CHAPTER 4

SUPERCRITICAL FLUID CHROMATOGRAPHY

4.1 Introduction

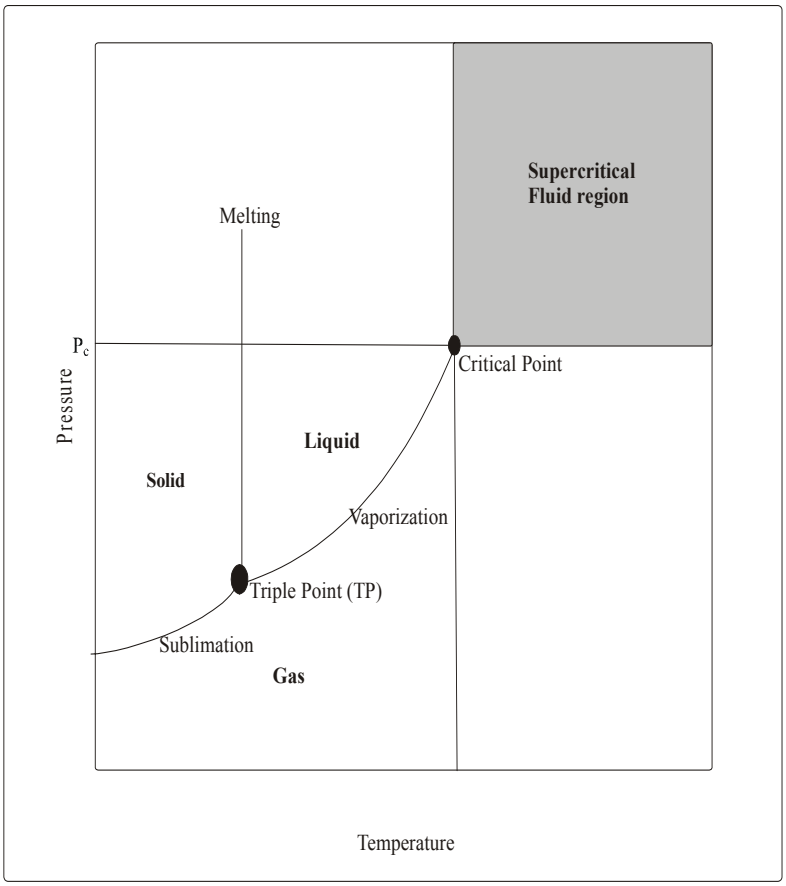
Chromatographic separation of highly complex samples is often impossible using single columns in a one-step separation process. The same problem applies to essential oil analysis. The complex molecular composition of many essential oils demand that high-resolution separation techniques be used to characterise the range of compounds making up the oils. Since essential oils are composed of different chemical classes such as terpene hydrocarbons, esters, ketones, aldehydes and alcohols, it will be advantageous to employ a separation technique that has capabilities of chemical class separation prior to high-resolution analysis. SFC can be used to offer improved group separation of complex mixtures of compounds compared to liquid chromatography on normal phase columns. The separation of complex sample mixtures such as essential oils into different chemical classes is one of the best methods to reduce the sample complexity. In Chapter 5 and 6 of this thesis, group-type separation of essential oils by SFC is reported.

4.2 Separation with supercritical fluids

4.2.1 Definition

A substance is said to be in the supercritical fluid state when heated above its critical temperature and compressed above its critical pressure and is referred to as a supercritical fluid. This can be seen as a very dense gas with a solvent strength comparable to that of liquids.

Figure 4.1 is a typical phase diagram for a pure substance that shows the temperature and pressure region where the substance occurs as a single phase (solid, liquid or gas)[1]. Three curves describe sublimation, melting and vaporization processes. They intersect at a point known as the triple point (TP). At this point the three phases co-exist in equilibrium. In this region phase transitions take place when the temperature and/or pressure of the substance are changed. The vaporization curve starts at the triple point (TP) and ends at the critical point (CP) with co-ordinates, the critical pressure P_c and critical temperature T_c. The melting point curve starts at the triple point and rises steeply with increasing temperatures and pressures. Above the critical point the liquid and gas have the same density and no longer exist as separate phases. A further increase in pressure will result in an increase in density but no phase transition will take place. By increasing the temperature at constant pressure above the critical point there is a continuous transition from liquid to supercritical fluid or from gas to supercritical fluid by increasing the pressure of a gas at constant temperature. The region of pressures and temperatures above P_c and T_c in figure 4.1 is called the supercritical region and in this region a substance is said to be in a supercritical phase[1]. Table 4.2 lists the critical pressure and temperature for various solvents including the fluid density at the critical point known as the critical density (p_c).



