

CHAPTER 5

SFC GROUP SEPARATION: EXPERIMENTAL

5.1 Introduction

Supercritical fluid chromatography (SFC) can be defined as a form of chromatography (i.e. physical separation method based on partitioning of an analyte between the mobile phase and the stationary phase) in which the mobile phase is subjected to pressures and temperatures near or above the critical points for the purpose of exploiting the mobile phase solvation power¹. Supercritical fluid chromatography can most easily be described as an adaption of either liquid chromatography (LC) or gas chromatography (GC) where the major modification is the replacement of either the liquid or gas mobile phase with a supercritical fluid mobile phase. SFC instruments employ almost all components normally used in conventional LC systems, including high-pressure pumps, stainless steel tubing, injection valves and columns with a few modifications or none at all². By the 1980s dedicated SFC instruments were commercially available from a number of manufactures. However, in recent years the use of SFC has declined.

In SFC the mobile phase is initially pumped as a liquid and is brought into the supercritical region by heating it above its critical temperature before it enters the analytical column. It passes through an injection valve where the sample is introduced into the supercritical stream, then into the analytical column. It is maintained at supercritical conditions as it passes through the column and into the detector by a pressure restrictor placed before a GC detector like the FID or after the LC type UV detector. The restrictor is a vital component as it keeps the mobile phase in the supercritical state throughout the separation. It often has to be heated at the exit to prevent clogging. Both variable and fixed restrictors are available.

In this chapter the application of SFC to separate compounds into different chemical classes using supercritical fluid CO₂ as the mobile phase is demonstrated with analysis of four different types of essential oils : *Tagetes minuta*, *Pelargonium*, *Artemisia afra* and *Cymbopogon*.

5.2 Instrumentation for SFC

The analytical system consists of a Lee Scientific (Salt Lake City, Utah, USA) Model 501 SFC pump to deliver supercritical fluid CO₂ (SFC grade, Air Products Sandown, South Africa) without helium head pressure to a Chrompack silica-gel PLOT column. An integral restrictor, prepared in the laboratory³ was used at the column exit to the FID to maintain supercritical fluid pressure conditions. The isothermal column conditions were maintained by a PYE-Unicam GCD gas chromatograph. The FID was maintained at 280 °C. Chromperfect software (Justice Innovations California) was used for data acquisition. An actuated internal loop injector (Vici C14-W, Valco, Switerland) with an 0.2 µL internal loop was used for sample injection. All connections were made of 1/16" o.d. 120 µm i.d. stainless steel (SS) tubing with electropolished ends and connected with SS ferrules and connectors.

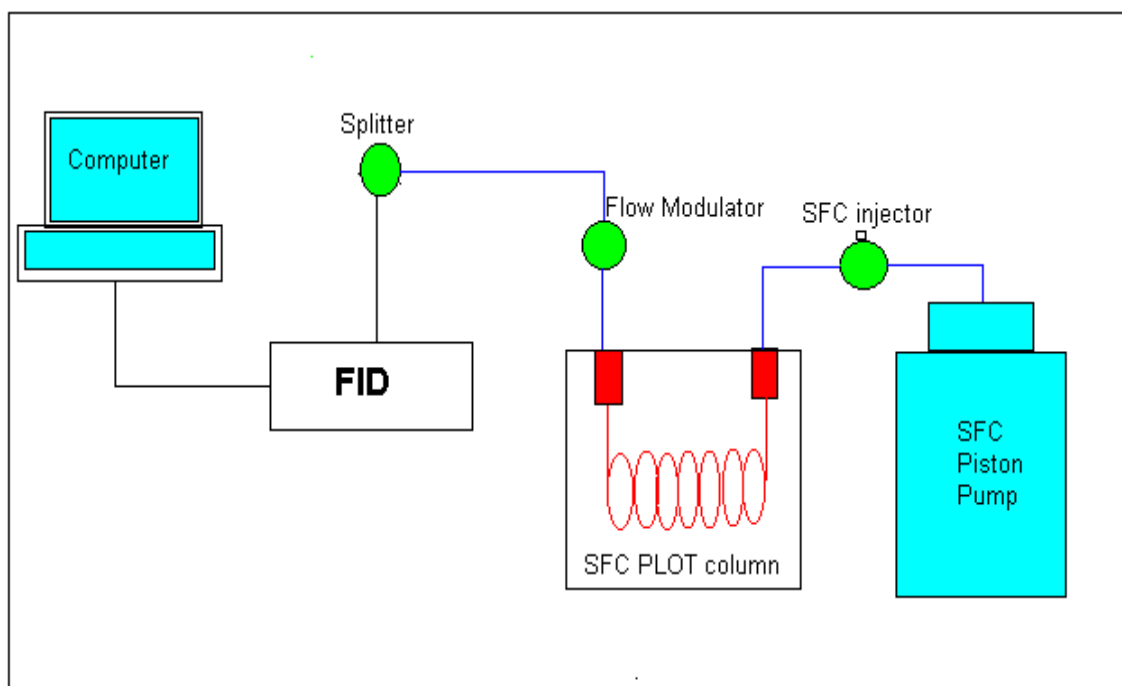


Figure 5.1. Schematic diagram of SFC instrument for group-type separation using a silica-gel PLOT column.

5.2.1 Restrictors for SFC

The use of either packed or capillary columns under SFC conditions requires a flow restrictor at the outlet of the column to maintain supercritical conditions of the mobile phase throughout the column. The ideal restrictor has to possess the following features⁴:

1. *At the column or detector interface, the restrictor must effectively transfer the mobile phase and solute materials from a supercritical phase into a gaseous phase compatible with the detection mode.*
2. *The desired restrictor should produce negligible extra-column zone broadening effects.*
3. *Should also allow uniform, pulse free flow.*

Other important practical aspects to be considered include the ease of restrictor fabrication and the restrictor mechanical durability. In SFC, the mobile phase pressure or density controls the solute partitioning phenomena while the restrictor geometry controls the mobile phase linear velocity⁴.

Increased restrictor temperature reduces the flow rate (mass flux) if the fluid is a gas. If the fluid is cooled to the liquid state in the restrictor, mass flux will increase with temperature. This behaviour is consistent with the temperature dependence on fluid viscosity. Studies have shown that at higher temperature (>120 °C) the linear velocity of supercritical CO₂ has nearly a direct dependence on pressure. If the SFC separation column and restrictor are independently thermostated, SFC mass flux (linear velocity times fluid density in the column) should be independent of the column temperature¹.

The integral restrictors were fabricated according to the Guthrie and Schwartz process³ depicted in figure 5.2. The capillary tube was heated so that the fused silica slowly drew closed by surface tension of the molten silica, producing a well defined conical closure using an oxygen-butane welding torch. The closed end of the capillary tube

was then gently abraded by hand using a wet abrasive sheet in a container of water, carefully removing the excess fused silica from the terminal end of the column until the conical closure was reached. The escape of gas bubbles from the pressurised column indicated when the conical closure point had been reached and the flow rate of the escaping gas was then measured. While polishing the capillary end, gas flow rate was measured until the desired flow rate was achieved. After the desired mobile phase flow rate was obtained the outlet of the restrictor column was inserted into the heated split/splitless injector of a GC (to prevent condensation and blockage of restrictor) and the other end into the FID for SFC analysis.

