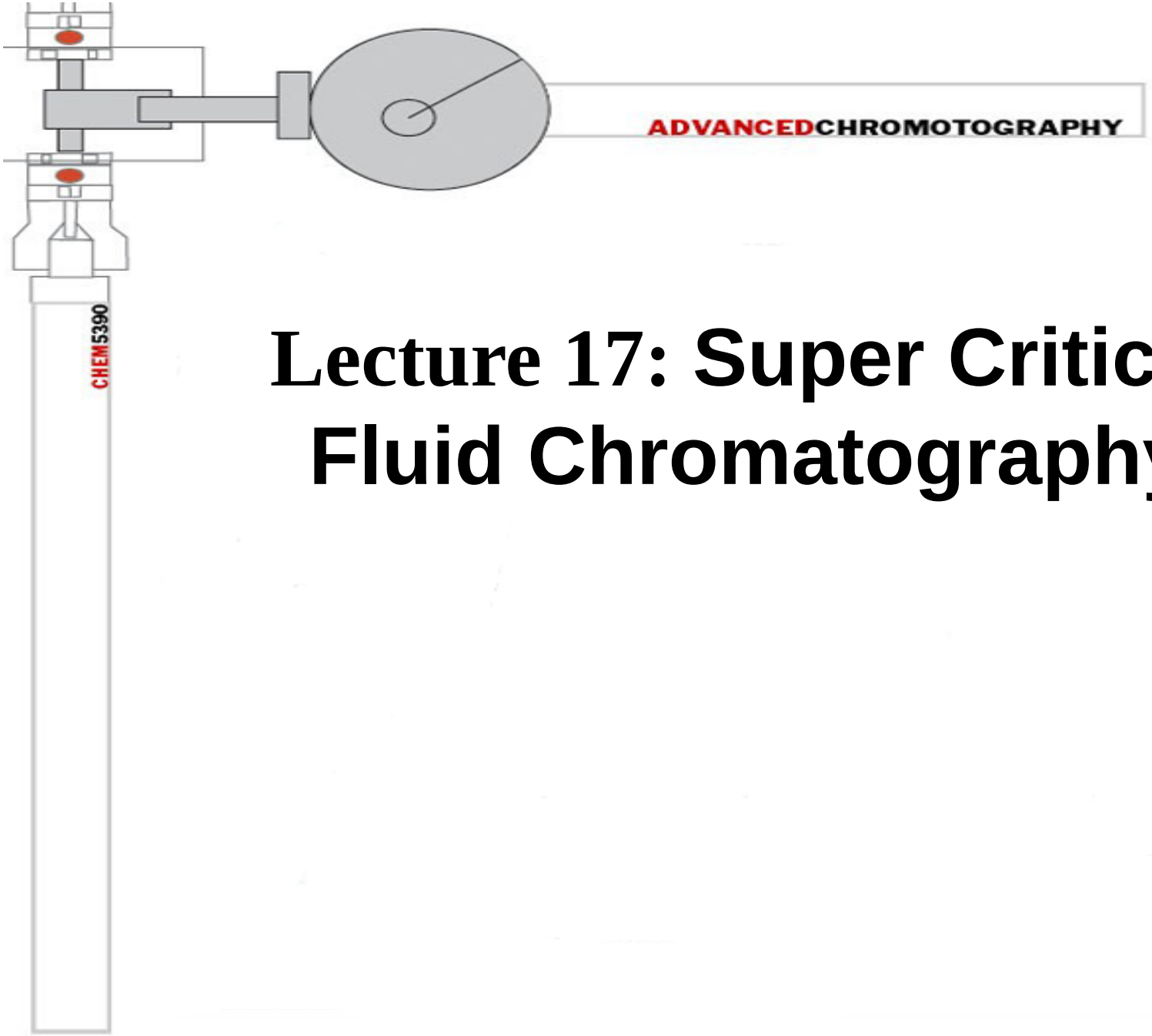
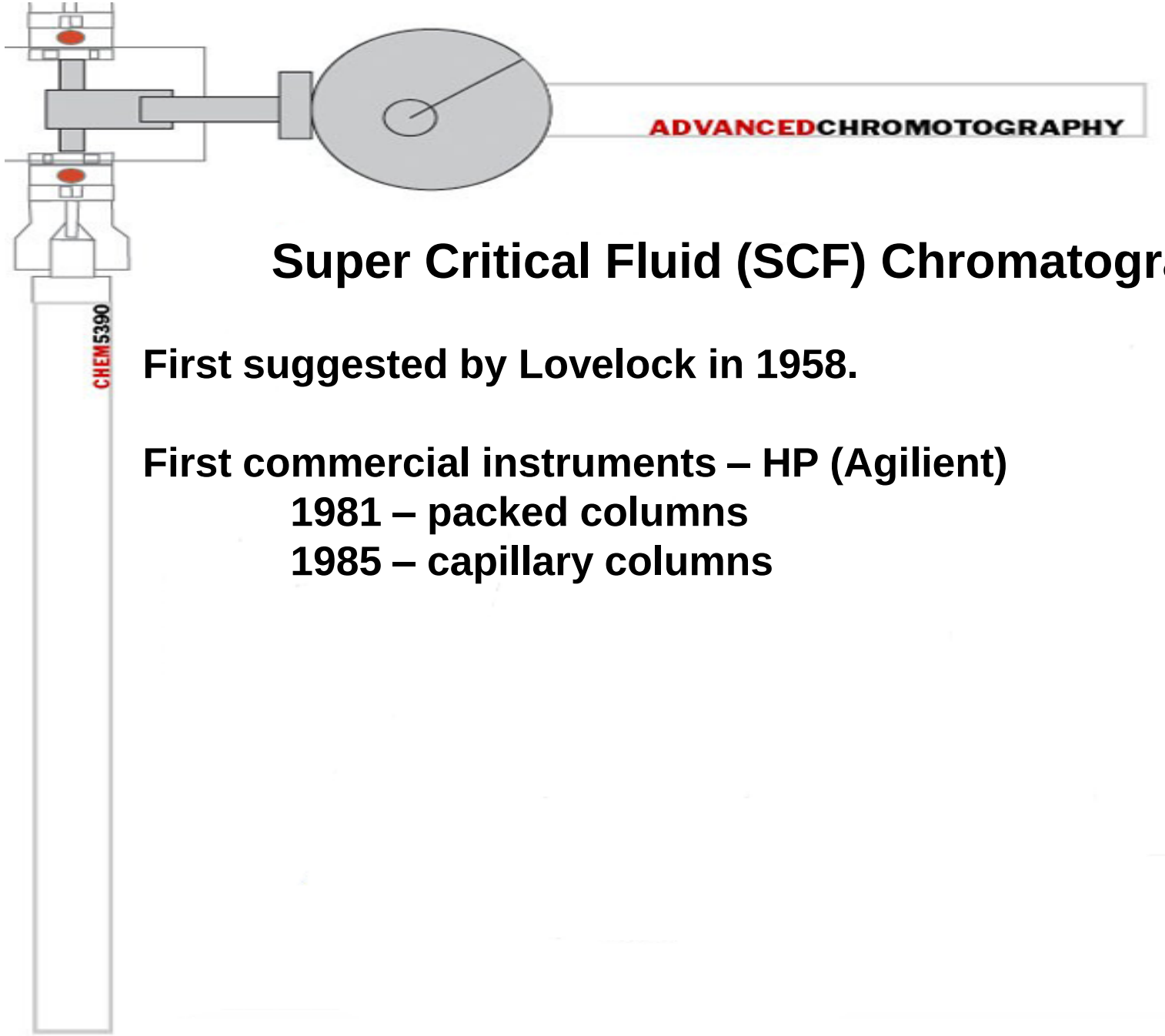


Lecture 17: Super Critical Fluid Chromatography



Super Critical Fluid (SCF) Chromatography

First suggested by Lovelock in 1958.

First commercial instruments – HP (Agilient)

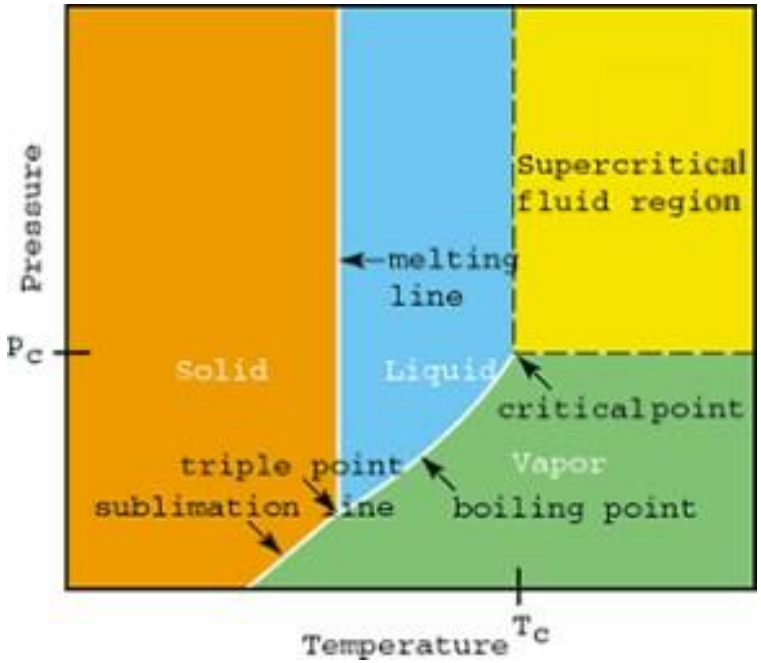
1981 – packed columns

1985 – capillary columns

Super Critical Fluid (SCF) Chromatography

What is a supercritical fluid?

Above a certain temperature, a vapor can no longer be in the liquid state regardless of pressure, critical temperature, Tc.



Super Critical Fluid (SCF) Chromatography

Examples

Fluid	T_c, °C	P_c, atm	d*
CO_2	31.3	72.9	0.96
N_2O	36.5	72.5	0.94
NH_3	132.5	112.5	0.40
n-C_5	196.6	33.3	0.51
n-C_4	152.0	37.5	0.50
CCl_2F_2	111.8	40.7	1.12
CHF_3	25.9	46.9	-----

*density in g/ml at 400 atm

Super Critical Fluid (SCF) Chromatography

Supercritical fluids have densities and diffusivities similar to liquids but viscosities comparable to gases.

Mobile Phase	Density (g/mL)	Viscosity (poise)	Diffusivity (cm^2/sec)
Gas	10^{-3}	$2x10^{-4}$	0.5
Liquid	0.9	$1.3x10^{-2}$	$1.25x10^{-5}$
SCF	0.5	$0.6x10^{-3}$	$1.8x10^{-4}$

Viscosity is a measure of the resistance of a fluid which is being deformed by either shear stress or extensional stress. In general terms it is the resistance of a liquid to flow.

While viscosity is concerned with the transfer of momentum and thermal conductivity with the transfer of heat, **diffusivity** is concerned with the transport of molecules in a mixture.