Fiqure 4.1. Typical (solid-liquid-gas -supercritical fluid) phase diagram

Table 4.1 Comparison of the properties of supercritical CO_2 and those of gases and Liquids[1].

		Density (g/cm^3)	Viscosity (g/cm·s)	Diffusion coefficient (cm^2/s)
Gases		0.0001-0.002	0.0001-0.0003	0.1-0.4
Supercritical CO_2	T_c, P_c	0.47	0.0003	0.0007
	T_c, $6P_c$	1.0	0.001	0.0002
Liquids		0.6-1.6	0.002-0.03	0.000002-0.00002

4.3 Supercritical fluid properties

Supercritical fluids exhibit physico-chemical properties intermediate between those of a liquid and a gas. SF properties include solvation, viscosity and diffusion coefficients. They are all influenced by density which is a function of applied pressure and temperature. These properties can be altered over a wide range by changing the pressure, the temperature or both simultaneously. A high density is responsible for increased solvating power of SFs, where interactions between the fluid and the solute molecules increase. At high densities, SFs have solvent strengths approaching those of liquids and they can dissolve many different types of solutes including thermally liable or high molecular mass and non-volatile compounds. Due to the lower densities of gases, they have no solvent action.

Supercritical fluids have more favourable hydrodynamic properties than those of liquids because supercritical fluid viscosity values are more like those of gases[2]. As a mobile phase in chromatography, gases have the fastest, liquids have the slowest and SFs have intermediate optimum flow rates. This is due to higher diffusion coefficients of analytes in gases as compared to supercritical fluids. Compared to LC, faster flow rates in SFC give rise to shorter analysis times. The diffusion coefficients of solutes in SFs are between those displayed for liquids and gases.

The solvent strength of supercritical fluids as a mobile phase in chromatography increases with compression. Densities may even approach those of liquids. The solvent strength depends on the average intermolecular distance, as defined by the density of the fluid. For liquid solvents, the density is generally constant with pressure and changes in the intermolecular distances of specific solvents can be considered negligible. The solubility power (δ), which was first introduced by Hildebrand and Scott[3] as a relative scale for solvent strength and a function of the cohesive energy density, c, is given as:

$$\delta = c^{\frac{1}{2}} = (\Delta u^{evp} / v)^{\frac{1}{2}} \qquad \textbf{[4-1]}$$

Where Δu^{evp} is the evaporation energy and v is the molar volume. Giddings *et. al.*[4] extended this theory for its application in representing the solvating power of supercritical fluids as :

$$\delta = 1.25\, P_c^{\frac{1}{2}} (P_r / P_{r,liq}) \qquad \textbf{[4-2]}$$

where P_c is the critical pressure, P_r the reduced density of the substance in the supercritical state and $P_{r,liq}$ is the reduced density of the substance in the liquid state.

The density of the mobile phase is the most important parameter to influence and optimise for separations in SFC. Density programming during an analytical run is as common in SFC as temperature programming in GC or programming of eluent composition (gradient elution) in HPLC[5]. The influence of density on the solvent properties is demonstrated using the concept of the solubility parameter, first introduced by Hildebrand and Scott[3]. The solubility parameter values vary from 0 up to liquid-like values of 10 at high densities. To solubilize a solute compound, the solubility parameters of both the solute and solvent need to be of equal values. To use a SF as solvent, the pressure of the fluid must be higher than the critical pressure, at which the density becomes similar to that of the liquid[2].

4.4 Supercritical Fluid Chromatography

Supercritical fluid chromatography (SFC) is defined as a form of chromatography (i.e. a 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 to or above the critical point for the purpose of enhancing the mobile phase solvating power[6]. The use of SFs as a chromatographic mobile phase was first reported in 1962 by Klesper[7].

Various fluids are used as supercritical mobile phases, for example, carbon dioxide, ammonia , sulfur dioxide , alcohols , chlorofluoromethanes and low boiling hydrocarbons. **Table 4.2** lists the critical pressure and temperature for various solvents including the fluid density at the critical point known as the critical density (p_c). The solvent properties of SFs that are relevent to chromatography are the critical temperature, critical pressure and polarity. Any specific solute-solvent intermolecular interaction such as hydrogen bonding which can enhance solubility and selectivity in a separation can be used to alter selectivity.

