFC$_{PLOT}$xGC$_{ftp}$ analysis of a commercial lead free petrol sample.

SFC: Pressure =150 atm, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 250°C at 450°C/min. Flow rate = 1m/sec H_2

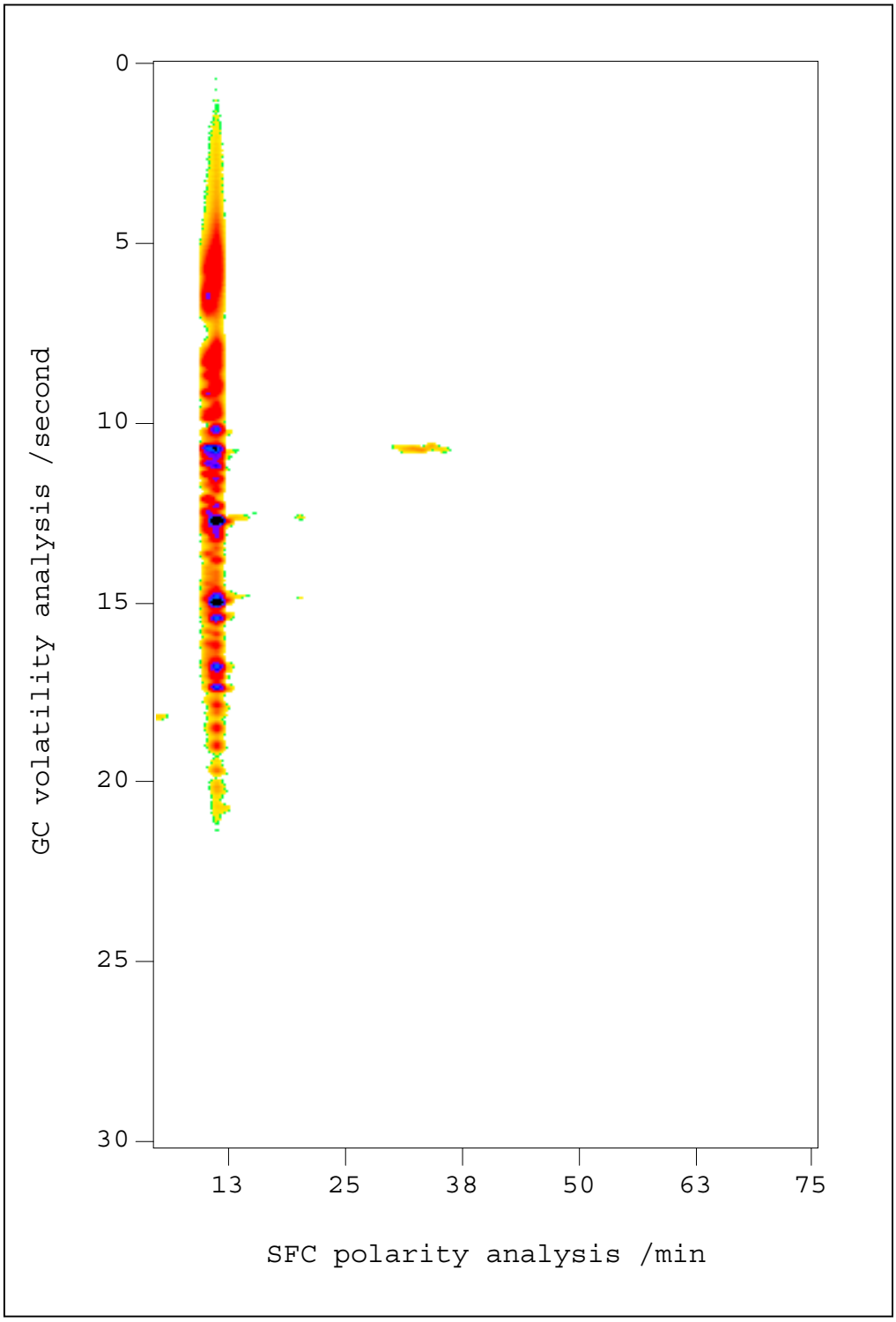


Figure 9-11 $SFC_{PLOT}xGC_{ftp}$ analysis of the Natref LCO diesel sample analyzed with packed column SFC in Chapter 6.
SFC: Pressure =150 atm, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 250°C at 450°C/min. Flow rate = 1m/sec H_2

