

Chapter 5

Supercritical Fluid Chromatography: Theoretical considerations

5.1 Introduction

In petrochemical analysis it is often not necessary to identify every compound in a sample. The boiling point distribution and group composition, e.g. aliphatic and aromatic groups or straight chain versus branched alkanes determine, to a large extent, the physical characteristics of fuels. The oxygenated group content of a fuel is another important parameter. It is believed that high oxygen content enhances fuel combustion and reduces harmful emissions, but oxygenates also end up as pollutants in water supplies. More effective ways of oxygenate analysis are required, both for fuel characterization and environmental monitoring.

Group selectivity and group resolution differs from the familiar definitions of selectivity and resolution. To obtain group resolution all members with the target functionality should elute close to one another without members of other groups in between. Quite often the resolution between individual members of a group is sacrificed. This type of separation is best achieved when the difference in polarity between the stationary and mobile phases is the greatest. Since polar mobile phases have impractical critical parameters, group separation is generally performed using a polar stationary phase. A very popular combination is packed column silica gel with sub-critical CO₂ as eluent. This separation of poli- aromatic hydrocarbons with SFC is an established method¹. With CO₂ as mobile phase, oxygenates and other polar compounds have unacceptably

high retention factors on silica gel. It has been shown that some oxygenates can be recovered when the column is back-flushed² but usually polar modifiers such as methanol are used to reduce the polarity of the stationary phase for oxygenate analysis. Unfortunately the use of modifiers precludes the use of a flame ionization detector.

This chapter provides background information on SFC in general and on group separation using packed polar columns. The ideas developed here are applied to both packed and porous layer open tubular columns in Chapter 6

5.2 Supercritical fluids defined

A supercritical fluid is a highly compressed gas that cannot be condensed into the liquid phase, yet with high density and therefore appreciable solvation strength. In more exact terms: A substance that is heated above its critical temperature and compressed above its critical pressure is said to be in the supercritical state and is referred to as a supercritical fluid.

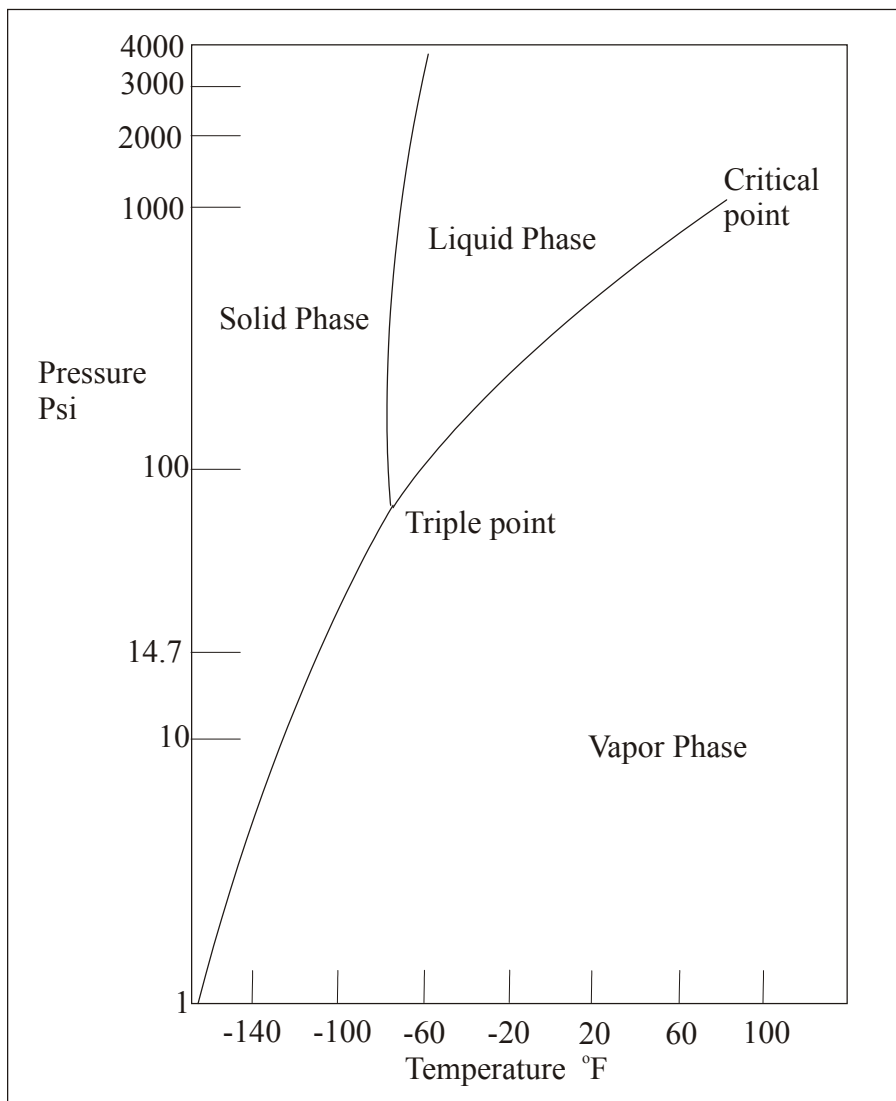
When a liquid sample is heated in a sealed container, the density of the vapor increases because it is confined to a fixed volume. The density of the liquid decreases when heated until the two densities are equal and the interface between the two phases disappear. This temperature where the interface between the two phases disappear, is called the critical temperature (T_c). The corresponding vapor pressure is called the critical pressure (p_c)^{3,4,5}.