Early developments in SFC were slow due to the experimental problems in using supercritical fluids, lack of commercially available SFC instrumentation and it being overshadowed by the simultaneous growth of LC[8]. The resurgence of interest in SFC was driven by the potential advantages afforded by the unique characteristics of the mobile phase in SFC over GC and HPLC, and more importantly it has been augmented by advanced technology in pumps and detectors for SFC.

The rapid mass transfer in a supercritical fluid mobile phase also attracted interest from researchers because it offers high speed separation capabilities. It has been used successfully with high resolution open-tubular capillary columns with internal diameters smaller than 100 μm which was not so successful in liquid chromatography due to slow mass transfer and high back pressures. SFC has also been achieved succesfully on packed capillary columns with internal diameters smaller than 1 mm[9]. One of the principal benefits of SFC is the flexibility of using both GC and HPLC detectors. For inorganic mobile phases such as carbon dioxide, ammonia and xenon, the universal flame ionization detector (FID) is commonly chosen. Modification of these supercritical fluids with more polar organic substances must be avoided since the modified phase will give a response in the FID. The absence of suitable pure supercritical fluids of high solvent strengths has probably been the main reason for a steady loss of interest in SFC.

Table 4.2 Features of various solvents at critical temperature, pressure and density.[10]

Solvents	T_c (°C)	P_c (bar)	ρ_c (g/mL)
Inorganic			
Carbon dioxide	31.1	72	0.47
Dinitrogen monoxide	36.5	70.6	0.45
Ammonia	132.5	109.8	0.23
Water	374.2	214.8	0.32
Sulfur hexafluoride	45.5	38	
Helium	-268	2.2	0.07
Xenon	17	56.9	1.11
Hydrocarbons			
Methane	-82	46	0.169
Propane	96.7	42.4	0.22
Ethylene	11	50.5	0.2
Benzene	288.9	98.7	0.302
Toluene	319	41.1	0.292
Alcohols			
Methanol	239	78.9	0.27
Isopropyl alcohol	235.3	47.6	0.273
Ethers			
Ethyl methyl ether	164.7	47.6	0.272
Tetrahydrofuran	267	50.5	0.32
Halides			
Trifluoromethane	26	46.9	0.52
Dichlorodifluoromethane	111.7	109.8	0.558
Chlorotrifluoromethane	28.8	214.8	0.58
Trichlorofluoromethane	196.6	28.9	0.554
Miscellaneous			
Acetonitrile	275	47	0.25
Pyridine	347	56.3	0.312

Compared to GC, capillary SFC can provide high resolution chromatography at much lower temperatures and allows fast analysis of thermally labile or high molecular mass compounds. Although GC and HPLC complement each other, they together are unable to cover all needs. A set of problems falls between the capabilities of GC and HPLC[11] :

1. *the analyte is either not volatile enough for GC or is thermally unstable and the analyte is present in a very complicated mixture (requiring a high efficiency separation) or*
2. *the analyte is either not volatile enough for GC or is thermally unstable and the analyte cannot be detected well enough in liquid mobile phase.*

In these cases, both HPLC and GC fail. The ideal technique to fill the gap should have a low temperature, solvating mobile phase of programmable strength, high chromatographic efficiency, and universal detection. SFC when used with CO_2 as mobile phase, is compatible with FID and has the charateristics needed to fill the gap with only one major restriction. The solute will have to have some minimum solubility in an FID compatible mobile phase[12]. Also the non-polarity of the CO_2 mobile phase poses some restrictions on analysing more polar compounds on packed silica gel columns[13].

4.4.1 Retention behaviour in SFC

The final resolution obtained in a chromatographic separation is a function of column efficiency, selectivity and retention as stated by the well known master resolution equation:

$$R_s = \frac{\sqrt{n}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right) \qquad \textbf{[4-3]}$$

where α, k are not constant along the column in SFC packed columns due to the pressure drop[14]. k is the retention factor and α is the relative retention also known as the selectivity.

The understanding of the solute retention mechanism of SFC is dependent on determining the complex interaction between multiple chemical processes. These physico-chemical processes involve the temperature, pressure, density and intermolecular interactions of the solute molecule with the mobile phase and stationary phase.