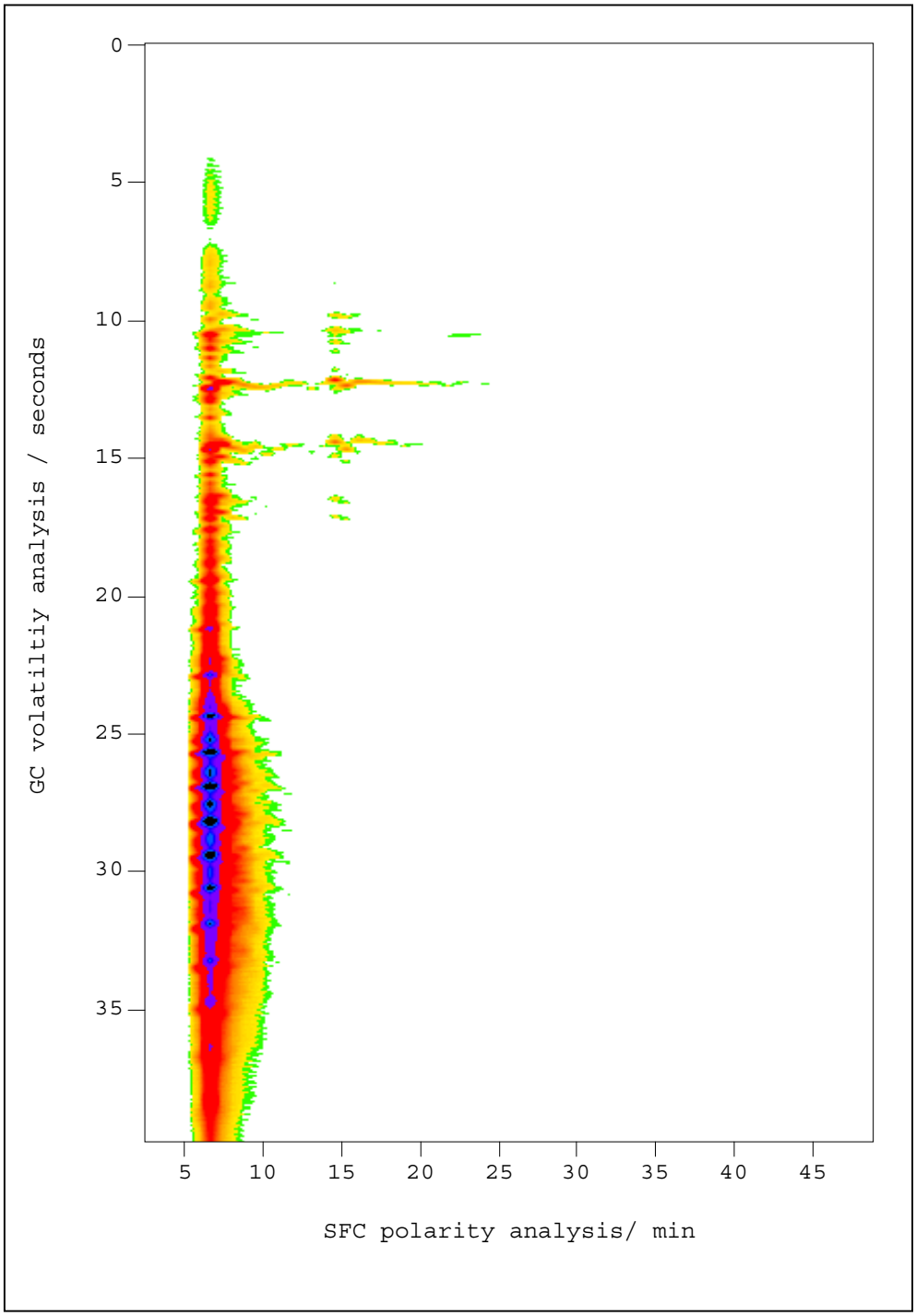
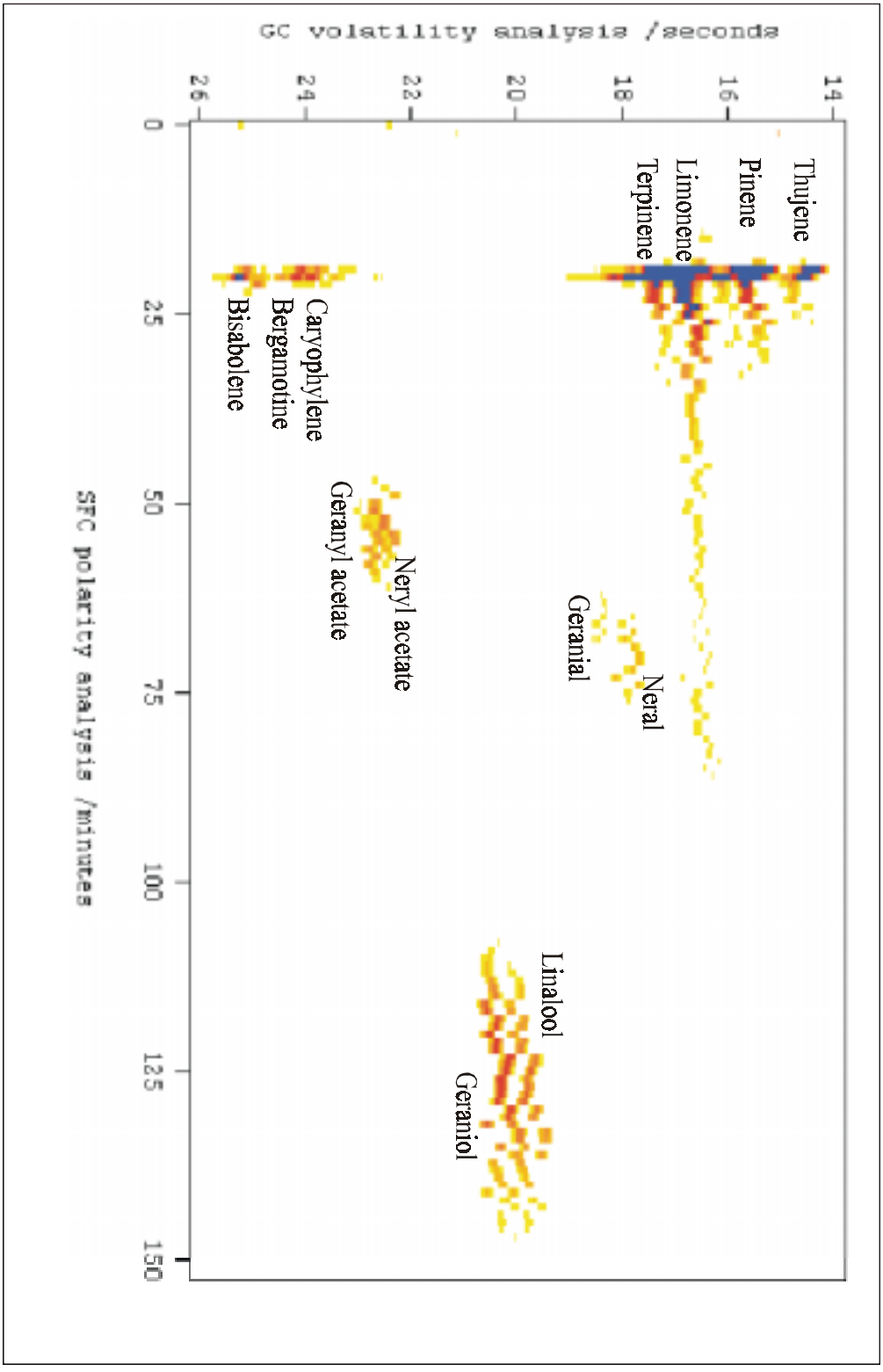