Super Critical Fluid (SCF) Chromatography

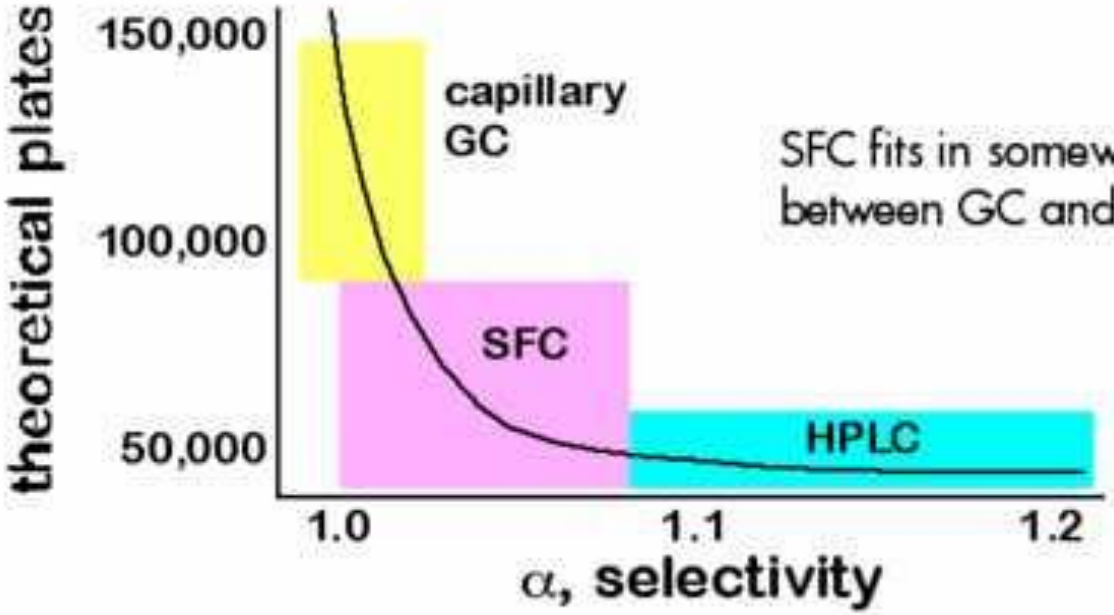
Supercritical fluids have densities and diffusivities similar to liquids but viscosities comparable to gases.

For SCF

- pressures and flows are similar to GC methods, due to low viscosities.

- efficiencies are similar to HPLC methods, due to low diffusion.

Supercritical fluid chromatography



SFC fits in somewhere between GC and HPLC.

Number of plates required to achieve a separation with $R_S = 1.5$ and $k' = 2$

Super Critical Fluid (SCF) Chromatography

Two types of techniques

- **Supercritical fluid extraction (SFE)**
- **Supercritical fluid chromatography (SCF)**

As a chromatographic method, it falls between HPLC and GC.

Super Critical Fluid (SCF) Chromatography

Supercritical fluid extraction (SFE)

Advantages as an extraction method:
- **can be “tuned”**
- **easy removal of extracting solvent**

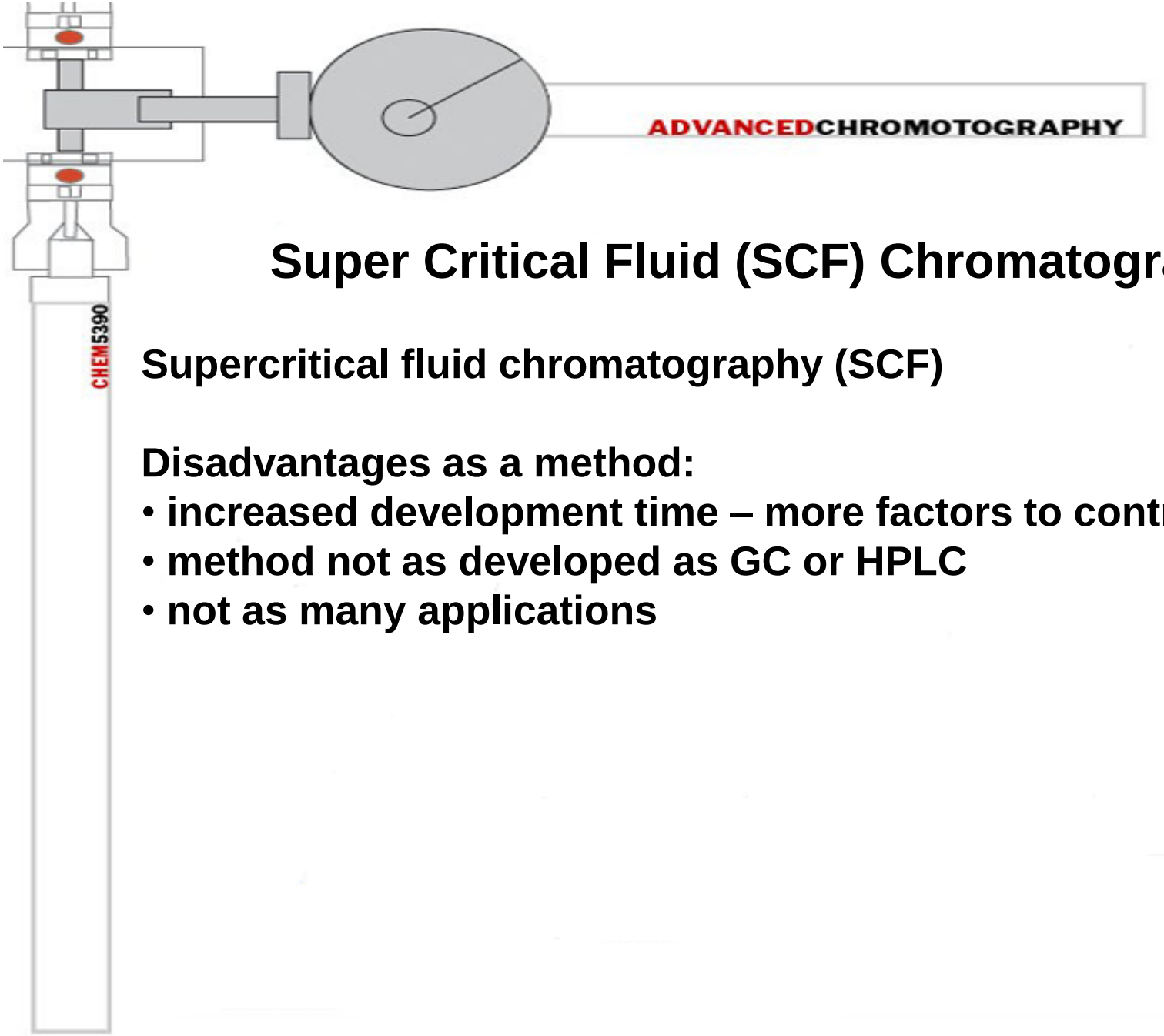
Applications include the decaffeination of coffee and extraction of crude oil from rocks.

Super Critical Fluid (SCF) Chromatography

Supercritical fluid chromatography (SCF)

Advantages as a method:

- **can use low temperatures for separation**
- **can use packed or capillary columns**
- **pressure and polarity of mobile phase can be “tuned” for optimum separation**



Super Critical Fluid (SCF) Chromatography

Supercritical fluid chromatography (SCF)

Disadvantages as a method:

- **increased development time – more factors to control**
- **method not as developed as GC or HPLC**
- **not as many applications**

Super Critical Fluid (SCF) Chromatography

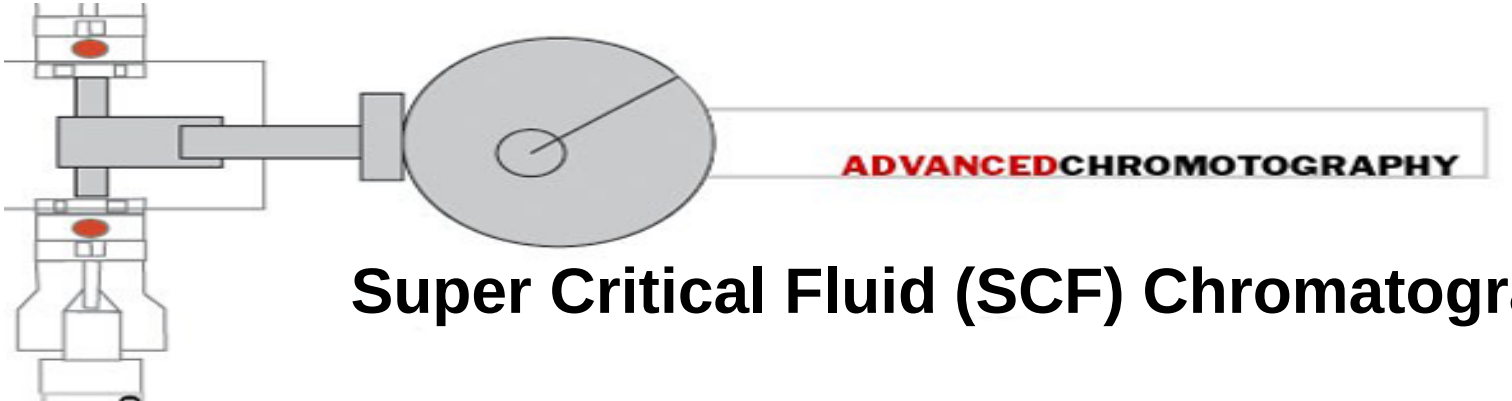
Instrumentation

Equipment is similar to HPLC and GC.

Use either a packed or capillary GC column.

Detectors can be GC or HPLC type and depends on sample and pressure.

Major differences is the ability to modify pressure or solvent during a run.



Super Critical Fluid (SCF) Chromatography

Instrumentation

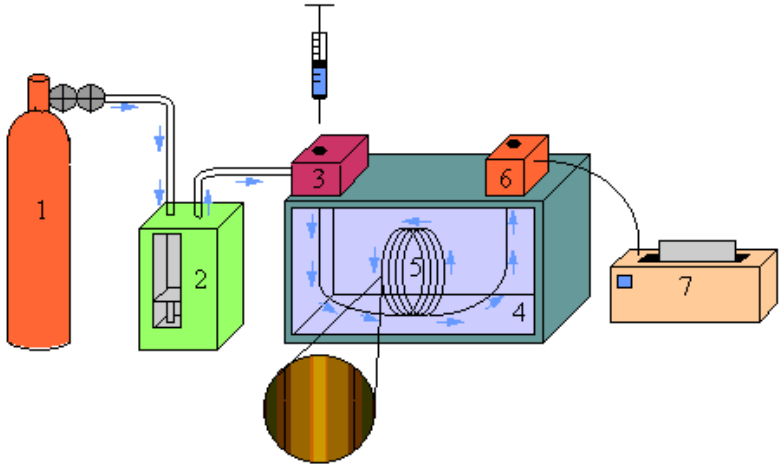
The most significant difference from HPLC is the replacement of most of the liquid mobile phase with a dense compressed gas, like carbon dioxide.
At high pressures such as greater than 80 bar, CO_2 acts as a solvent.

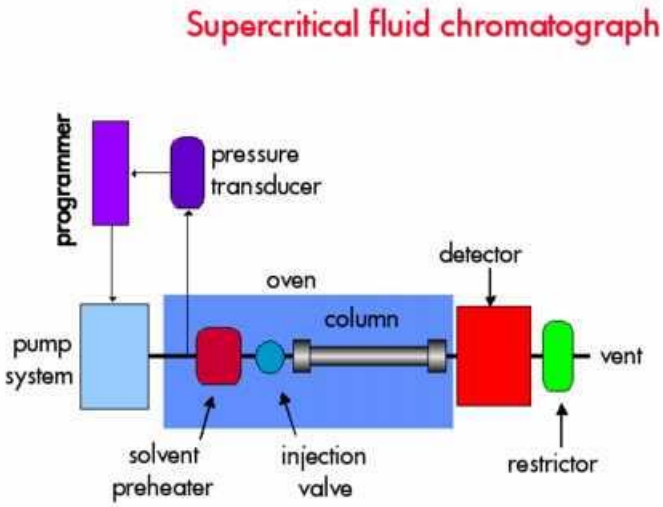
Because it is a compressed gas, a backpressure regulator is required on the system outlet to ensure the mobile phase remains a single dense phase throughout the run.

Requires some detectors, such as an ultraviolet (UV) detector, to be operated at elevated pressures.