The density of a supercritical fluid is the parameter that determines the solvation power of the mobile phase. If the density increases, then the solvent strength of the fluid is increased. Density programming during the analytical run is as common in SFC as temperature programming in GC or programming of the eluent composition (gradient elution) in HPLC. Pressure is also one of the fundamental properties that influences solvation strength of a fluid. Pressure is the physical property that is directly measured by supercritical fluid delivery systems. At a fixed temperature, when the pressure is increased, the density and solvent strength of the mobile phase increases. On the other hand, an increase in temperature at a fixed pressure causes the solvation strength of the fluid to decrease as the density decreases and this in turn increases the retention times. Temperature has an important influence on selectivity as far as group-type in SFC on silica gel is concerned. It has been shown that group-type separation strongly depends on temperature and that the best group separation is obtained at low temperatures[15].

4.4.2. Packed *vs* Capillary SFC columns

Both packed and capillary columns can be used in SFC to elute a wide range of compounds with some modifications on the system to suit conditions for a particular column. The total surface area of the packing in a packed column is much greater than the surface area of the capillary tube, giving it a larger sample capacity. Larger amounts of analytes can be separated and can be collected. Due to the shorter diffusion interparticle distances in packed columns, higher linear flow rates may be used.

Packed columns generate a greater number of theoretical plates per unit length, which-together with the high linear flow rates permits faster analysis than in a 50-100 μm i.d. capillary column. Due to smaller channel dimensions, decrease in the number of theoretical plates with an increase in flow rate is less for packed than for capillary columns[16]. For constant column dimensions, the pressure drop along a SFC column is typically ten times smaller than in liquid chromatography, however, ten times greater than in gas chromatography. The primary advantage of capillary columns in SFC is that they offer a greater number of theoretical plates than packed columns due to the long lengths that can be used with a given pressure drop. The low pressure drop and open-tubular nature of the capillary column allow very long columns to be employed, whereas only a limited length for packed columns can be used due to the high pressure drop occuring. However, Berger[5] demonstrated that a very high number of theoretical plates may be achieved by coupling eleven packed-columns in series and 220 000 theoretical plates were achieved with early-eluting peaks producing up to 298 plates/second.

There are two general types of partition methods in liquid chromatography unlike in SFC: reversed-phase and normal-phase. In reversed-phase LC, the stationary phase is non-polar (chemically modified silica) and the mobile phase is polar (water + organic modifier). This is an excellent set-up for solubilizing and separating polar solutes. In normal-phase LC, the stationary phase is polar (silica or chemically modified surface)

and the mobile phase is non-polar (hexane, ether). Nowadays packed columns (such as normal phase LC chiral columns) are widely applied in SFC for racemic mixture separation. Packed column SFC is considered to be a suitable replacement for normal-phase liquid chromatography, mostly for the separation of polar compounds. In normal-phase LC and in SFC silica gel and porous silica are often used. This silica gel is made by poly-condensation of silicic acid[18]. Further gelation and drying of silica gel leads to porous silica particles (microbead), available as spherosil, porasil or others. They often contain many surface silanols which are removed by heat treatment or deactivation agents such as inorganic salts[19].

In reversed phase packed column SFC two types of sites contribute independently to retention with non-polar supercritical fluids such as carbon dioxide. These are the surface of chemically bonded packings which is always heterogenous, containing different concentrations of chemically bonded and free silanol groups[20]. It has been established that the interaction of sample proton donor / acceptor and dipolar functional groups with free silanol groups of the column packings causes the characteristic peak tailing and sample adsorption or degradation that occurs in packed column SFC with relatively non-polar fluids[21,22].

4.4.3 Group separation by PLOT column

Essential oils contain oxygenates such as aldehydes, ketones, esters and alcohols. These compounds have large retention factors on silica gel and are therefore difficult to elute. However, with a backflush method[13] or the addition of the modifier to the pure supercritical CO_2 mobile phase[21] it is possible to elute them. Alternatively, it has been shown that the reduced phase ratio of a porous layer open-tubular (PLOT) column allows elution of oxygenates without modifier or back-flush methods[22].