If excessive heat is used, then a rounded, rather than the proper conical, closure would be produced. The rounded closure is difficult to use, since when the polishing process reaches the fused silica inner wall, small polishing increments will produce rapid increases in the orifice diameter and flow rate.

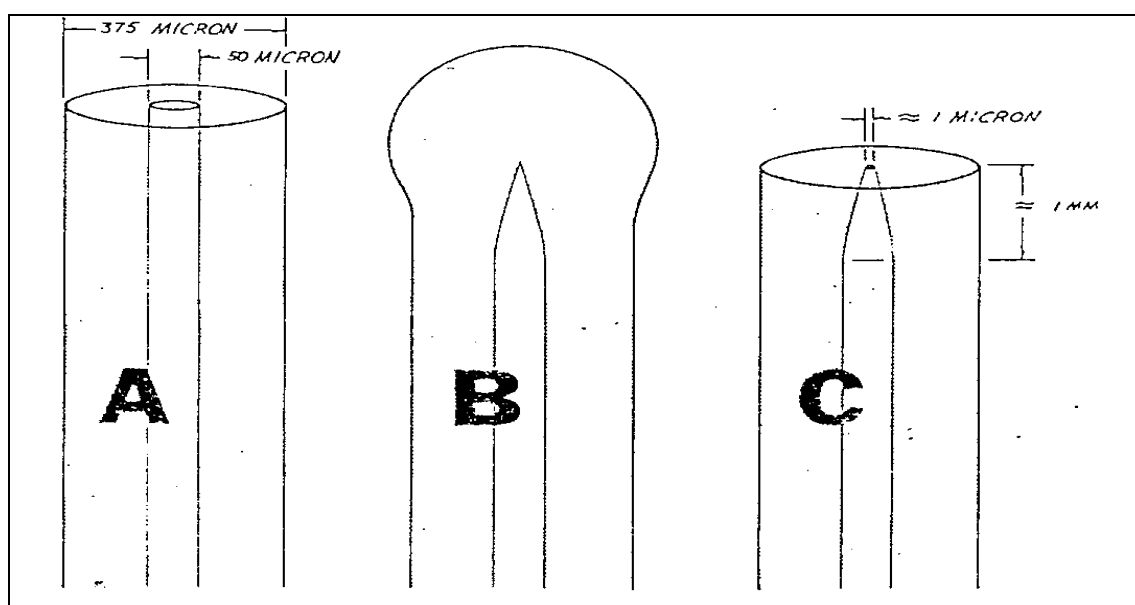


Figure 5.2. Restrictor fabrication sequence process. (A) Capillary before heating. (B) Capillary after heating with microtorch. (C) Capillary terminus and orifice after wet polishing³.

5.2.2. Supercritical fluid CO₂ mobile phase

There are a number of possible fluids which may be used in SFC as the mobile phase. Pure carbon dioxide (CO₂) is not polar, making it a mobile phase most appropriate for the elution of low to moderately polar solutes⁵. However, based on its low cost, low interference with chromatographic detectors, and good physical properties (non-toxic, non-flammable, low critical values), CO₂ is the standard mobile phase. The main disadvantage of CO₂ is its inability to elute very polar or ionic compounds. This can be overcome by adding a small portion of a second fluid called a modifier fluid. This is generally an organic fluid (alcohols, cyclic ethers). The addition of the modifier fluid improves the solvating ability of the supercritical fluid and sometimes enhances selectivity of the separation. It can also help improve separation efficiency by blocking some of the highly active sites on the stationary phase⁵. Modifier fluids are commonly used, especially in packed column SFC.

Sulphur hexafluoride (SF₆) was investigated as an alternative to CO₂ as mobile phase for group separation⁶. SF₆ is a very weak solvent and less polarizable than CO₂. Hydrogen fluoride (HF) produced when SF₆ decomposes in the flame is very corrosive to the FID. However, flame ionisation detection was made possible by gold plating of the detector. Xenon showed resolution of chemical groups comparable to that of CO₂, with superior coupling to the fourier transform infra-red (FTIR) detector due to the absence of infra-red absorption bands from the mobile phase⁷. The fluid was however, found to be too expensive for routine analysis. Ammonia is suitable for more polar compounds, however, it is not compatible with the FID.

5.3 Optimization of SFC group separation

Optimization of the fluid for chromatographic separation can be obtained by changing a variety of parameters. In GC by changing or programming the analysis temperature and choosing a suitable stationary phase, the separation efficiency can be altered. On the other hand, in liquid chromatography, the variation and programming of the eluent composition or gradient elution are the vital tools for optimization beside the choice of the stationary phase, which is packed into a column as particles.

In SFC all parameters mentioned above for GC and LC have an influence on the separation efficiency. These are: (1) selection of a stationary phase (in either a packed or capillary column), (2) selection of a mobile phase and (3) optimisation of the analysis temperature. Further, variation of the eluent density and the working pressure are of great importance in optimizing the SFC separation. Eluent composition, temperature, pressure (and therefore also density) may be varied individually or simultaneously. In the following paragraphs a brief and tentative optimisation study for group separation is reported. A detailed study would involve not one compound per class type but a volatility range of compounds for each class.

5.3.1. Determination of the optimum flow rate

Optimization of the separation conditions in chromatography requires careful evaluation of the influence of mobile phase flow rate on the peak resolution. The flow rate of the mobile phase is one of the most important factors to be taken into account in order to properly design and operate an SFC system.

5.3.1.1 Experimental

Before starting with the investigation of the influence of pressure and temperature on the alkanes, ethers, esters, alcohols and aldehydes resolution, the PLOT column

optimum performance was tested. For this reason the Van Deemter curve was drawn up at a constant pressure of 150 atm and 28 °C near critical temperature with different sets of restrictors. Dodecanal in CS₂ solvent was injected using a different set of restrictors. The restrictor flow rate was changed at constant pressure of 150 atm and temperature 28°C.

5.3.1.2 Results and Discussion

Figure 5.3 shows the plate height, measured for the dodecanal peak versus volume flow rate. It can be seen HETP increases slightly with increasing flow. However, the optimum point of HETP could not be achieved despite using a very slow rate of 20 ml/min that ended in a long analysis time of about 9 hrs. This slow chromatography is expected due to the large inner diameter (0.32 µm) of the open tube by SFC standards, where 50-100 µm i.d's are the norm. To avoid the long analysis time resulting from the slow flow rate, the SFC separation was done at the flow rate of 480 ml/min (atmospheric pressure) measured at the restrictor exit. This corresponds to a linear flow rate of 7.7 cm/sec in the column.