Super Critical Fluid (SCF) Chromatography

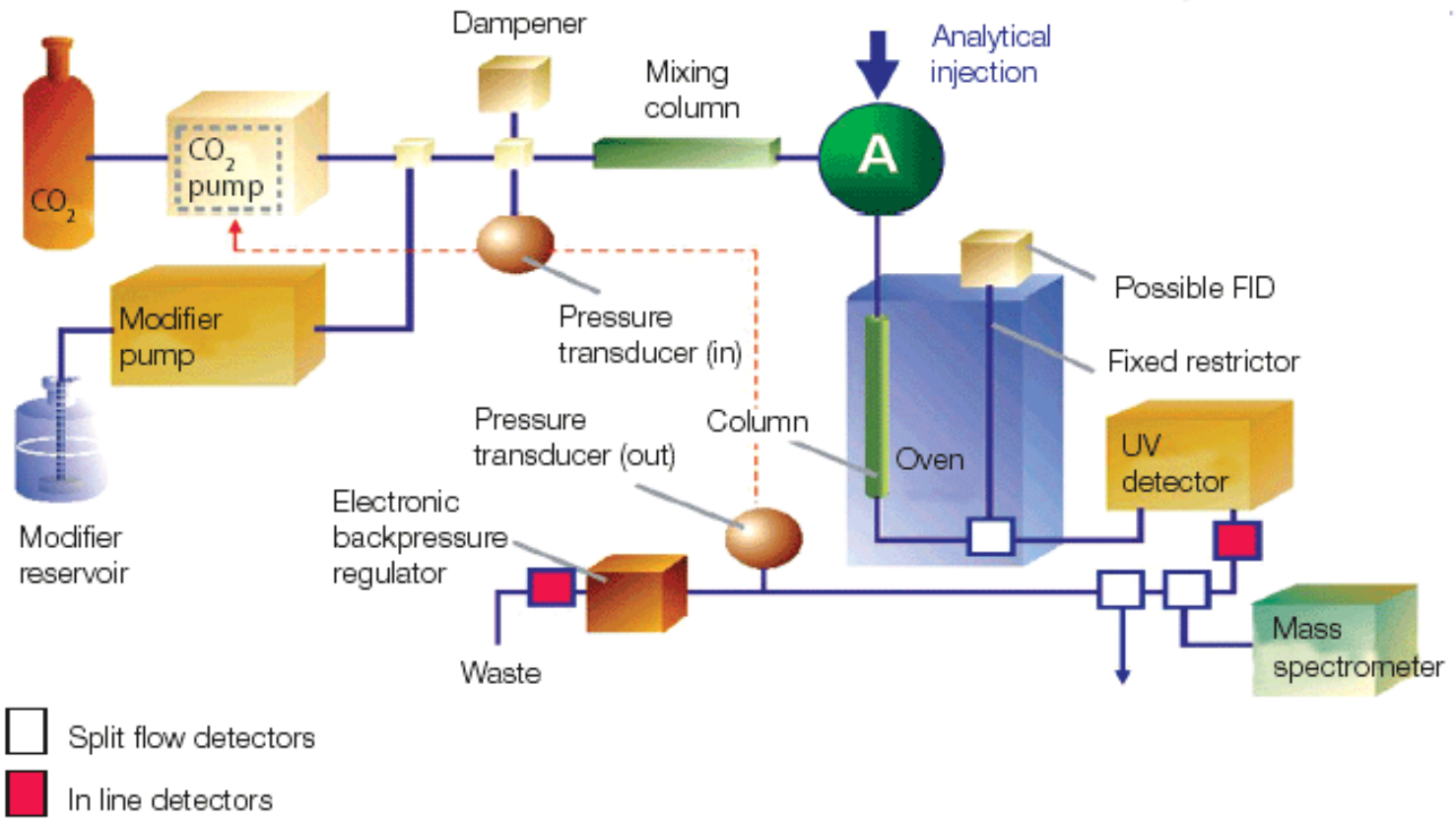
Instrumentation – mixture of GC and HPLC





Super Critical Fluid (SCF) Chromatography

Instrumentation



Analytical SFC diagram

Super Critical Fluid (SCF) Chromatography

Instrumentation - Pump

Functions to maintain a suitable, precise mobile phase flow, and used to apply pressure to keep the mobile phase in the supercritical state.

Both pressure and temperature must be precisely controlled so use an oven also.

Mobile phases enter the pump as a liquid from a cylinder and is pumped to the column where it is heated to the supercritical state.

Super Critical Fluid (SCF) Chromatography

Instrumentation - Pump

On-line filters and activated carbon or alumina adsorption cartridges are employed to purify the mobile phase prior to entering the pump.

Pump design and seals are selected to tolerate very high solvent strengths and pressures.

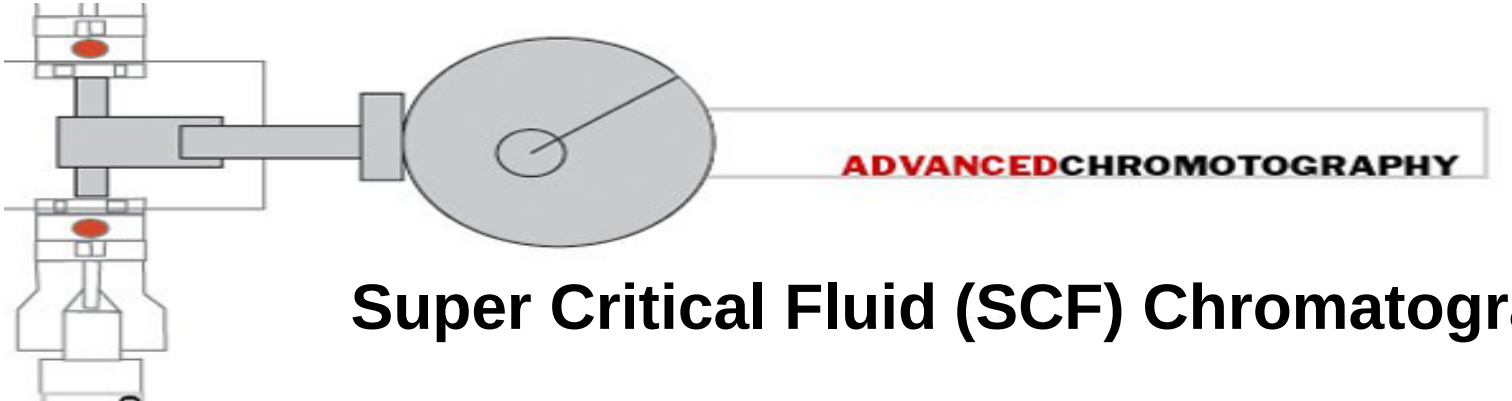
Typically syringe pumps or reciprocating pumps are used for 1ul/min to 10 ml/min flow rates.

Super Critical Fluid (SCF) Chromatography

Instrumentation - Pump

Syringe pumps deliver pulseless flow, but have a fixed delivery volume and require a refilling cycle.

Reciprocating pumps need no refilling but require a pressure dampening device.



Super Critical Fluid (SCF) Chromatography

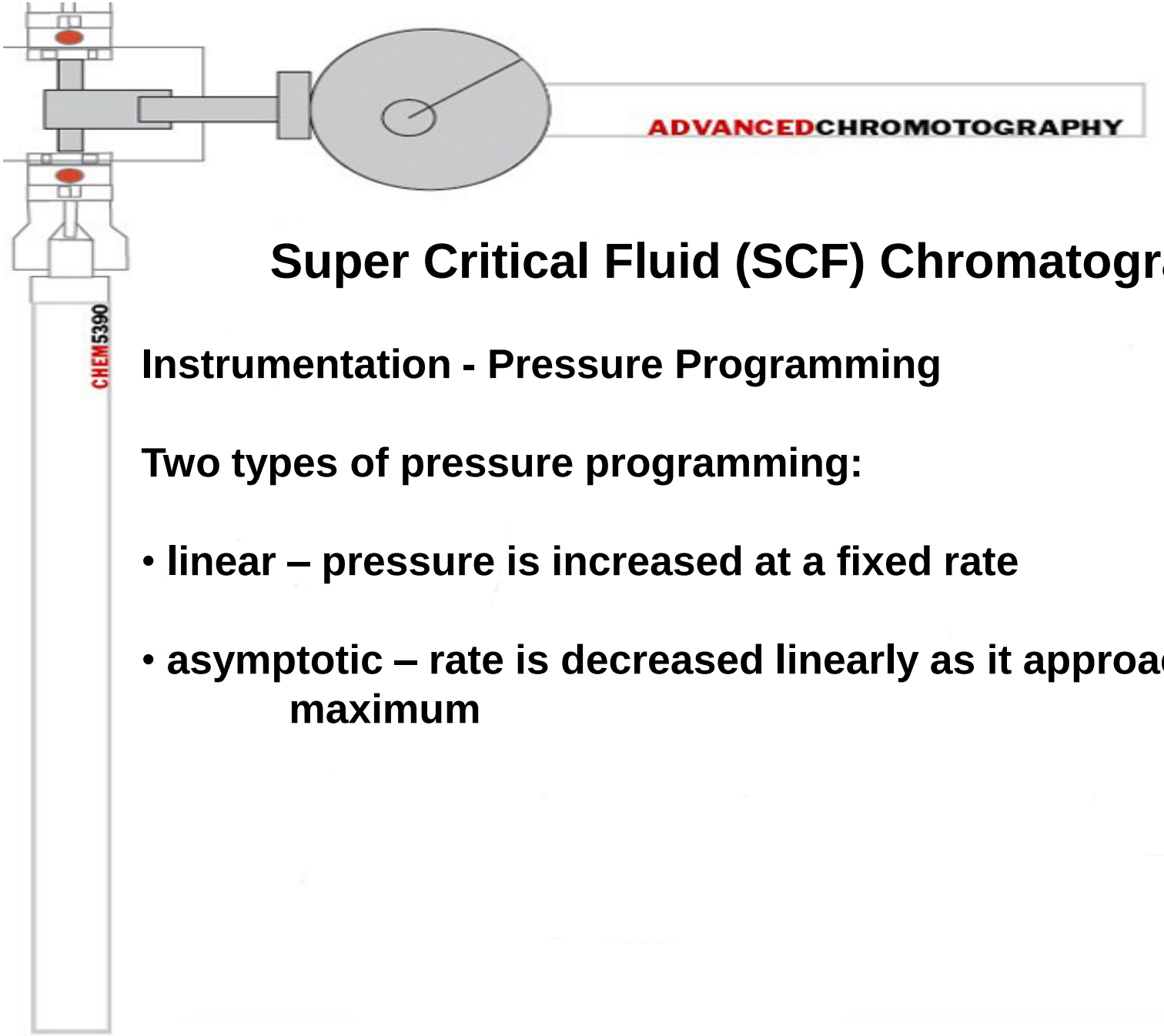
Instrumentation - Pressure Programming

Similar to temperature programming for GC or gradient elution for HPLC.

As pressure increases, so does the density – method may be called density programming.

Density programming results in increasing the solubility of the solutes in the mobile phase.

<u>As P increases, retention decreases.</u>



Super Critical Fluid (SCF) Chromatography

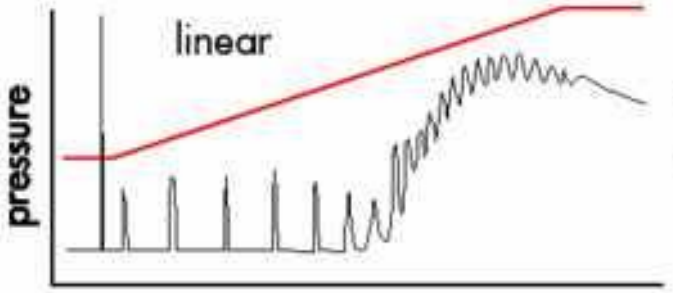
Instrumentation - Pressure Programming

Two types of pressure programming:

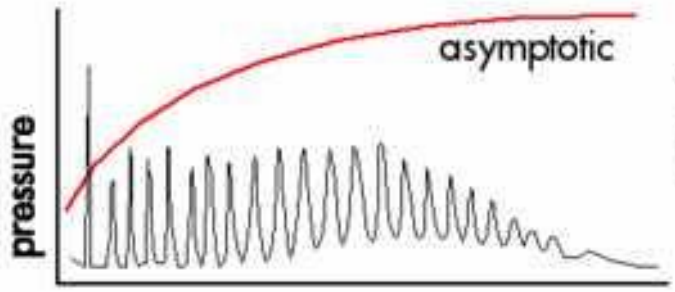
- **linear – pressure is increased at a fixed rate**
- **asymptotic – rate is decreased linearly as it approaches a maximum**