Figure 9-12: SFC$_{PLOT}$xGC$_{ftp}$ analysis of a lemon essential oil.
SFC: Pressure =110 to150 atm at 1 atm/min, Temperature= 28°C
Modulation: Collection time=5 seconds, Equilibration time=5 seconds
GC: -50 to 250°C at 450°C/min. Flow rate = 1m/sec H_2



9.3.2.4 Analysis of a lemon essential oil

The volatility axis greatly improves the group analysis of commercial lemon oil. When the PLOT column alone was used, groups could be identified but no baseline group resolution was obtained. Apart from improved group separation by the combination SFC$_{PLOT}$xGC$_{ftp}$, additional groups could also be identified. A clear distinction between mono- and sesquiterpenes was observed. While there is a large volatility gap between the mono terpenes(C_{10} compounds) and sesquiterpenes (C_{15}-compounds) the distinction is not normally so obvious in GC analysis because the oxygenated compounds are of intermediate volatility. Apart from improved group separation, the constituents of each group are also separated according to volatility. Thus, for the alcohol group, linalool and geraniol are seen to be present at a high level. Citral and geranial are separated, as is citral acetate and geranyl acetate. For the terpene group, however, the peak capacity of the boiling point separation is not high enough to clearly separate every compound. This is especially a problem for the mono-terpenes. Having both the group separation and boiling point distribution available on one chromatogram greatly simplifies compound identification.

9.4 Conclusions

The chromatograms obtained with SFCxGC$_{ftp}$ strongly resemble the results obtained with GCxGC. A high degree of order is obtained. Due to the high polar selectivity by the low temperature SFC separation, a more orthogonal separation space is obtained. Unfortunately, the peak capacity of the volatility dimension is much less for SFCxGC$_{ftp}$ than for GCxGC. This is due to the order of separation. For GCxGC the more complex volatility chromatogram is the slower, first dimension. This same separation (though slightly simplified by the preceding group separation) needs to be achieved in a very short time with SFCxGC$_{ftp}$. Apart from the lower peak capacity other practical problems like retention time irreproducibility were encountered.

Operation of the modulator that turns the SFC and GC into a comprehensive chromatograph is simple relative to the thermal and cryogenic modulators. It allows independent optimization of the two separation dimensions. This simplifies method development, as a change in one dimension does not alter the operation of the other e.g. no changes to the GC separation were needed when exchanging the packed column for the PLOT.

The first results obtained with the comprehensive $SFCxGC_{ftp}$ are very promising. This technique is sure to find its rightful place among the comprehensive chromatographic techniques. The potential to analyze a wide boiling point range of samples, the mild conditions compounds are subjected to, as well as the high selectivity of the low temperature first separation, are bound to give $SFCxGC_{ftp}$ an advantage over other available analysis techniques.

Chapter 9

[1] Annual Book of ASTM standards, Vol 05.03. American society for testing and materials, Philadephia (1991) Method ASTM D5186

[2] E.J.Guthrie, H.E.Swartz, J.Chrom.Sci. 24 (1986) p236

[3] G.S.Frysinger, R.B.Gaines, HRC 23 (2000) p197