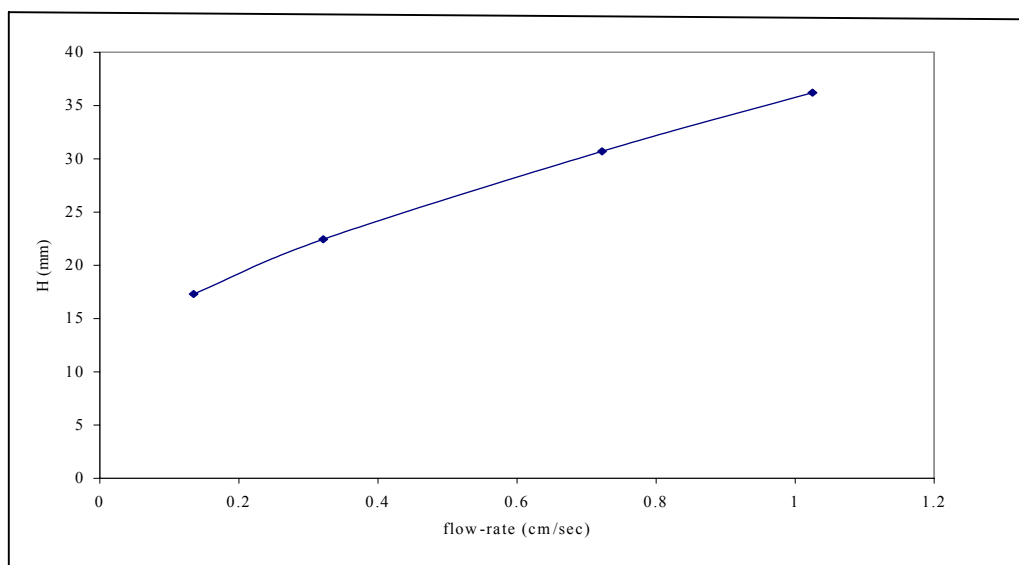


Figure 5.3. Van Deemter curve for dodecanal flow rate vs Plate height at constant pressure of 150 atm and temperature 28 °C with different restrictor flow rates.

5.3.2 Effect of temperature on SFC group separation

The effect of temperature at constant pressure is even more complicated than the effect of pressure at constant temperature. Increasing temperature decreases fluid density and solute-fluid interaction, which results in a decreased solvation power. At the same time it also decreases solute-solute interaction, which results in an increased solubility. Therefore, in terms of temperature, two competing factors affect the capacity factor (k) of solutes in the SFC separation¹. In general there are two ways in which a change in temperature can influence resolution in SFC. Firstly, changing the temperature alters the density and consequently the solvent power of the fluid so that the capacity factors (k) of solute changes, secondly, temperature can play a deciding role in selectivity (α).

It has been found that a small change in temperature can result in large changes in resolution and retention^{8,9}. Further, the selectivity (α) changes with temperature in open-tubular SFC⁵. This behaviour is important because it provides the means to adjust the selectivity over a fairly wide range. The suitable temperatures for the PLOT column separation were investigated in relation to the elution and resolution of groups in essential oils samples. It is important that a suitable temperature is determined where only polarity and not volatility is expressed.

5.3.2.1. Experimental

A 0.2 μ l standard mixture containing limonene, methyl nonanoate, iso-amyl ether, dodecanal and linalool was prepared in 500 μ l CS₂ and injected at a constant pressure of 110 atm and temperatures (20 °C, 28 °C and 40 °C). To reduce long analysis times, a high flow rate of 480 ml/min (atm) measured at the column exit was used.

5.3.2.2 Results and Discussion

Figures 5.4 to 5.6 show the SFC chromatograms of the standard sample at different temperatures (20 °C, 28 °C and 40 °C). The pressure of 110 atm was used to investigate the effect of temperature on group separation. These chromatograms were obtained in about 20 minutes run-time. This was achieved by very fast linear flow rates measured as 480 ml/min decompressed CO₂ at the column exit which correspond to a linear flow rate of 7.7 cm/sec. It has been documented that operation below the critical temperature is beneficial for group-type separation in SFC^{10,11}. At a temperature 40 °C the ester and the aldehyde co-elute as shown in figure 5.4. The trends of increased group selectivity at the lower temperature (30 °C) is followed as predicted by the literature^{10,11}. However, it appears from the observations that group selectivity of esters and aldehydes is more efficient at the temperature of 28 °C compared to both 20 °C and 40 °C. Table 5.1 shows the calculated capacity factor [$k=(t_r - t_m)/t_m$] values as the function of temperature of the methyl nonanoate and dodecanal by taking limonene as the unretained molecule.

Table 5.1. Change in capacity factor of esters and aldehydes groups as a function of temperature.

Compounds	20 °C	28 °C	40 °C
Limonene t_m	0	0	0
Methyl nonanoate	0.31	0.33	0.27
Dodecanal	0.45	0.61	0.39

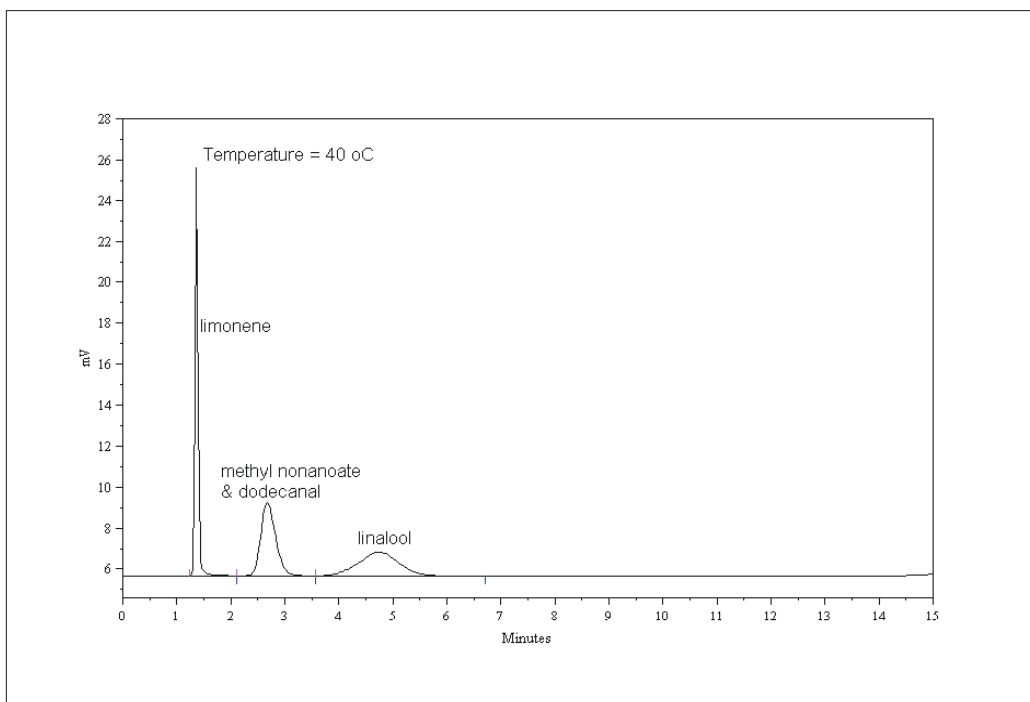


Figure 5.4. SFC_{PLOT} group separation. Pressure 110 atm and temperature 40 °C.