Figure 5-1 shows a temperature-pressure phase diagram of carbon dioxide.

The solid, liquid and gas phases are indicated. Below the critical point, phase transitions take place whenever, by changing the temperature or pressure, a solid line is crossed. Above the critical point the liquid and vapor 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.

Above the critical point there is a continuous transition from liquid to supercritical fluid by increasing the temperature at constant pressure or from gas to supercritical fluid by increasing the pressure of a gas at constant temperature.

Figure 5-1: Phase diagram for Carbon dioxide.



5.3 Supercritical fluids as mobile phase in chromatography.

Concise and useful information on the use of supercritical fluids as mobile phases in chromatography is available in the literature^{4,6}.

Properties such as solvation, viscosity and diffusion coefficients of supercritical fluids are intermediate between those of liquids and gasses, making SFC superior for some

separations despite kinetic advantages of gas chromatography and the greater flexibility of solvents available to HPLC.

Solvation, viscosity and diffusion coefficients in SFC depend on density, which is a function of the applied pressure and temperature.

5.3.1 Diffusion coefficients

Despite the fact that diffusion coefficients in SFC are closer to those of liquids than gases, much faster separation times compared to liquid chromatography can be achieved. Minimum plate heights for packed column SFC and liquid chromatography are similar except that the same plate height in SFC is reached at a faster flow rate.

Because of increased diffusivity of solutes in supercritical fluids compared to liquids, fast mass transfer between stationary and mobile phases allows much faster flow rates to be used before band broadening (column efficiency) becomes detrimental to resolution.

Column efficiency is related to flow through the Van Deemter equation, simplified as

$$H = A + B/u + Cu \quad \text{[eq 5-1]}$$

Where H is the total column efficiency also called the height equivalent of a theoretical plate (HETP), A arises due to the unequal lengths of flow channels in a packed bed and B is the contribution to band broadening due to longitudinal diffusion, C the contributions due to slow mass transfer and u is the flow rate. (Also see Chapter 3)

The greater the diffusivity, the faster the exchange of solutes between the stationary and mobile phases and the smaller the value of C . A small value of H implies a high number of theoretical plates. This increases resolution at high flow rates for SFC compared to the resolution in liquid chromatography on the same column.

5.3.2 Viscosity and pressure drop effects

High viscosities are responsible for high pressure drop due to flow resistance.

Gasses generally have very low viscosities so that the pressure drop across a column is inconsequential. Liquids experience a much higher pressure drop than supercritical fluids but, since liquids are virtually incompressible, this has little effect on separation. A large column pressure drop in SFC has the effect of decreasing the density along the length of

the column and increasing mobile phase linear velocity. A changing linear velocity means that the theoretical minimum in plate height cannot be achieved over the length of the column. An increase in pressure drop causes a decrease in density when pressure is controlled at the pump or an increase in density at the column inlet when pressure is controlled at the column outlet. In both cases, the capacity factors of solutes (k') change over the length of the column. Recent publications^{7,8} have found the effect of pressure drop on capacity ratios, selectivities and plate numbers to be negligible for most SFC systems. This was tested for pressures up to 300 atm and three 25cm packed silica gel columns connected in series.