Super Critical Fluid (SCF) Chromatography

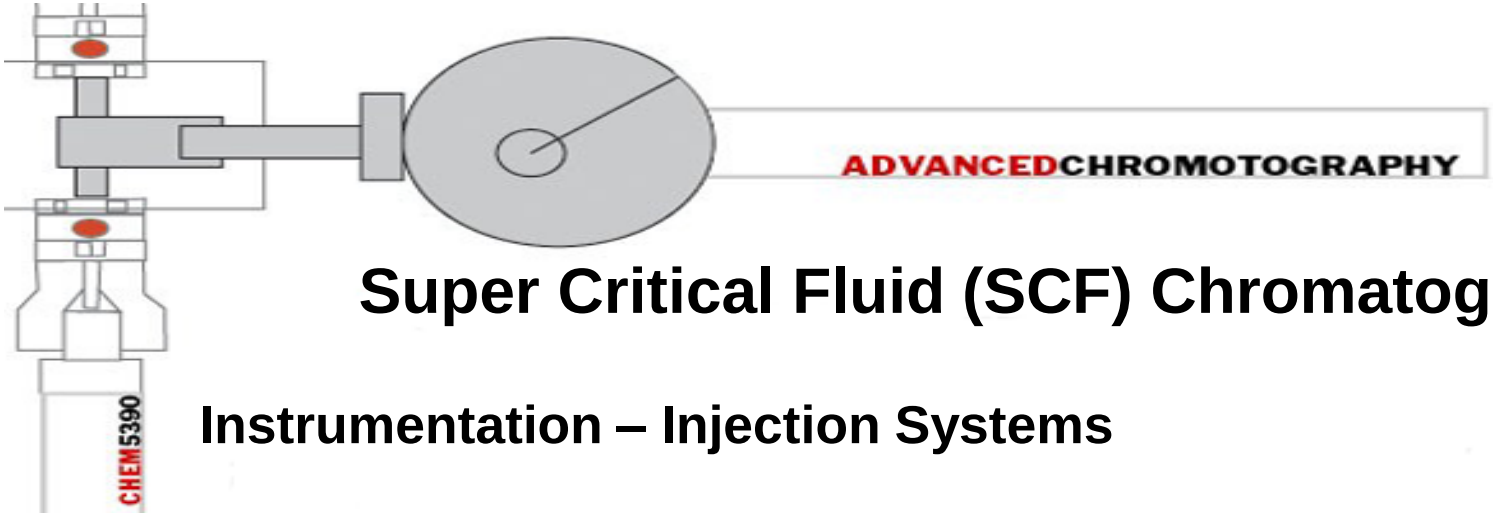
Pressure Programming



Example. Analysis of polystyrene monomers.



Asymptotic programs typically result in a better separation.



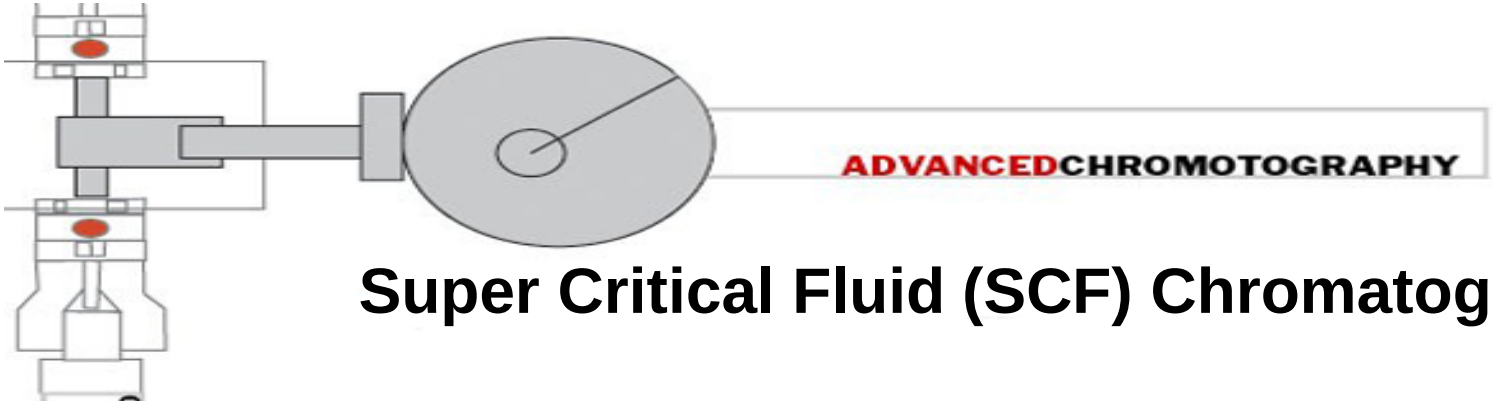
Super Critical Fluid (SCF) Chromatography

Instrumentation – Injection Systems

Sample introduction is based on the high-pressure rotary valve system used for HPLC.

Problems with injection devices for SCF include;
- **peak distortion and loss of resolution**
- **discrimination and memory effects**
- **poor reproducibilty**
- **lack of concentration sensitivity**

To overcome these problems several approaches have been used, including;
- **splitting devices**
- **solvent venting**



Super Critical Fluid (SCF) Chromatography

Instrumentation – Injection Systems

Split/Splitless Valve Injector

Similar to GC type also called dynamic split system.

When valve is in sampling position, the sample is injected under ambient conditions into the sample loop.

When valve is switched to load position, sample is forced into the splitter by flow of high pressure mobile phase.

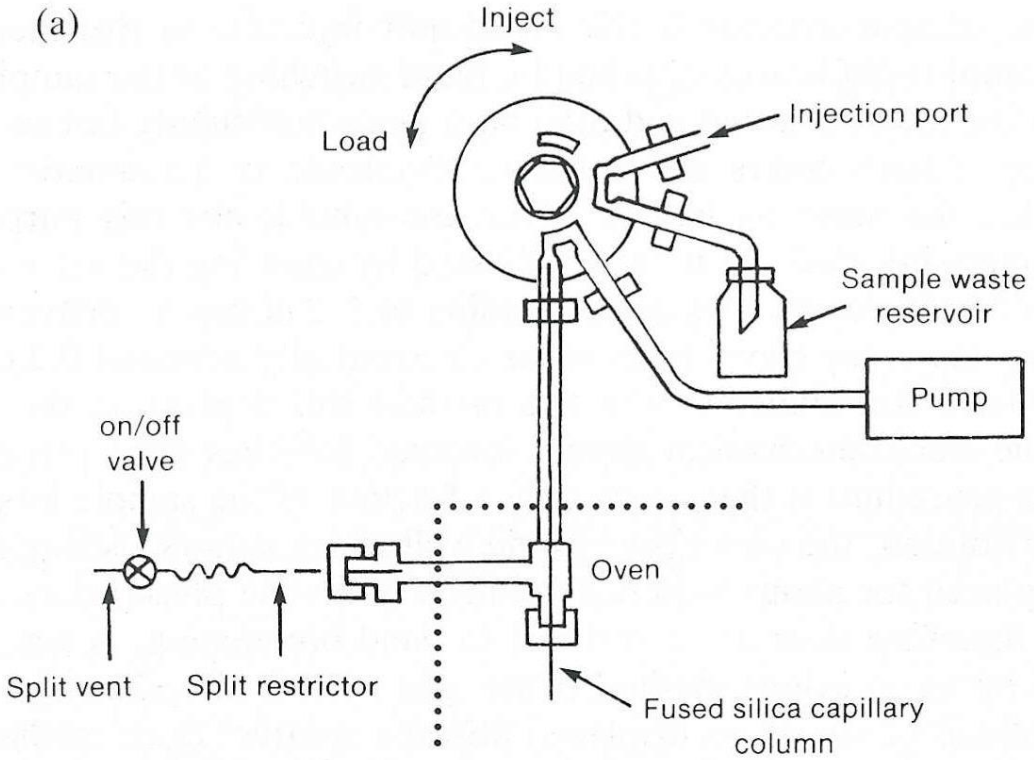
Split vent is open and sample is split with a small fraction entering the column.

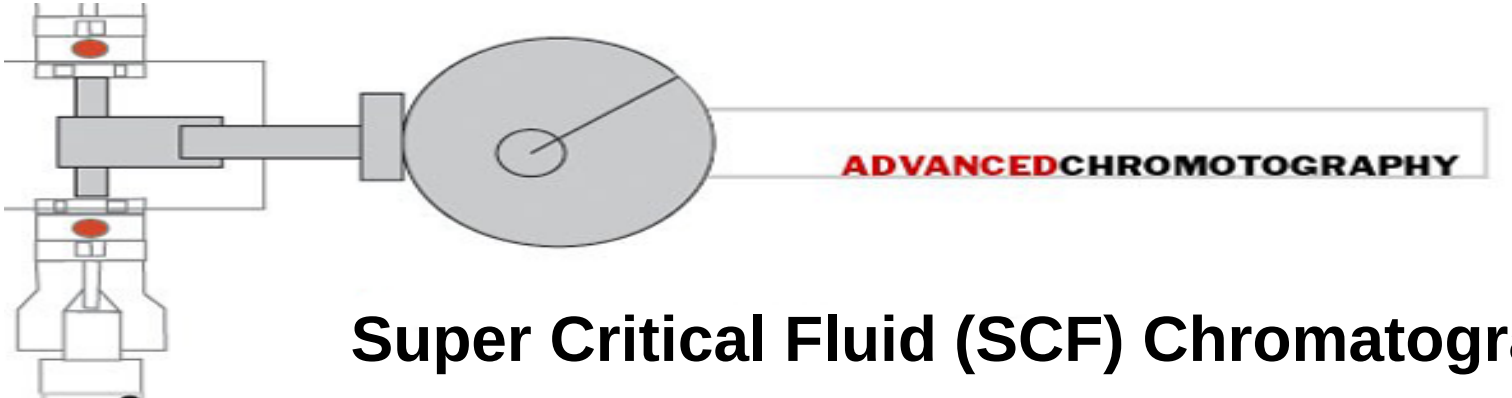
Splitless mode is when the split vent valve is kept closed.

Super Critical Fluid (SCF) Chromatography

Instrumentation – Injection Systems

Split/Splitless Valve Injector





Super Critical Fluid (SCF) Chromatography

Instrumentation – Injection Systems

Timed-Split Injector

Splitting of sample is done by quickly switching back and forth between load to inject position so that only a fraction of the sample enters the column.

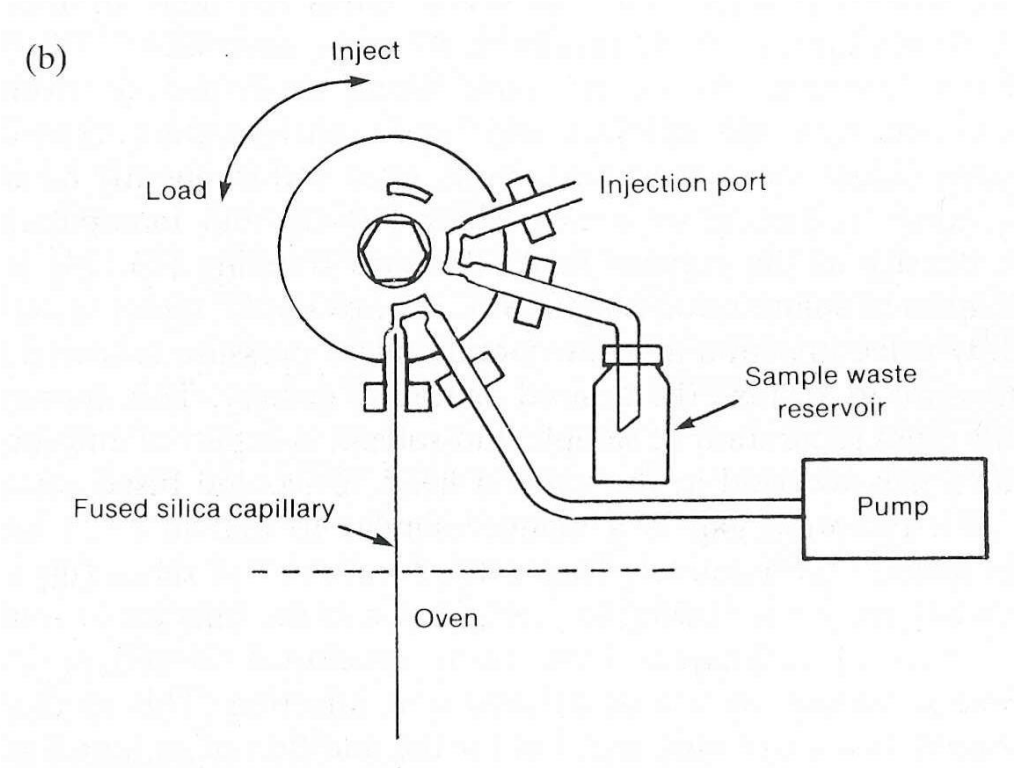
Electronic or pneumatic actuators can move the valve as fast as 10 ms injecting 1-2 nL of sample.