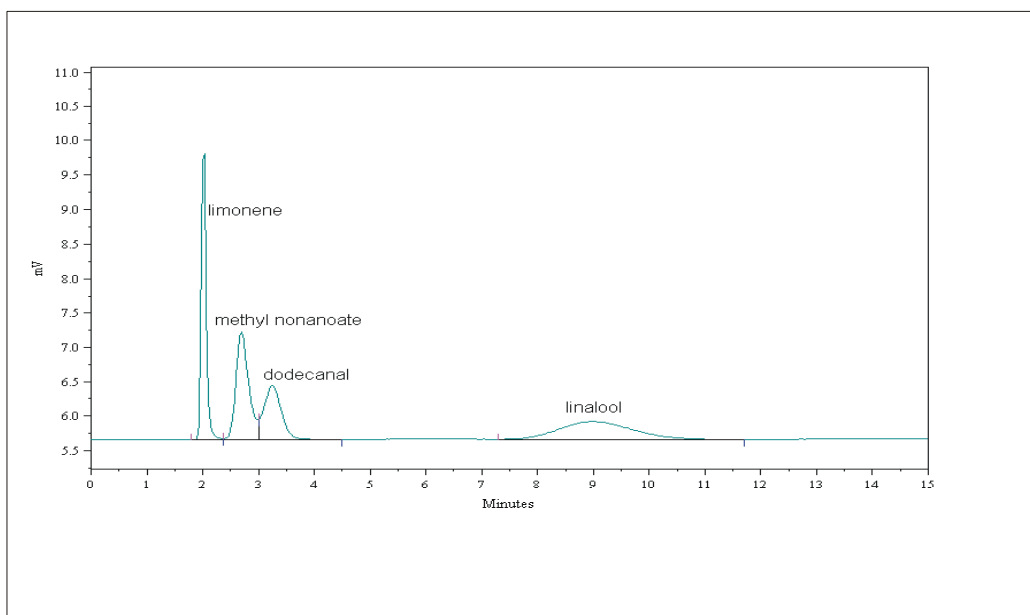


Figure 5.5. SFC_{PLOT} group separation. Pressure 110 atm and temperature 28 °C.

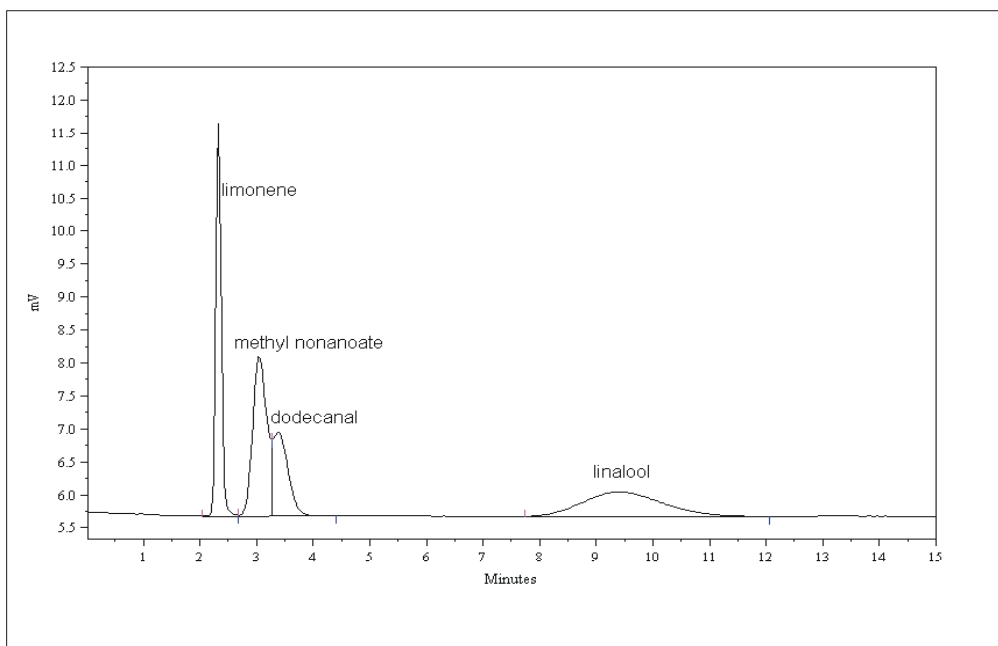


Figure 5.6. SFC_{PLOT} group separation. Pressure 110 atm and temperature 20°C.

5.3.3 Effect of pressure on SFC group separation

While density is the fundamental property that influences solvation strength of a supercritical fluid, pressure is the physical property that is directly measured by supercritical fluid delivery systems. In SFC retention of the solute is closely related to the increase in the solvent strength of the mobile phase during the run. At fixed temperature, when the pressure is increased, the solvent strength of the mobile phase increases as the density increases.

5.3.3.1 Experimental

A standard sample mixture containing compounds of different chemical classes was prepared into 500 μl CS_2 solvent and injected at different pressures and a constant temperature of 28 °C to investigate the effect of the mobile phase pressure on group-type separation by SFC_{PLOT} column. Table 5.2 shows the standard compounds prepared.

Table 5.2. Different chemical compound class standard prepared for studying the pressure effect on SFC_{PLOT} group-type separation.

Hydrocarbon	Ester	Ether	Aldehyde	Alcohol
Limonene	Methyl nonanoate	Iso- amyl ether	Dodecanal	Linalool

5.3.3.2 Results and Discussions

Figure 5.7 to 5.11 shows the SFC group separation chromatograms of the standard sample mixture of different compounds. Under the chromatographic conditions mentioned above, limonene (terpene hydrocarbon) was eluted in the first fraction because of less molecular interaction with the silica gel PLOT column surface. Methyl nonanoate (ester) and iso-amyl ether (ether) co-elute in the second fraction while the aldehyde compound dodecanal eluted in the third fraction. An alcohol, linalool eluted last due to the strong retention on the silica-gel surface.

With a silica-gel PLOT column, hydrocarbons are well separated from the carbonyl and oxygenate compounds because of the differences in polarity of the stationary phase and the mobile phase. At lower pressures, below 120 atm, methyl nonanoate and iso-amyl ether are better separated from dodecanal. Compounds are ordered according to the functional group i.e. group selectivity is strongly expressed. The situation becomes slightly worse as the pressure increases. The resolution of methyl nonanoate and iso-amyl ether with dodecanal is slightly reduced.