5.3.3 Solvation

A solute in a chromatographic system is subject to a number of forces. In liquid and supercritical fluid chromatography the solvent competes with the stationary phase for interactions with the solute as it is swept along towards the exit of the column. In liquid chromatography the type of interaction and selectivity of separation can be controlled by varying the identity of the liquid. Solvent power depends heavily upon intermolecular distances, which depends on the density of the liquid. In the case of a liquid solvent the density is generally constant and the intermolecular distances of a specific solvent is fixed. Other factors that influence solvent - solute interactions are the polarizability of the solvent, the ability for a solvent to participate in dispersive and orientation interactions, to induce a dipole moment in surrounding molecules and to function as a proton donor or acceptor. Ideally in a comprehensive multidimensional approach only one of these interaction types should be active in a particular column or separation axis. In practice it is near impossible to obtain pure separation based on a single sample dimensionality (see Section 2-7) in any one axis.

The identity of solvents are also important for selectivity in SFC but since only a few compounds have critical parameters that are moderate enough to be useful and are unreactive towards solutes and instrumentation at these conditions, the choices are relatively limited. In SFC, because of high kinetic energy of molecules heated above the critical temperature, the molecular distances (density) can be controlled as a function of

pressure to be more liquid or gas-like. Since solvation is directly related to density, the solvation strength of a supercritical fluid is adjustable by density control.

The solubility parameter (δ) was first introduced by Hildebrand and Scott as a relative scale for solvent strengths. δ is a function of the cohesive energy density c :

$$\delta = c^{1/2} = (\Delta u^{evp} / v)^{1/2} \quad [\text{eq 5-2}]$$

where Δu^{evp} is the vaporization energy and v is the molar volume. Since the molar volume is inversely proportional to the density of the fluid, the solvent parameter will vary as the density varies.

This theory was extended by Giddings et al⁹ for use in supercritical fluids :

$$\delta = 1.25 P_c^{1/2} (\rho_r / \rho_{r,liq}) \quad [\text{eq 5-3}]$$

where P_c is the critical pressure(atm), ρ_r is the reduced density of the substance at the supercritical state and $\rho_{r,liq}$ is the reduced density of the substance in the liquid state.

Table 5-1: Critical temperatures, critical pressures and solubility constants at the critical state of fluids commonly used in SFC.

Compound	T_c ($^{\circ}\text{C}$)	P_c (atm)	δ (cal/cm^3) ^{1/2}
CO ₂	31.1	72.9	10.7
N ₂ O	36.5	71.9	10.6
Xe	16.6	57.7	9.5
SF ₆	45.6	37.1	7.5
Hexane	230.9	31.3	7.0

Group selectivity requires a maximum difference in polarity of stationary and mobile phases. Since polar mobile phases have very high critical parameters, polar stationary phases like silica gel and alumina are commonly used together with less polar mobile

phases for group separations. For maximum group selectivity, polar stationary phases have to be complemented with the least polar mobile phase.

Carbon dioxide is the most popular fluid used as eluent in SFC. It shows little FID response, is non-toxic, has moderate critical parameters and is relatively inexpensive. Yet it is perhaps not the ideal eluent for many separations. It is not the least polar fluid available nor is it polar enough to elute many functionalized analytes from polar stationary phases.

For CO₂ the solubility parameter is calculated to vary with density from 0 to liquid-like values of 10 (cal/cm³)^{1/2} at high pressures. This is explained by the fluid free volume. The closer the molecules are to each other, the higher will be the intermolecular interactions. While several researchers¹⁰⁻¹¹ found the solubility of carbon dioxide to be similar to hexane, Randall¹² found strong dipole selectivity tendencies. This was confirmed by Philips and Robey¹³ who added that CO₂ has proton acceptor properties.

Other fluids were also investigated as alternatives to CO₂ for group separation. Sulphur hexafluoride (SF₆)¹⁴⁻¹⁵ is a very weak solvent, less polarizable than CO₂. Separation of alkane and alkene groups without the use of a silver column was observed for samples in the gasoline boiling range¹⁶. FID detection was made possible by gold plating of the detector to withstand the corrosive action of hydrofluoric acid formed when the mobile phase decomposes in the flame.

Xenon shows comparable resolution of groups to CO₂, but has no IR absorption, providing superior Fourier transform infrared spectra of solutes¹⁷. However, this fluid was found too expensive for routine analysis.