Reproducibility is ~ 4%.

Super Critical Fluid (SCF) Chromatography

Instrumentation – Injection Systems

Timed-Split Injector



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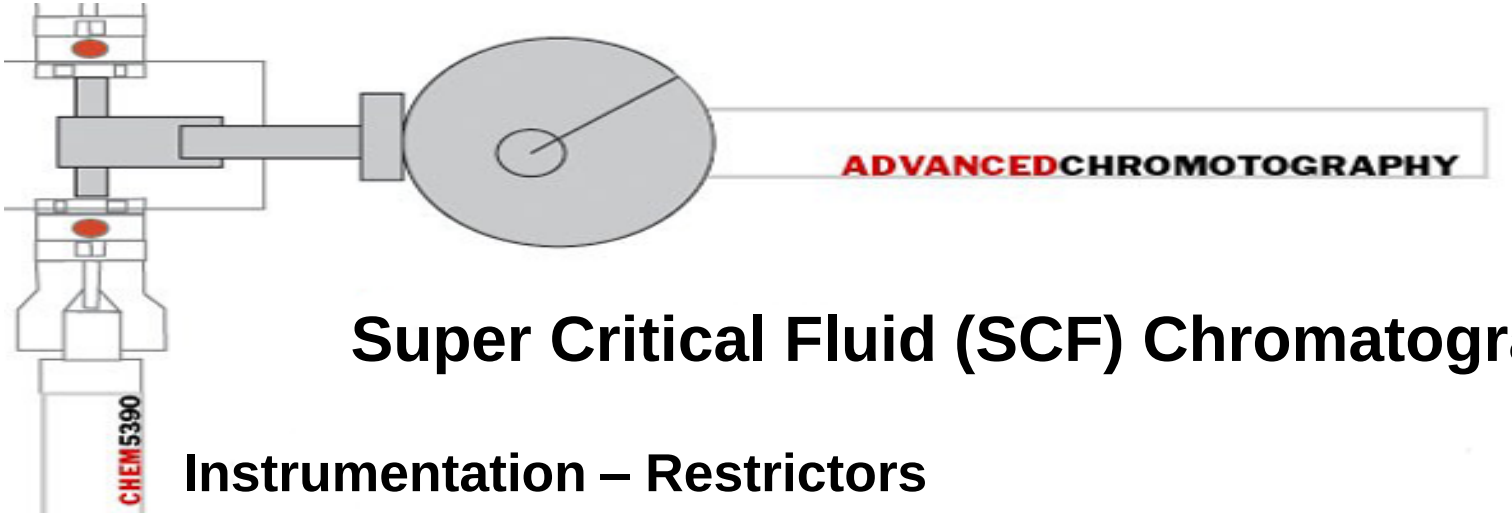
Super Critical Fluid (SCF) Chromatography

Instrumentation – Injection Systems

Other injection techniques have been used including;

- solute focusing – separating solute from solvent at column
- pressure trapping – low pressure injection of sample while venting mobile phase



Super Critical Fluid (SCF) Chromatography

Instrumentation – Restrictors

Placed between the column and detector or after the detector to maintain fluid flow and supercritical fluid conditions along the length of the column but affect rapid decompression to atmospheric pressure before detection.

The difficulty is transferring the eluent from the supercritical phase to the gas phase without compromising column efficiency by introducing excessive dead volume or causing sample components to condense.

Super Critical Fluid (SCF) Chromatography

Instrumentation – Restrictors

Ideal restrictors do not exist but need to have some of the following properties:

- **inert**
- **immune from plugging**
- **adjustable**
- **easily replaced**
- **effective for all samples**

Super Critical Fluid (SCF) Chromatography

Instrumentation – Restrictors

Linear - Initial restrictors were short lengths of narrow-bore fused silica connected to the column end. Generate a linear pressure gradient and work well for low molecular mass analytes and gas phase detectors.

Tapered and integral – restrictors which help with rapid decompression over a short path length. Tip is heated to avoid condensation of analyte, better for polar and high molecular mass analytes.

Super Critical Fluid (SCF) Chromatography

Instrumentation – Restrictors

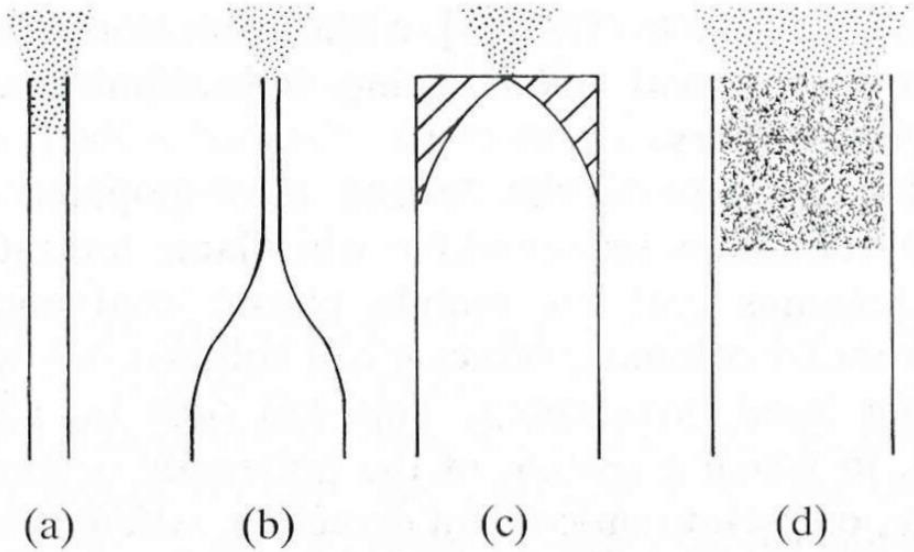


Fig. 7.4. Different designs of flow restrictor: (a), linear; (b), tapered; (c), integral; and (d), frit.

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Super Critical Fluid (SCF) Chromatography

Instrumentation – Detectors

Most popular – FID and UV

Super Critical Fluid (SCF) Chromatography

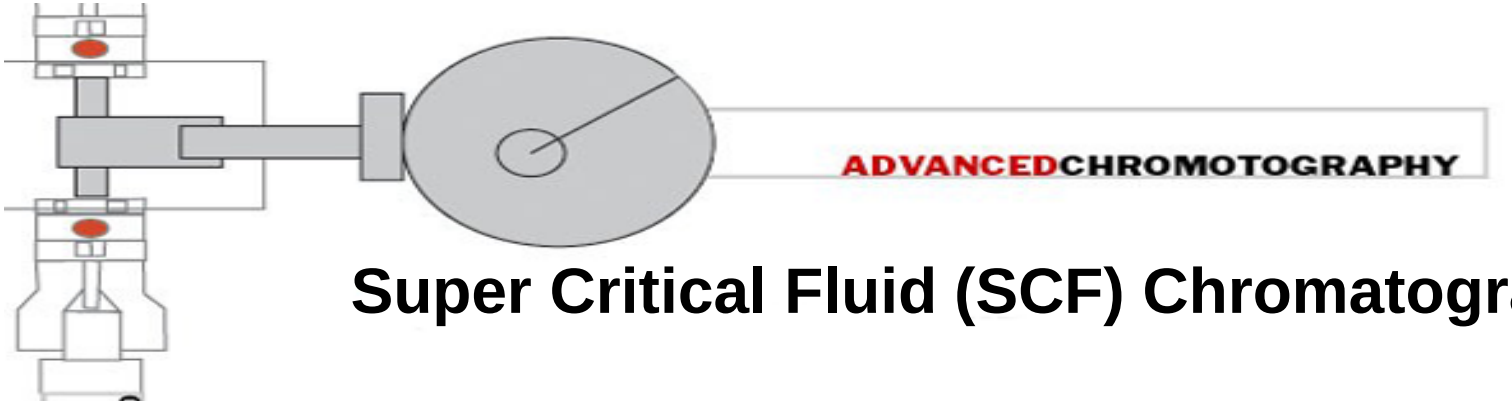
Instrumentation – Columns

Two types of columns used;
- Open tubular
- Packed

Open tubular is preferred because pressure control is easier.

Table 7.2 Columns used for supercritical fluid chromatography.

Parameter	Packed column			Open tubular column
	Conventional	Narrow-bore	Micro	
Internal diameter (mm)	4.6	1.0	<0.5	0.025–0.1
Length (cm)	10–30	10–30	50–150	100–5000
Column material	Stainless steel	Stainless steel	Fused silica	Fused silica
Film thickness (μm)	–	–	–	0.05–0.5
Particle size (μm)	3–10	3–10	3–10	–
Stationary phase	Chemically bonded silica, alumina, polymeric resins			Polysiloxanes
Pressure drop (MPa)	7	–	–	0.01



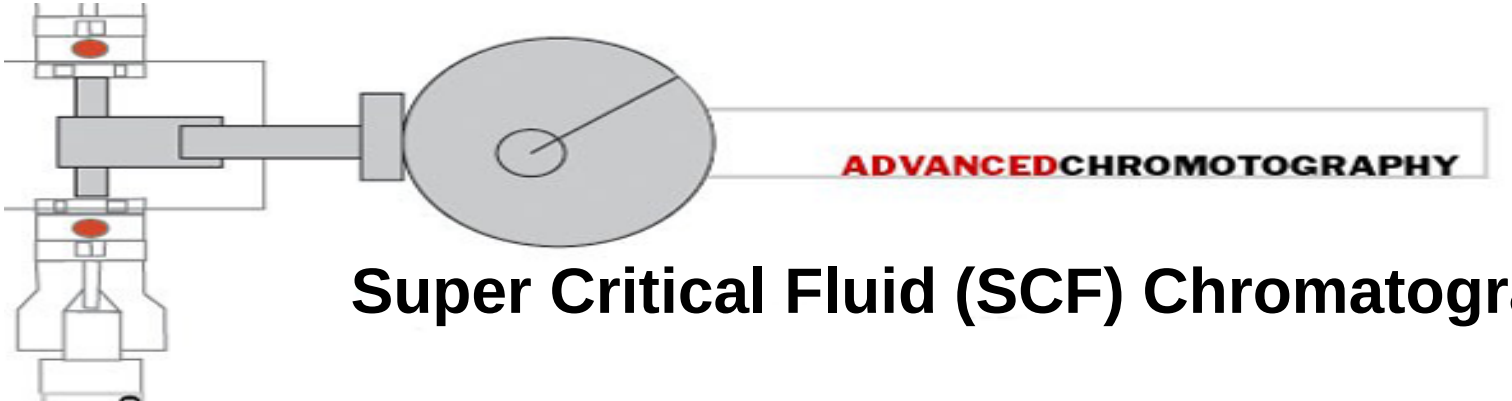
Super Critical Fluid (SCF) Chromatography

Instrumentation – Columns

Open tubular columns for SFC have smaller diameters than those used in GC.

However, column preparation and stationary phases are the same as used in GC.

Polysiloxanes are the most popular stationary phases, since they have high thermal stability and low viscosity.
Polarity is varied by incorporating different functional groups into the polymer and cross-linking is essential to ensure stability.