It appears from the observations in figure 5.7 to 5.11 that analytes elute earlier as the solvent strength of CO₂ increases with increased pressure as expected (also because of a slight increase in mobile phase linear flow rate). In this case, the use of density programming of the SFC mobile phase like temperature programming in GC and gradient elution in LC will solve the problem. The group selectivity between ester and aldehydes seems better at lower pressure, so it could be better to start the separation at

lower pressures and ramp to higher pressure gradually. By increasing the pressure using the pressure or density programming, both flow rate and the solvent strength will be enhanced to elute certain compounds. Thus, polar compounds that are strongly retained on the phase can be chromatographed in relatively short time without the loss of resolution for the earlier eluting compounds.

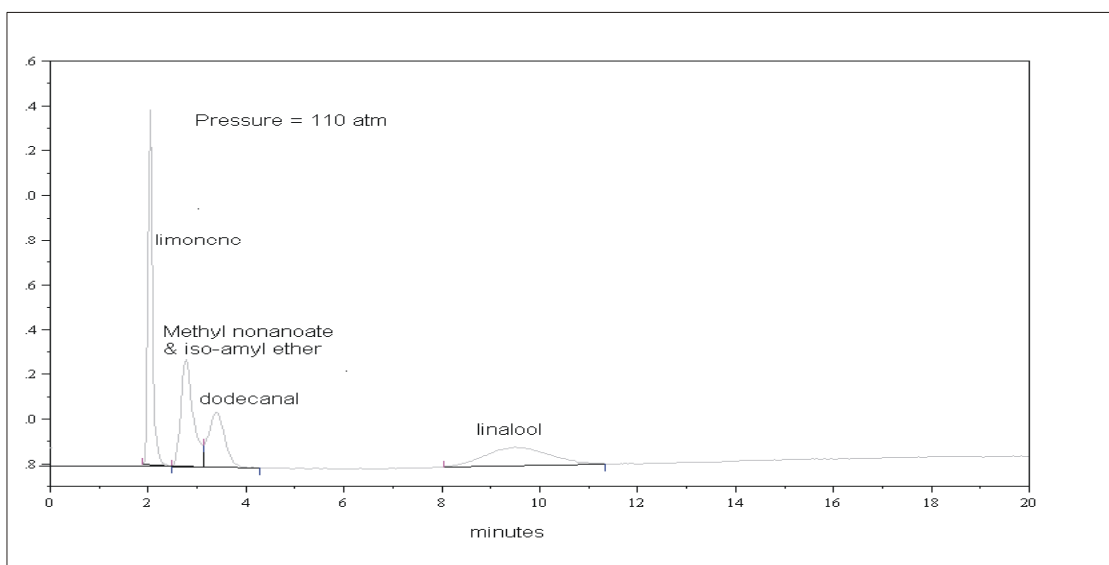


Figure 5.7. SFC_{PLOT} chromatogram of standard sample at pressure 110 atm.

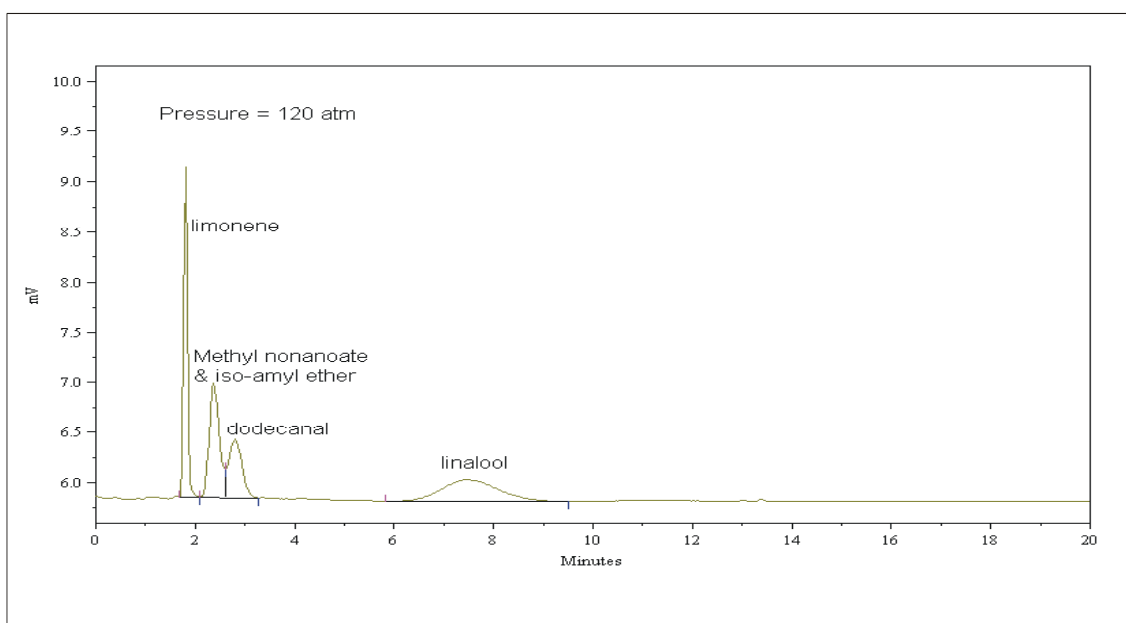


Figure 5.8. SFC_{PLOT} of standard sample at pressure 120 atm.

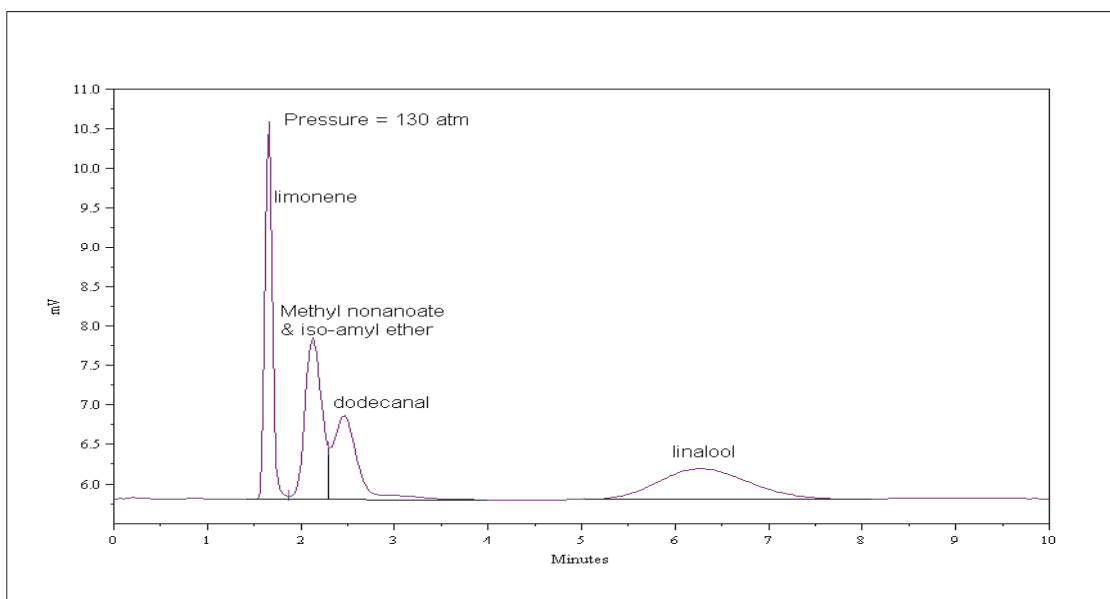


Figure 5.9. SFC_{PLOT} of standard sample at pressure 130 atm.