Because of the relatively strong interaction of the sorbents inside PLOT and packed columns with polar molecules of the analyte, the kinetics of adsorption and desorption

is slower for the polar than for the less polar analytes. Thus, the column efficiency for less polar molecules would be higher than for polar molecules[23]. Column selectivity is classified approximately by three types of interaction of sorbent with analyte: size, strong dipole (polarity) and polarizability. Interaction of analyte with porous silica PLOT columns involves polarizability selectivity. Therefore, the separation of polar molecules such as light alcohols, thiols, esters, ketones, ethers and aldehydes can be achieved[24].

The capability of a PLOT column to separate the oxygenates has been demonstrated [25]. Figure 4.2 shows the difference between the packed and PLOT column in terms of the dimensions and phase ratio. The phase ratio, β, is defined as the amount of stationary phase relative to mobile phase.

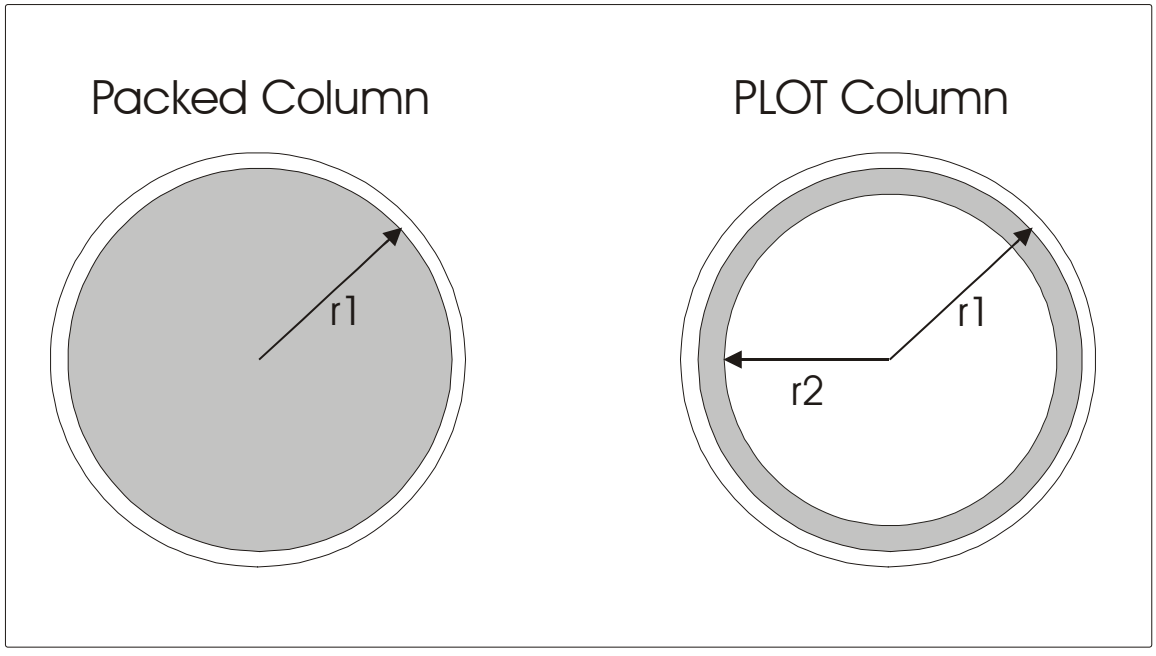


Figure 4.2 Schematic diagram showing the phase ratio difference in Packed and PLOT (specifically 0.3 mm id, 1.5 μm porous layer) columns[25]. r1 (Packed) = 1 mm, r1 (PLOT) = 0.1500 mm and r2 (PLOT) = 0.1485 mm.

In PLOT columns most of the central volume available contributes to the volume filled with the mobile phase, this reduce the phase ratio. Since k= βK, retention factors are reduced 116 times[25]. The reduction in **k** makes it possible to elute polar oxygenated compounds with pure CO_2 on silica gel in PLOT columns.

4.5. Conclusions

The advantages of supercritical fluids as mobile phase in chromatography have been shown in this chapter. These include the capability to work at room temperatures to express group-type separation of compounds at a given pressure range. It is important to note that separation temperature plays a role in order to achieve group separation. Group-type separation can be very important if applied to very complex mixtures, thus reducing sample complexity prior to second dimension analysis. With the opportunity to work with density programming, the mobile phase solvation strength can be manipulated. All these advantages of SFC will be applied in chapter 5 and 6 for group-type separation on essential oil samples.

4.6 References

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