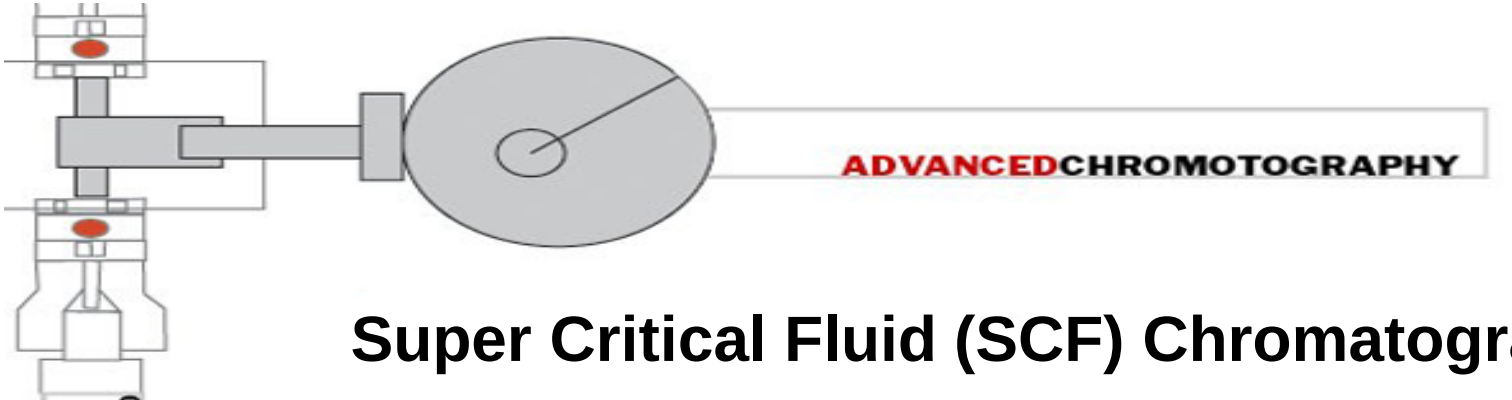


Super Critical Fluid (SCF) Chromatography

Instrumentation – Columns

Packed columns

Most common stationary phase is octadecylsilyl (ODS or C18) modified silica.



Super Critical Fluid (SCF) Chromatography

Factors affecting retention

Dependent on temperature, pressure, mobile phase density, and composition of the stationary and mobile phases.

Supercritical fluids most important properties are viscosity, diffusivity, and solvating power.

Solvating power relates to the capacity of the supercritical fluid for specific intermolecular interactions.

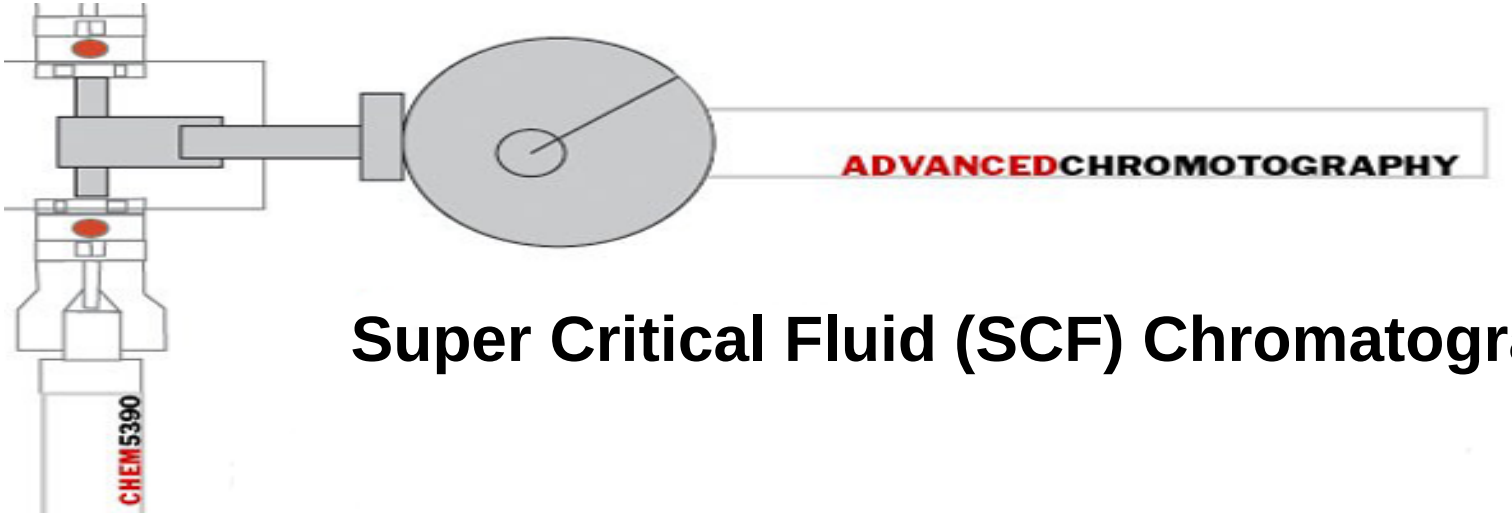
Solvating power for solvents is expressed by the Hildebrand solubility parameter, δ.

Super Critical Fluid (SCF) Chromatography

Table 7.5 Solvent strength data for conventional liquids and fluids. Solubility parameter data are quoted for a reduced temperature T_R (= T/T_c) of 1.02 and reduced pressure P_R (=P/P_c) of 2 and Kamlet–Taft constants for a T_R of 1.02 and a reduced density of 1.02.

Substance	Hildebrand solubility parameter, δ (J cm^{-3})$^{1/2}$	Kamlet–Taft scale π^*	Dipole moment (D)
n-Hexane	10.0	−0.03	–
n-Pentane	10.4	–	–
but-1-ene	10.6	–	0.34
n-Butane	10.8	–	0.05
Ethoxyethane	11.0	–	1.15
Freon 13	11.0	−0.46	–
Sulphur hexafluoride	11.2	−0.54	–
Propane	11.2	–	0.08
Dichloromethane	11.5	–	1.60
Ethyl acetate	11.7	–	1.78
Ethane	11.9	−0.48	0
Ethene	11.9	–	0
Xenon	12.5	−0.38	0
Nitrous oxide	14.7	−0.27	0.17
Carbon dioxide	15.3	−0.19	0
Benzene	–	0.62	0
Methanol	18.2	–	1.70
Ammonia	19.0	0.20	1.47
Ethanol	26.0	0.54	1.69
Water	27.6	–	1.85

Data from Schoenmakers and Uunk [1] and Smith *et al.* [8].

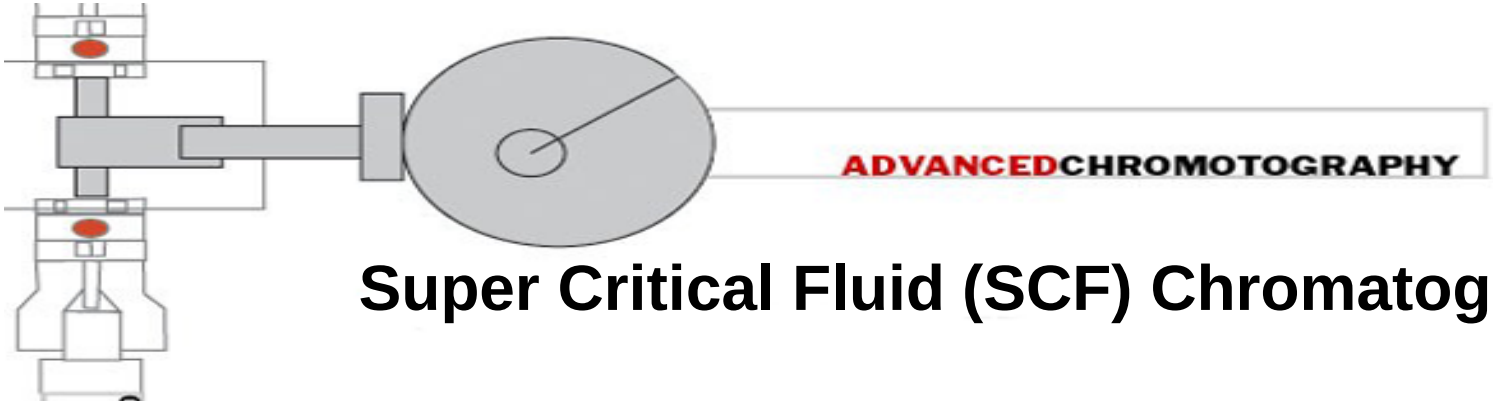


Super Critical Fluid (SCF) Chromatography

Supercritical fluids are an intermediate between gases and liquids, but closer to liquids.

They have greater solubilizing power than gases but do not have an advantage over liquids.

The advantage is that solvent strengths of supercritical fluid can be varied by controlling density through changes in applied pressure and temperature.



Super Critical Fluid (SCF) Chromatography

Mobile Phases

Non-polar or low-polarity compounds have been explored for use as mobile phases in SFC.

These include: CO_2, N_2O, SF_6, xenon, ethane, propane, pentane

Separations are a combination of low polarity mobile phases with relatively polar stationary phases, so SFC typically functions in the normal-phase mode.

Elution is a function of molecular mass and polarity. Larger or more polar compounds have longer retention times.

Super Critical Fluid (SCF) Chromatography

Mobile Phases

Most common mobile phase is carbon dioxide (with and without polar additive).

Carbon dioxide is nontoxic, nonflammable, noncorrosive, available in high purity, low cost, inert to most substances, and does not interfere with most detection methods.

Under normal SFC operating conditions, CO_2 has a wide density range which provides optimum solubilizing power.

CO_2 must be 99.9995% pure for FID (no hydrocarbons) and 99.8% pure for UV.

Super Critical Fluid (SCF) Chromatography

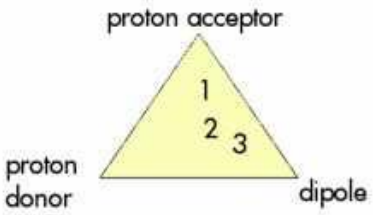
Mobile Phases

Small amounts of a modifier (organic solvent) can be added to the supercritical fluid. Most common are methanol and water.

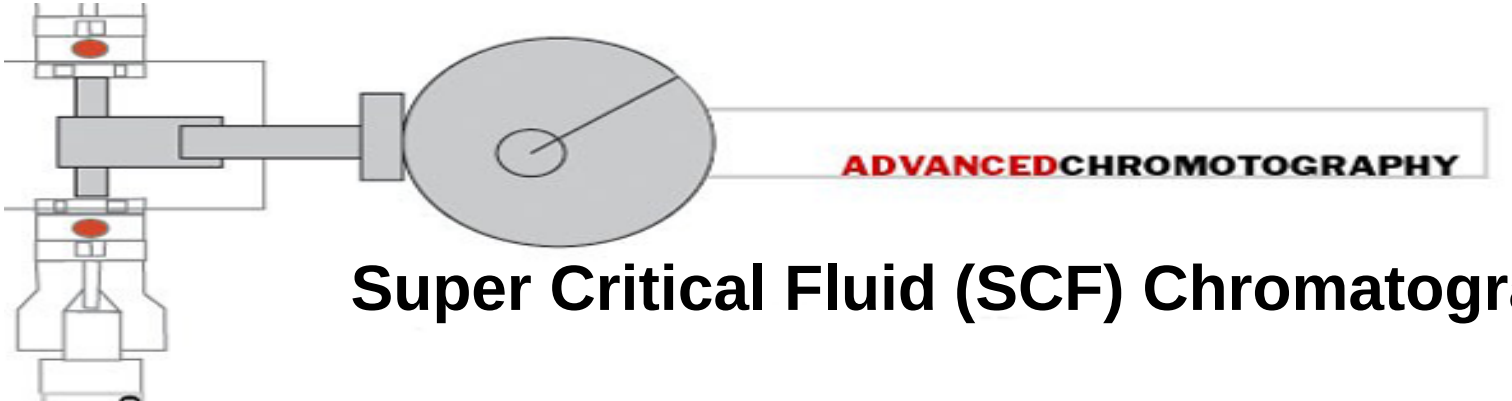
This results in a change in solvent polarity and nature and follows Snyder rules.

SFC modifier solvents

Small amounts of a second solvent can be added to the supercritical fluid. This results in a change in solvent polarity and nature - follows Snyder rules.