Both these two gasses are less polar than CO₂, have moderate critical parameters (**Table 5-1**) and have low FID responses. However, both are very expensive compared to CO₂. Nitrous oxide is less compatible with the FID but was found useful for the reduction of tailing when resins are back flushed as the final step in crude oil analysis¹⁸ and might prove useful when sample components like amines react with CO₂.

5.4 Parameters affecting retention in SFC

Retention in SFC is a complex function of the experimental parameters and not as easy to explain as in gas or liquid chromatography. Retention in SFC is dependent on temperature, pressure, density, composition of the mobile phase and the stationary phase.

5.4.1 Density

The density of a supercritical fluid determines the solvation power. If there is an increase in density, then the solvation strength of the fluid is increased. This is similar to what is achieved in liquid chromatography by solvent programming using solvents with increasing solvent strength. One very important difference to solvent programming is that while the solvation power of the fluid is increased, the chemical selectivity stays unaltered. Only selectivity due to differences in solubility of analytes is affected, because only the number of molecules are altered and not their identity. Instantaneous density change over the length of the column is another difference to solvent programming but reminds one of temperature programming in gas chromatography. In fact one of the benefits of density programming is that for an optimized density program of homologs, later eluting peaks have the same width as earlier peaks in the chromatogram.

5.4.2 Pressure

While density is the fundamental property that influences solvation strength of a fluid, pressure is the physical property that is directly measured by supercritical fluid delivery systems. A number of papers^{19,20} have appeared that relate retention to pressure. Isothermal pressure programming is most often used to increase solvent strength during a run²¹. At a fixed temperature, when the pressure is increased, the solvent strength of the mobile phase increases as the density increases.

Pressure can be controlled before or after the analytical column. With most SFC instruments the upstream mode is used. This means that the pump delivers a continuous flow of fluid, at the set pressure, to the front of the column. With the fixed restrictors commonly used, an increase in volume flow is observed when a positive pressure program is applied. Inter-diffusion coefficients decrease with an increase in pressure⁹,

making the faster flow rate unfortunate. The increased flow increases the pressure drop over the column so that the exit pressure is lower than the set pressure.

Modern instruments offer pressure control before the column for capillary and narrow bore packed columns as well as pressure control after the column²² for larger diameter packed columns. In the latter case with pressurized UV detectors a variable restrictor that can be configured before the column in the upstream mode or after the column for the downstream mode allows for independent control of flow rate and pressure.

5.4.3 Temperature

Temperature does not only influence diffusivity of solutes but also the retention mechanism. The influence of temperature on retention is a function of free energy changes in the interaction between the analyte and the stationary phase²³. This can have a major effect on the selectivity of the system.

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. At higher temperatures more gas chromatography like mechanisms are observed and the volatility of solutes play a bigger role in retention.

Increasing the temperature increases the diffusivity of solutes. This is one of the major advantages of SFC over LC and has a major influence on the optimum flow rate that can be used.

For density to appreciably change with pressure, the fluid has to be heated above its critical temperature. Some researchers reported that temperatures below the critical temperature are beneficial for petrochemical group separations with carbon dioxide as mobile phase^{24,25,26}. This is sometimes called subcritical fluid chromatography.

Group selectivity requires that the non-specific type interactions between the stationary phase and the saturated carbon chain be negligible but the specific induction interactions between the double bonds (or other functional groups) and the stationary phase need to be as strong as possible.

It has been shown²⁷ that the non-specific London type dispersion forces are independent of temperature. On the other hand the orientation forces experienced by unsaturated π -systems due to the dipole of the hydroxyl groups on the silica gel stationary phase depends strongly on temperature according to the following equation:

$$E_k = -\frac{2}{3} \left(\frac{(\mu_1 \mu_2)^2}{r_d^6 k' T} \right) \quad [\text{eq 5-4}]$$

where

μ_1, μ_2 = dipoles of the solutes and the stationary phase

r_d = distance between dipoles

T = absolute temperature

E_k = interaction energy resulting from the orientation forces (after Keesom²⁸)

Thus, the orientation forces are weak at high temperatures and increases as the temperature is decreased. The best group separation should then be obtained at low temperatures where the orientation forces are maximized. This was demonstrated by Lee²⁴ who obtained the best resolution between docosane and toluene at 28°C (the lowest temperature studied).

Tagaki and Suzuki²⁶ investigated the influence of temperature on paraffin-olefin separation and found that the best selectivity (α) and resolution (R) of homologs differing only in number of carbon atoms were nearly independent of temperature, whereas the α and R of homologues differing in number of double bonds increased as the temperature was reduced. They concluded that separation by carbon number is controlled by entropy differences, whereas separation by double bonds is based on enthalpy contributions.