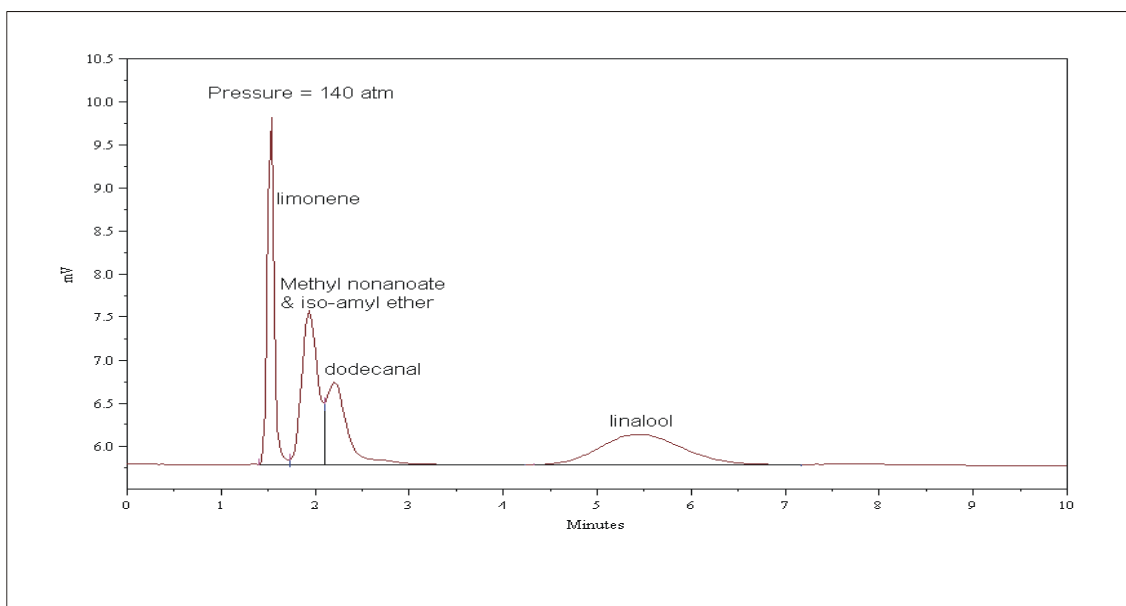


Figure 5.10. SFC_{PLOT} of standard sample at pressure 140 atm.

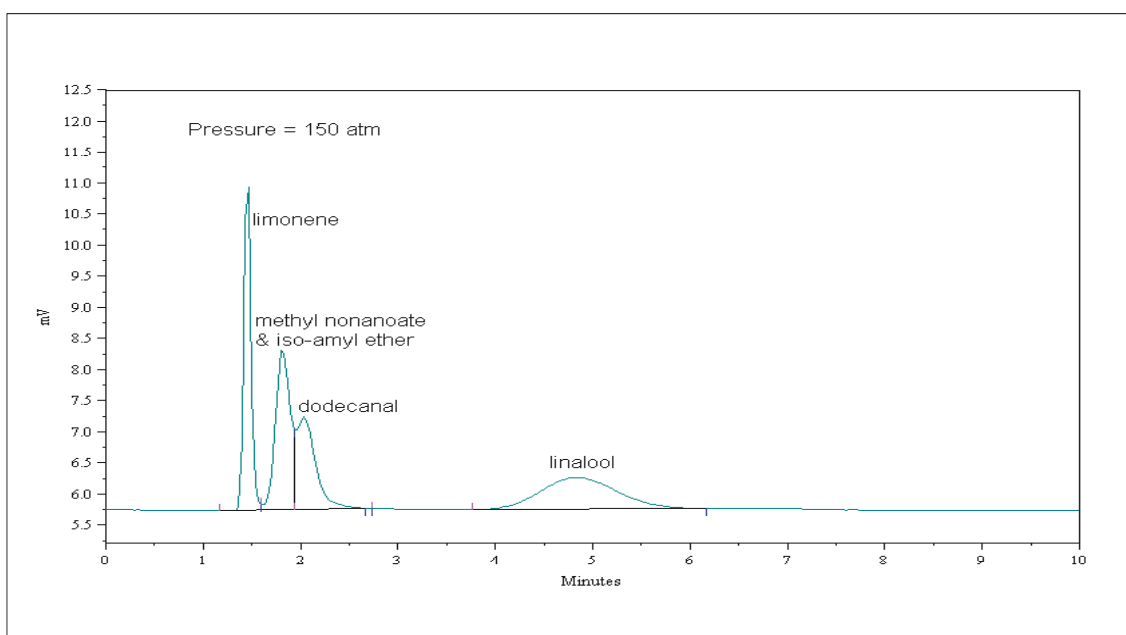


Figure 5.11. SFC_{PLOT} of standard sample at pressure 150 atm.

5.3.4 Analysis of essential oil sample

5.3.4.1 Experimental

Five pure samples of essential oils (*Tagetes minuta*, *Artemisia afra*, *Pelargonium raden X capitatum*, *Cymbopogon citratus*, and *C. flexuosus*) were obtained from the Lowveld College of Agriculture. A 0.2 μ l of real sample was injected undiluted into the optimized SFC for group separation. SFC conditions for group separation of the oil samples were used as outlined below.

Table 5.3. SFC_{PLOT} optimized chromatographic conditions for group separation of essential oil samples

Temperature	28 °C
Pressure	110 atm
Flow rate	480 ml/min (measured at restrictor outlet)

5.3.4.2 Results and Discussions

Four essential oils samples : *Cymbopogon citratus* and *C. flexuosus*, *Tagetes minuta*, *Artemisia afra* and *P. radens X capitatum* were successfully separated into different chemical classes of the terpene hydrocarbons and the oxygenates on a silica-gel PLOT column. The oxygenate derivatives were further separated into aldehydes, ketones, esters and alcohols using the CO₂ mobile phase. Figure 5.12 to 5.15 shows the SFC_{PLOT} column chromatograms of the four different oils. The isobaric pressure of 110 atm and constant temperature of 28 °C conditions indicated in table 5.3 were used for this analysis. A common observation about all four oils is that they all contain terpene hydrocarbons, esters, ketones and aldehydes.

The absence of the alcohol chemical class in *T. minuta* and *A. afra* oils distinguish them from the other two oils (*Pelargonium* and *Cymbopogon*). This information could easily be obtained by SFC with a silica-gel PLOT column. The separation of compounds into different chemical classes is its main advantage compared to other separation techniques. Under the separation conditions in table 5.3 the elution order of the chemical classes is based on the polarities of the solutes and is similar to that in normal-phase liquid chromatography.