1 - 2-propanol
2 - acetonitrile
3 - dichloromethane



Super Critical Fluid (SCF) Chromatography

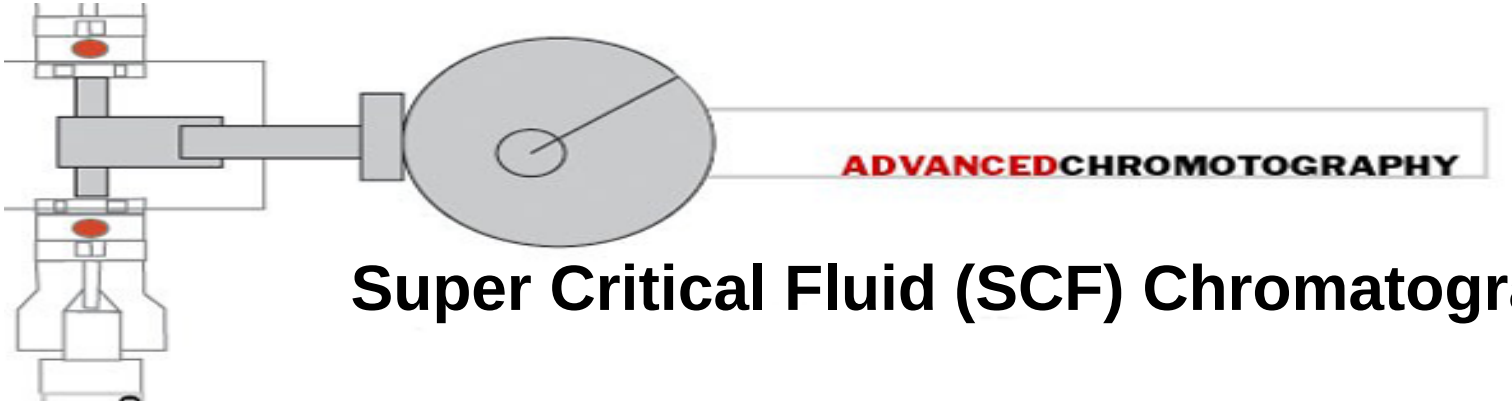
Mobile Phases

CO_2 is a highly nonpolar solvent, similar to a hydrocarbon but in a different solvent family.

So for more polar solutes, an organic modifier, sometimes called a cosolvent (often an alcohol), is added to the mobile phase.

Gradient elution from low to high modifier concentration is the norm.

Peaks elute from lower to higher polarity.

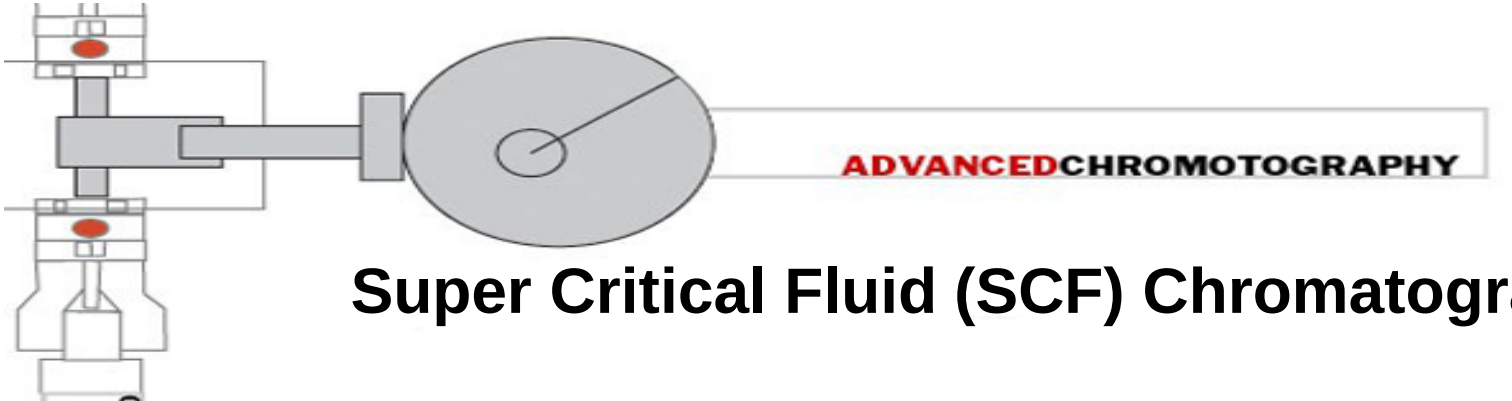


Super Critical Fluid (SCF) Chromatography

Mobile Phases

For highly polar analytes, the interactions with the stationary phase are too strong, and the solutes often fail to elute, or elute with poor peak shapes.

This problem can usually be solved by including a highly polar additive in the mobile phase such as a strong acid or base dissolved in the modifier.

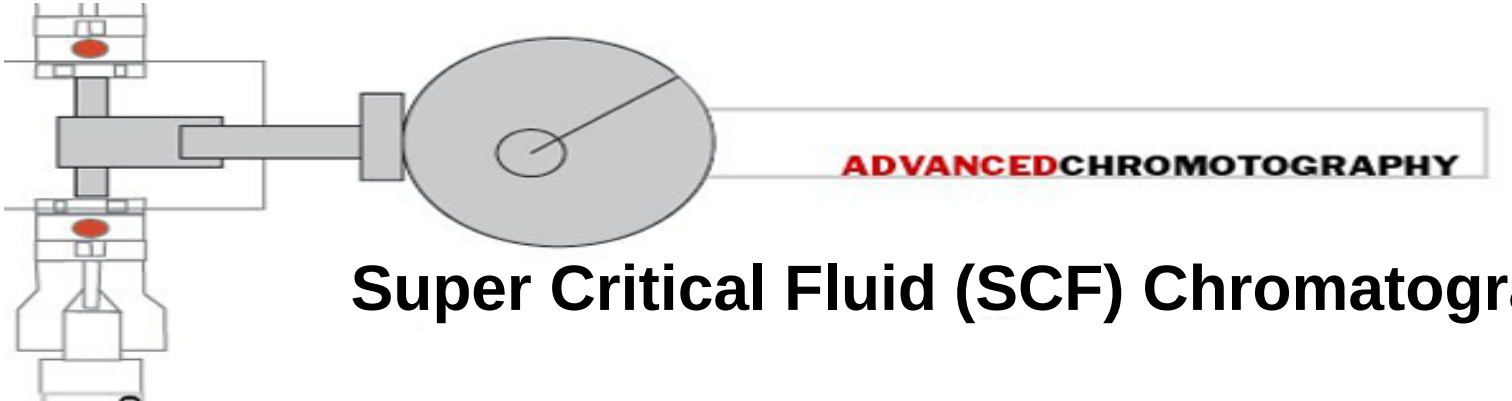


Super Critical Fluid (SCF) Chromatography

Mobile Phases

SFC is usually a normal phase technique because composition is programmed from low to high polarity.
However, SFC has some advantages compared to normal phase HPLC.

- Equilibration is extremely fast (runs are shorter)
- reproducibility is excellent
- aqueous-based samples can be injected.
- mobile phase can be more environmentally friendly (green chemistry)



Super Critical Fluid (SCF) Chromatography

Stationary Phases

For polar solutes, polar stationary phases are used.
Classical polar phases included bare silica, cyano, diol and amino.

New stationary phases have been developed specifically for SFC.
These phases include several ethylpyridines and a number of proprietary phases.

For low polarity solutes, reversed phase columns such as C18, C8, C4, and methyl are sometimes used.

Super Critical Fluid (SCF) Chromatography

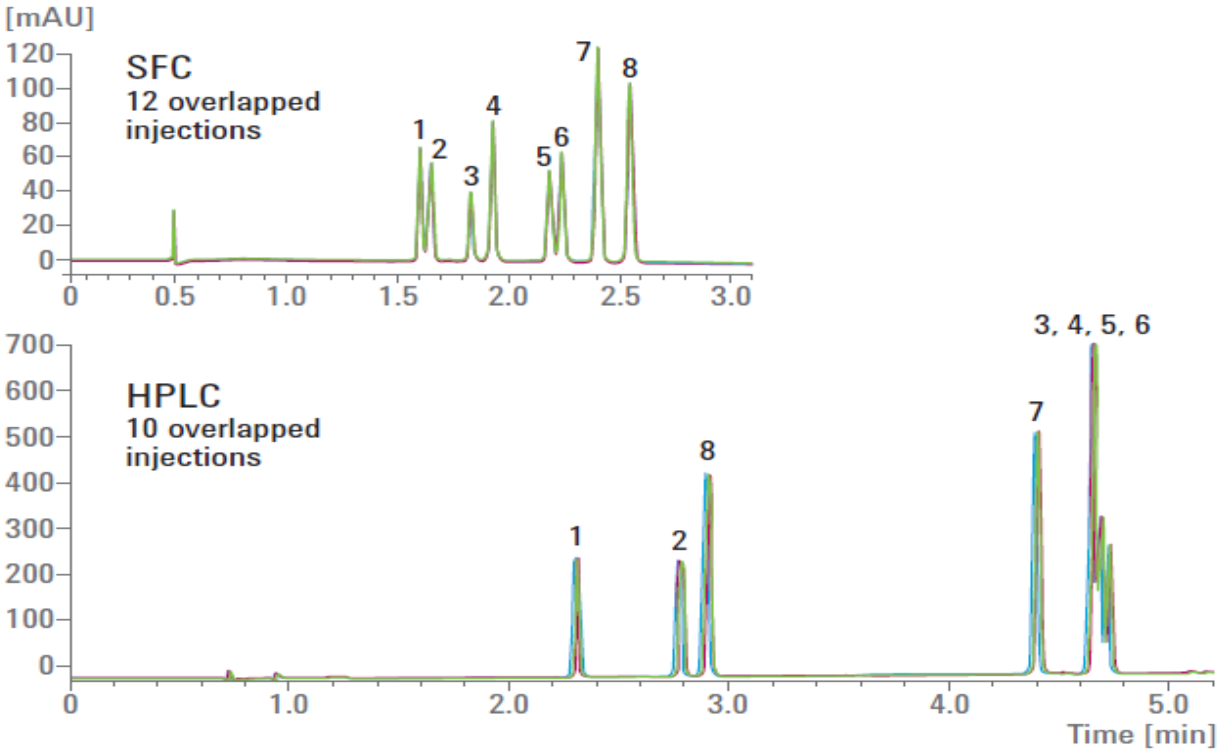
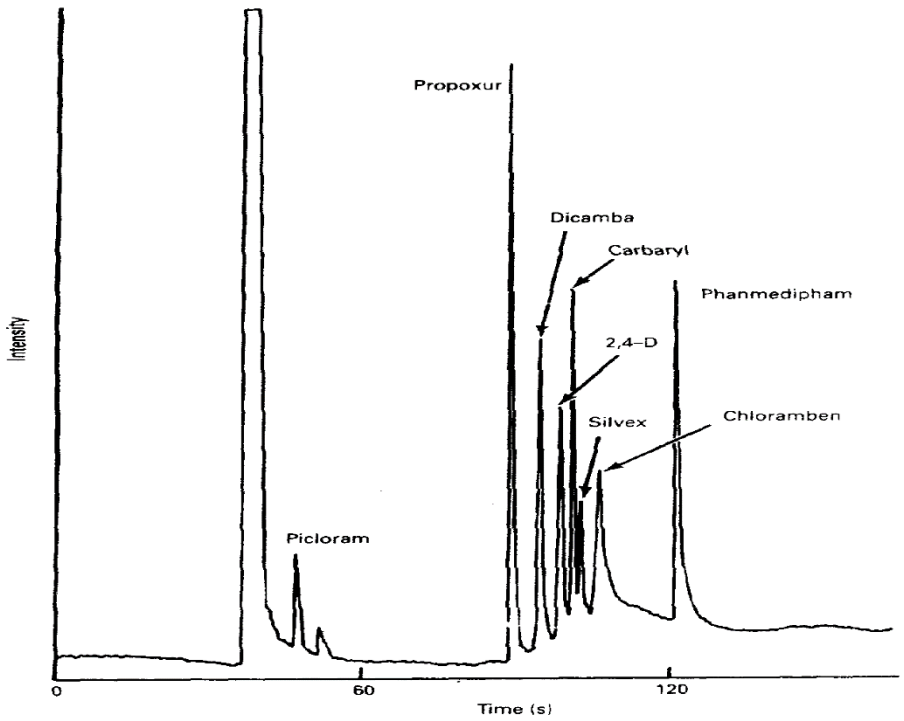


Figure 1.5 SFC is orthogonal to reversed phase HPLC. Comparison of SFC and HPLC chromatograms for a mixture of: 1. caffeine, 2. theophyline, 3. cortisone, 4. prednisone, 5. hydrocortisone, 6. prednisilone, 7. sulfamerazine, 8. sulfaquinoxaline. SFC Conditions: 4 mL/min of 5 to 25 % methanol in CO2 in 3 minutes. 150-bar outlet pressure with a 4.6 by 150 mm, 5 µm, RX-SIL column. HPLC conditions: 1.5 mL/min of 10 to 90 % methanol in water in 4.5 minutes, 40 °C, on a 4.6 by 150 mm, 2.7 µm, Poroshell C18 column[15].