Squicciarini²⁵ confirmed these results by noting that at higher temperatures, separation was increasingly governed by boiling point. At temperatures as low as 0°C, he observed a

substantial decrease in resolution due to peak broadening. He too, suggested the use of 29°C as optimum for group separation of gasoline and jet fuel samples.

5.5 Stationary phases used with SFC

Packed column SFC adopted the stationary phases generally used for liquid chromatography. These include silica gel and other polar stationary phases, reversed phase columns, size exclusion and columns made of chiral selectors. While the solvent strength of supercritical fluids can be adjusted when the fluid is heated above its critical temperature by changing pressure, the choice of practically useful fluids for packed column SFC is limited by the low temperature stability of many HPLC stationary phases (<80-100°C)²⁹.

Later, when SFC grew into the realm of capillary chromatography, the stationary phases that are commonly used for GC were adopted³⁰. For capillary SFC, analysis times are much longer compared to gas chromatography, due to the slower diffusion coefficients in the dense fluids. Thus, very narrow capillaries are called for. Typically columns with internal diameters less than 100µm are needed. Practical column dimensions of 50µm i.d. and lengths of 20 to 30m have been accepted as a good compromise between performance and ease of sample introduction and detection³⁰.

Reverse phase columns provide selectivity mostly due to non-specific London type interactions that are closely related to the size and hence the volatility of compounds and are therefore of little use for comprehensive two-dimensional SFCxGC_{ftp}. This is because the retention in GC is primarily governed by boiling point and use of reversed phase columns will lead to a high level of sample dimensionality correlation with a corresponding loss in peak capacity.

Apart from very polar stationary phases like silica gel, other stationary phases that show good promises for use in SFCxGC_{ftp} are chiral and liquid crystal stationary phases.

The comment has been made that the use of smaller diameter columns will increase the possibility of eluting very polar analytes with strong affinity for polar stationary phases. This was made on the premise that when the column surface area is decreased, the adsorptive activity of the column will decrease³¹. However in this statement the effect of the phase ratio (β) was neglected.

5.6 Using phase ratio (β) to reduce retention of oxygenates

The retention ratio (k') is an indication of the time solutes spends absorbed on the column material relative to the time they travel down the column. It is expressed as the product of the phase ratio (β) and the distribution coefficient (K):

$$k' = K \beta \quad [\text{eq 5-5}]$$

The 'stationary time' is determined by the relative strength of interaction between the solute and the stationary and mobile phases. This is expressed in terms of the relative distribution of the solute between the stationary and mobile phases:

$$K = \frac{C_S}{C_M} \quad [\text{eq 5-6}]$$

C_S = solute concentration on the adsorbent surface.

C_M = solute concentration in the mobile phase.

The distribution coefficient (K) becomes a thermodynamic quantity when the concentrations are multiplied with their respective activity coefficients. It can then be used to calculate the heat of interaction between stationary phase and solute through the temperature dependence of the retention times³². This can in turn be used to predict retention when the phase ratio is known.

With highly polar molecules like alcohols and carboxylic acids, strong hydrogen bonding is present with polar stationary phases like silica gel. Other oxygenated compounds such as ethers and carbonyl compounds are subjected to strong dipole-dipole and proton donor

and -acceptor interactions. This implies a large value for K with most of the solute adsorbed to the stationary phase at any specific time.

These compounds have very high retention factors on packed column silica gel columns where the β -value is also very high.

$$\beta = \frac{V_s}{V_m} \quad [\text{eq 5-7}]$$

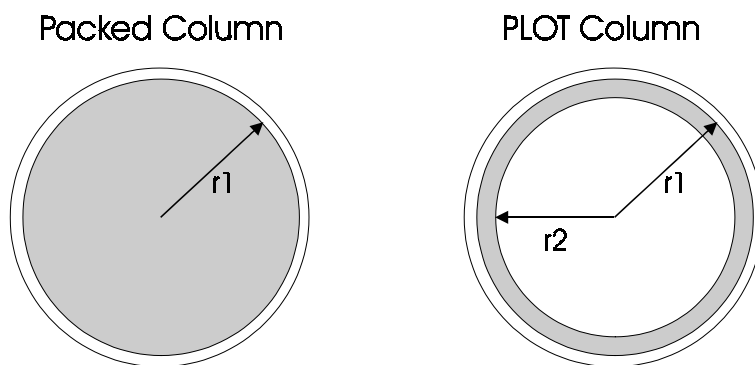
V_m = the volume available to the mobile phase.