The compounds which belong to the same chemical class should ideally produce a single peak on the SFC with FID quantitation. Fraction I belongs to the terpene hydrocarbons group, the least retained on silica-gel PLOT column, followed by the carbonyl compounds. Information such as the quantitative amount of each group in the oil can readily be obtained in SFC. Note that in fig 5.14 and table 5.4 lemongrass shows a higher percentage of alcohols than other oils. This cannot be as this oil mainly consists of the aldehydes neral and geranial: more about this contradiction in chapter 6. This is consistent with the SFC data shown by Yamauchi where neral and geranial elute slightly before terpineol from the short (5 cm) packed silica gel column with CO₂ as mobile phase.

Table 5.4. Qualitative chemical group analysis in *Artemisia afra*, *Tagetes minuta*, *Pelargonium* and *Cymbopogon* oils. (SFC Temperature = 28 °C and Pressure =110 atm)

Fraction	RT(min)	Chemical Class	Artemisia	Tagetes	Pelargonium	Cymbopogon
1	0-2.5	Terpenes	7.08	61.17	28.87	5.29
2	2.5– 12.0	Esters	16.87	5.99	18.20	0.40
		Ketones	61.170	5.48	8.90	4.57
		Aldehydes	14.88	27.42	12.09	2.75
3	>12.0	Alcohols			31.94	86.99

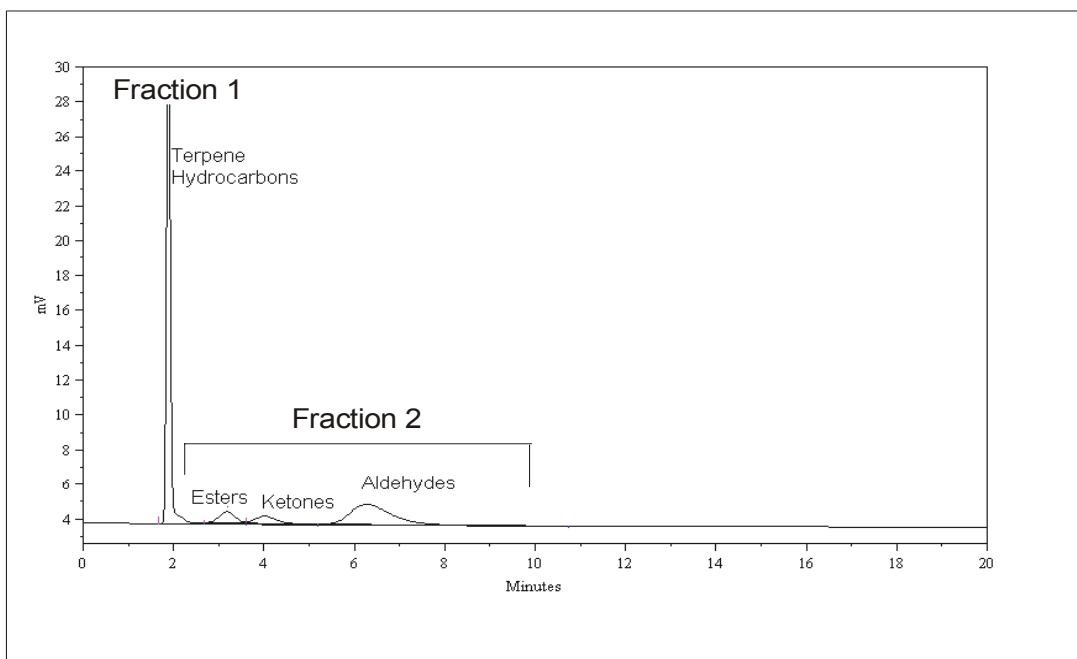


Figure 5.12. SFC_{PLOT} chemical class separation of *Tagetes minuta* oil.

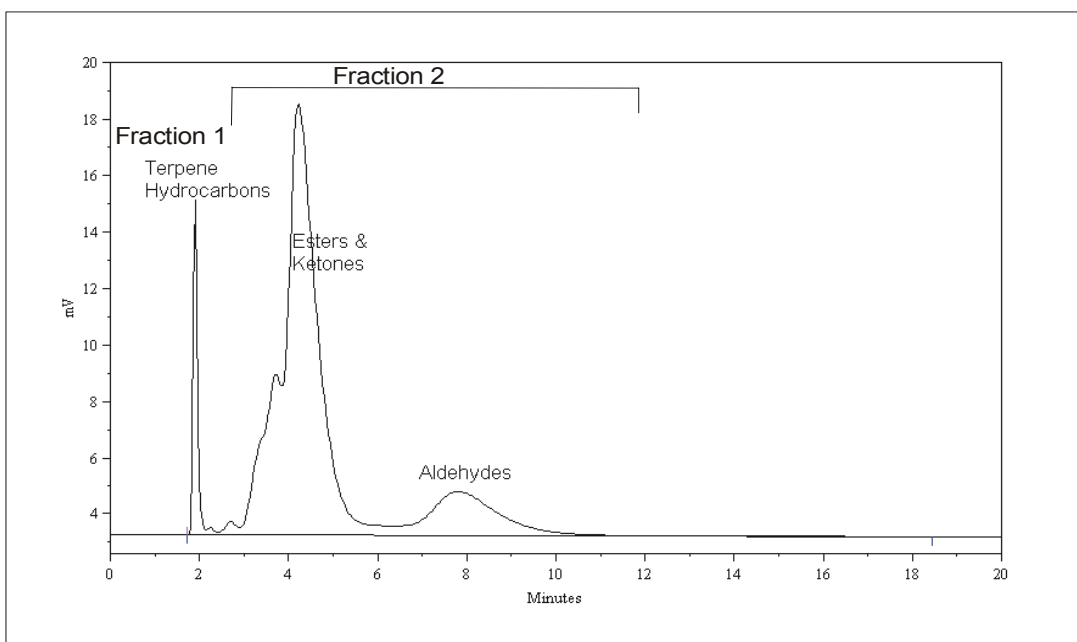


Figure 5.13. SFC_{PLOT} chemical class separation of *Artemisia afra* oil.