Super Critical Fluid (SCF) Chromatography

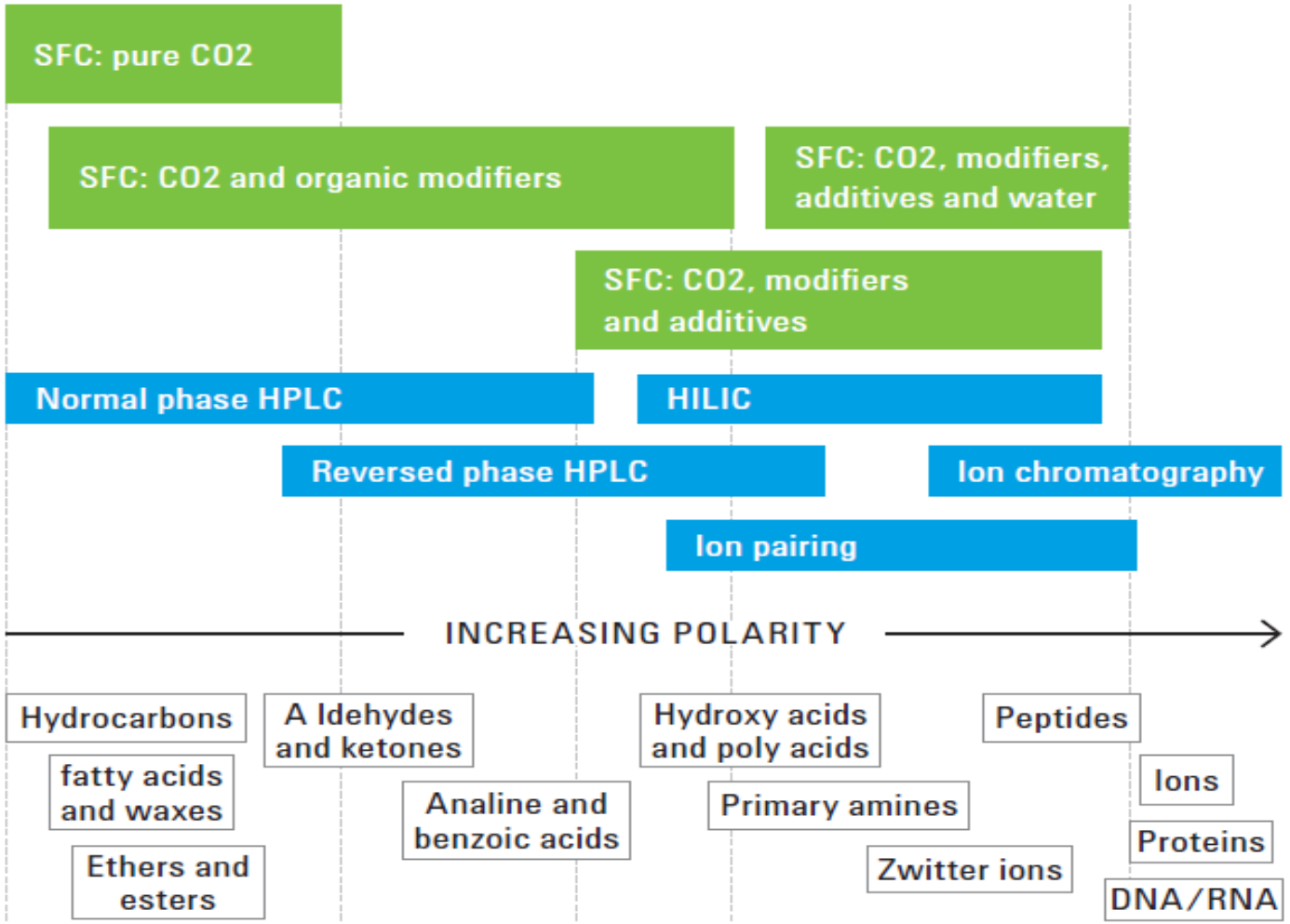
Applications

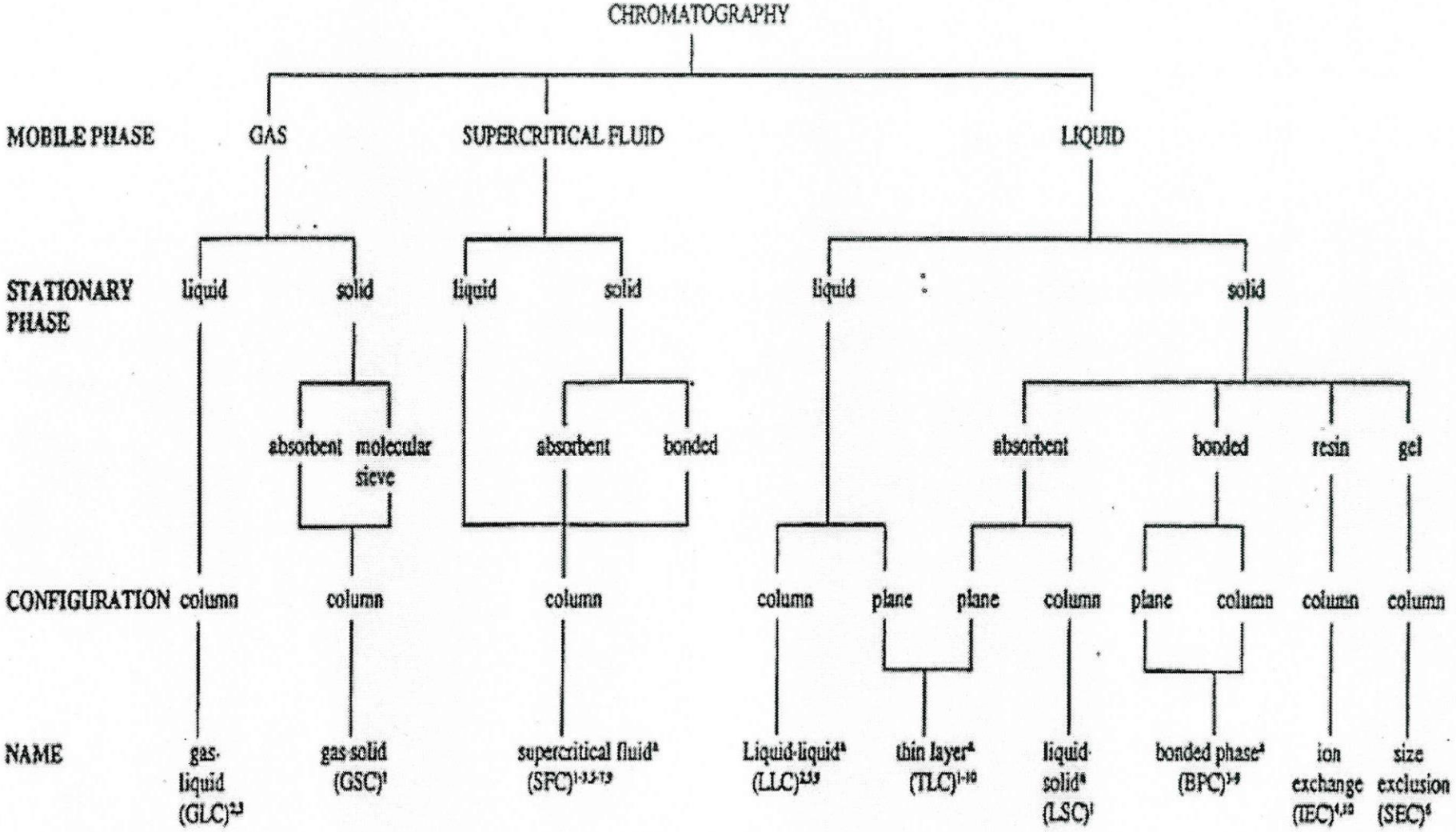
SFC Separation of Thermally Labile Pesticides



Carbamate and acid pesticides
SE-54 1.5m x 25um ID x 0.15um Df
Carbon dioxide 100 C, 135 bar

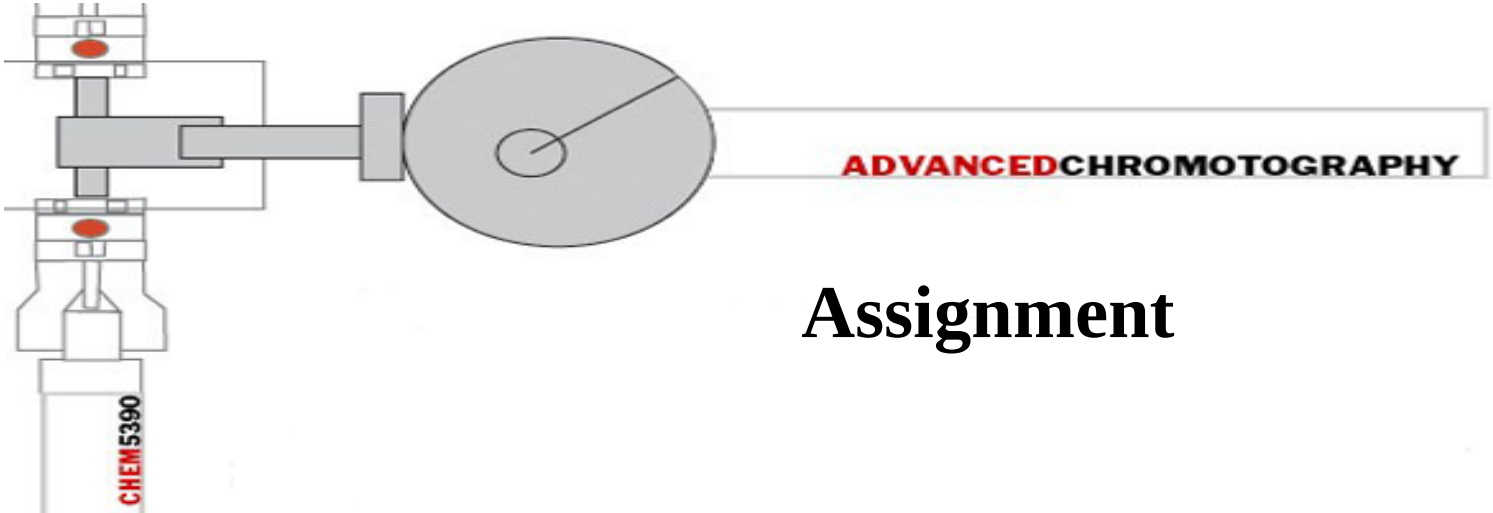
Where Does SFC Fit Relative to HPLC?





[a]For these techniques the combination of mobile and stationary phase can be varied to generate either a normal phase or reversed phase system. Mechanisms which have been exploited in the various techniques are identified as: [1]adsorption, [2]partition, [3]bonded phase, [4]ion exchange, [5]ion interaction, [6]size exclusion, [7]affinity, [8]micellar, [9]chelation, [10]ion exclusion.

Fig. 1.3. Classification of chromatographic systems.



Assignment

- Read Chapter 7: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.