V_s = the surface area of the stationary phase

The strength of interaction with the stationary phase can be reduced by the competitive interaction of the mobile phase with the stationary phase, for example when methanol is added as a *modifier* to the CO_2 mobile phase. But this increases instrumental complexity and precludes the use of the FID.

As a novel, alternative approach, reduction of the retention factor by a dramatic increase in V_m with a consequent reduction in the phase ratio, will be theoretically described in this chapter.

Figure 5-2 shows the difference between the volumes available for mobile phase in the packed and PLOT columns. With a packed column only the open spaces between stationary phase particles are filled with mobile phase. For a PLOT column the large central volume contributes to the volume filled with the mobile phase and drastically reduces the phase ratio i.e. the ratio of stationary phase to mobile phase.

Figure 5-2: Phase ratios for Packed and PLOT columns

$$V_s = L\pi r_1^2$$

[eq 5-8]

$$V_s = L\pi (r_1^2 - r_2^2)$$

[eq 5-9]

$$\frac{V_{s,packed}}{V_{s,PLOT}} = \frac{L\pi(r_1)^2(1-V_m)}{L\pi(r_1^2 - r_2^2)} = \frac{(1.0)^2(0.7)\pi L}{(0.15^2 - 0.1485^2)\pi L} = 1563 \quad [\text{eq 5-10}]$$

$$\frac{V_{m,packed}}{V_{m,PLOT}} = \frac{0.3L\pi r^2}{0.3L\pi(r_1^2 - r_2^2) + L\pi r_2^2} = \frac{0.3(1.0)^2}{0.3(0.15^2 - 0.1485^2) + 0.1485^2} = 13.5 \quad [\text{eq 5-11}]$$

$$\frac{\beta_{packed}}{\beta_{PLOT}} = \frac{\left[\frac{V_{s,packed}}{V_{m,packed}} \right]}{\left[\frac{V_{s,PLOT}}{V_{m,PLOT}} \right]} = \frac{V_{s,packed}}{V_{m,packed}} * \frac{V_{m,PLOT}}{V_{s,PLOT}} = \frac{V_{s,packed}}{V_{s,PLOT}} * \frac{V_{m,PLOT}}{V_{m,packed}} = \frac{1563}{13.5} = 116 \quad [\text{eq 5-12}]$$

L is any length element along the column. We assumed particles to be spherical and close packed, thus an inter-particle volume of 0.3 times the volume of the column was estimated for the mobile phase volume. In equation 5-10 the volume of the stationary phase was calculated as the empty volume of the column minus the volume of inter-particle space occupied by the mobile phase. A fundamentally more correct estimation of the retention ratio improvement will be obtained if the stationary phase surface area was used for calculation as opposed to the volume of stationary phase. However these values

are not readily available especially for the PLOT column. Off course the volume of stationary phase particles is not fundamental in adsorption systems like silica gel. We merely imply that the volume ratio of stationary phases is approximately equal to the surface area or activity. Furthermore, the purpose was to illustrate the improvement due to the increased volume of mobile phase and especially the decrease in the amount of stationary phase, all other factors being equal.

Equation 5-8 to 5-12 theoretically demonstrates that retention factors are reduced **116 times** due to the effect of β . In the next chapter it will be experimentally verified that this reduction in k' allows polar oxygenated compounds to elute from silica gel and that it is possible to obtain group information normally lost when oxygenated compounds are back flushed from packed silica gel columns.

5.7 Conclusions

This chapter described the various ways in which resolution and analysis times can be manipulated in SFC. For many analysis schemes the optimum conditions are well researched and reported. For example, it is well known that for group separation with packed column silica gel, CO₂ below its critical temperature and at a pressure of about 150atm provides the optimum separation. This type-analysis of complex mixtures of chemical compounds will be very useful for comprehensive SFCxGC_{ftp} as it is completely orthogonal to the boiling point separation typical of GC analysis.

Furthermore we have theoretically shown that by reducing the column phase ratio the analysis time of polar analytes can considerably be reduced. It remains to be practically demonstrated that group separation of polar oxygenated compounds is possible on very polar stationary phases. This is partly the purpose of the following chapter.

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