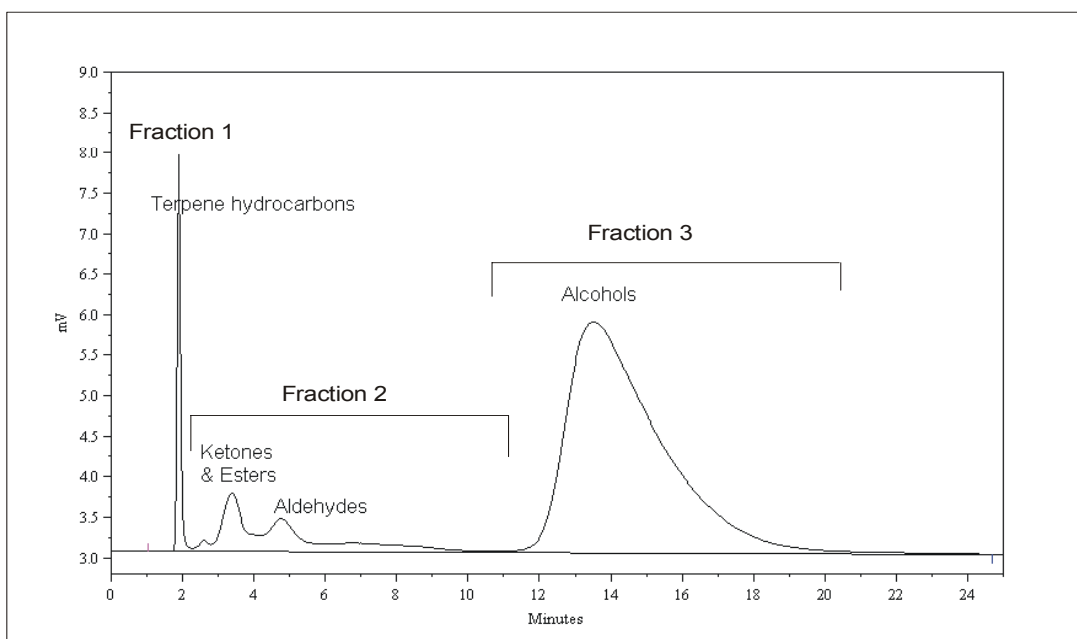


Figure 5.14. SFC_{PLOT} chemical class separation of *Cymbopogon* essential oil

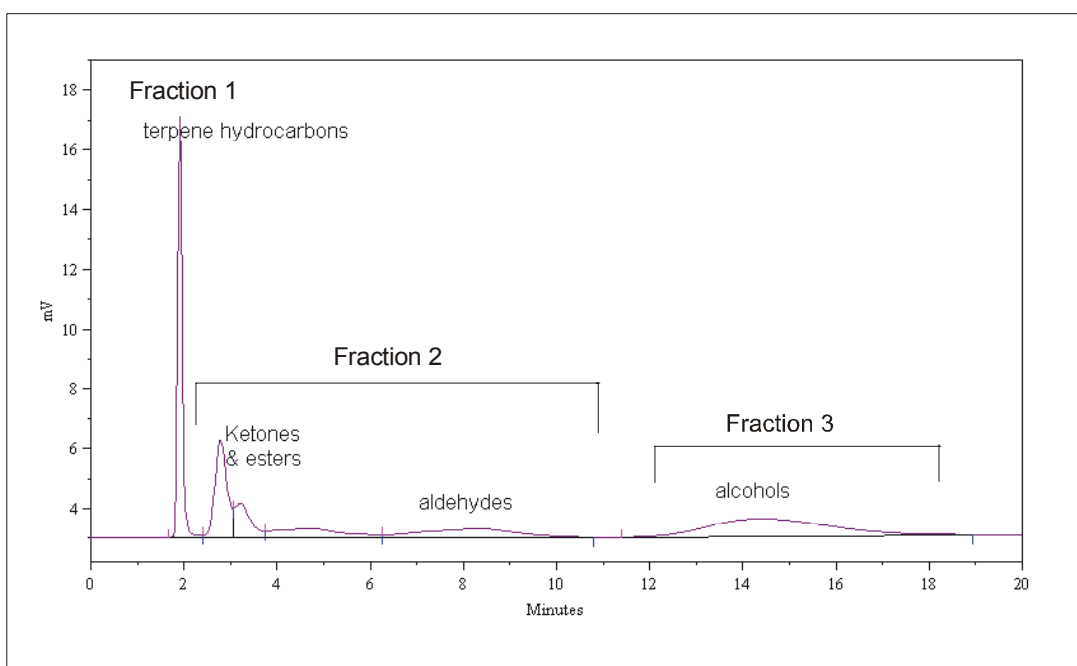


Figure 5.15. SFC_{PLOT} chemical class separation of *Pelargonium radens X capitatum* essential oil .

5.4 Conclusion

The results presented in this chapter shows that supercritical fluid chromatography with non-polar CO₂ mobile phase on a silica gel PLOT column is an appropriate technique for group-type separation. By using a silica-gel PLOT column, essential oil samples of *Cympogon flexuosus* and *C. citratus*, *Tagetes minuta*, *Artemisia afra*, and *P. radens X capitatum* are separated into terpenic hydrocarbons and oxygenate groups. The oxygenates are further separated into esters, aldehydes, ketones, aldehydes and alcohols. Group-type separation on a silica-gel PLOT column with SFC is obtained without the use of (1) the modifier or (2) backflush method.

For better group separation, it is important that the flow rate, separation pressure and temperature are investigated or optimized. Slightly, better group separation was found at a pressure of 110 atm and the near critical temperature of 28 °C. A flow rate of 7.7 cm/s [480 ml/min (atm) after expansion] was used to provide acceptable analysis times. The Van Deemter curve showed that higher chromatographic performance can still be expected at lower flow rates from the 0.32 i.d. PLOT column (optimum SFC is normally performed in 100 µm i.d. columns or smaller). Although a thorough and systematic optimization was not our aim, convenient separation conditions could be found to couple the PLOT silica gel SFC group separation system to a second dimension GC as reported in the next chapter.

5.5 References

1. M.L. Lee, K.E. Markides, *Analytical SFC and SFE*, ed. 1990, Chromatography Conferences Inc., Provo, Utah.
2. M.Saito, Y.Yamauchi, T. Okuyama, *Fractionation by Packed-Column SFC and SFE Principles and Applications*, 1994, USA, VCH, publishers
3. E.J. Guthrie, H.E. Swartz, *J. Chromatogr. Sci.*, 24 (1986) 236-241
4. C.M. White, *Modern Supercritical Fluid Chromatography*, (Edited by C.M. White), *Chromatographic Methods*, Heidelberg, Bsel, New York: Huthig, 1988, 189-210
5. T.A. Berger: In *Supercritical Fluid chromatography with Packed-Column, Techniques and Application* (K. Anton, T. Berger eds.), *Chromatographic Science Series*, Vol.75 Chapter 2, 19-58, Marcel Dekker, New York, 1997.
6. J.W. Helgeth, M.G. Fessehaie, L.T. Taylor, *Chromatographia*, 25 (1988) 172-177.
7. C.H. Kirschner, L.T. Taylor, *HRC.*, 17 (1994) 61-67.
8. P. Petrsson, N. Lundell, K.E. Markides, *Chromatographia*, 35 (1993) 486-492.
9. J.P. Foley, J.A. Crow, *ACS symp. Ser.*, 488 (1992) 304.
10. M.P. Squicciarini, *J. Chrom. Sci.*, 34 (1996) 7.
11. T. Takagi, T. Suzuki, *J. Chromatogr.*, 625 (1992) 163.