Supercritical Fluid Chromatography

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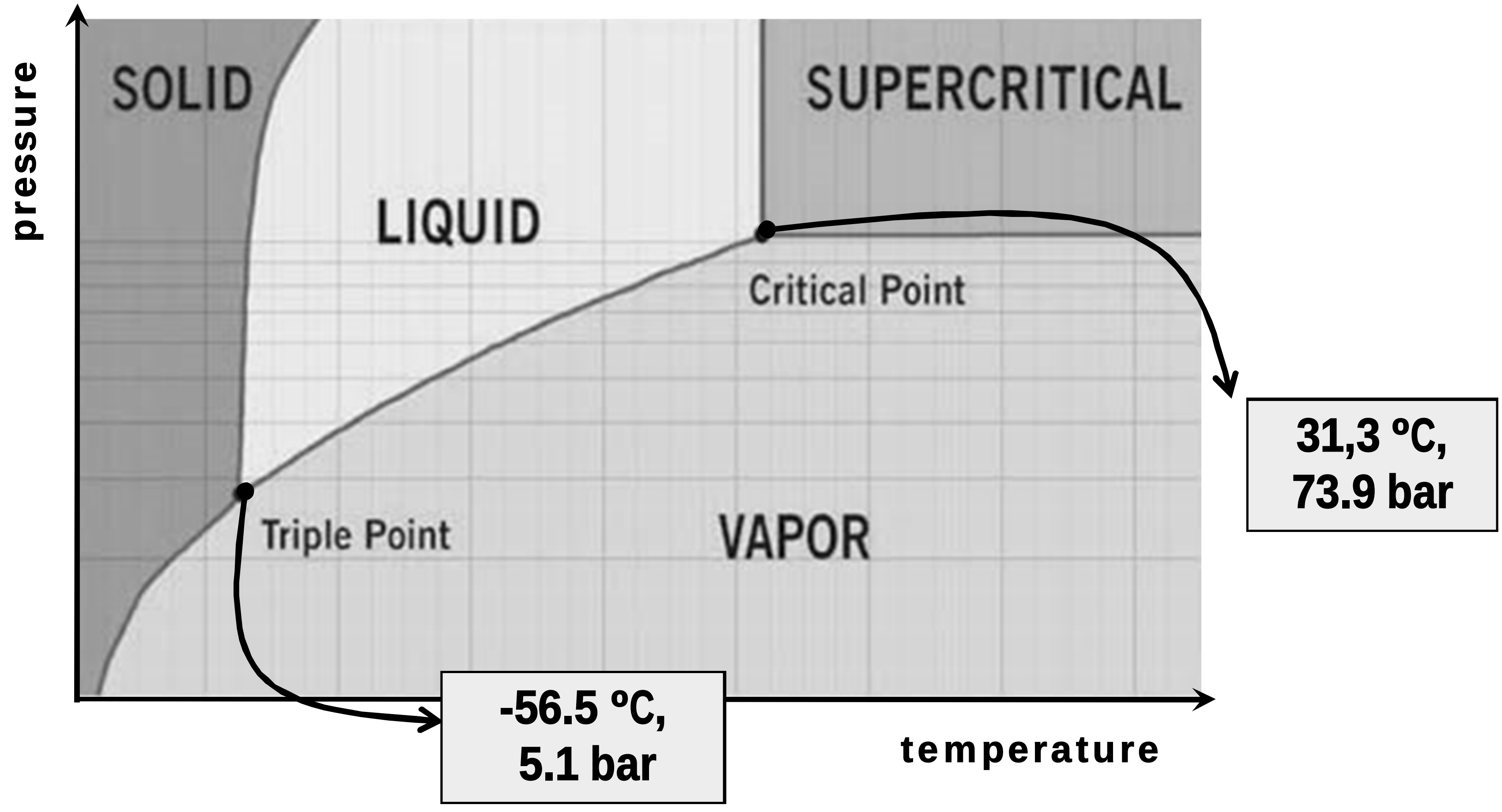
<u>Mobile phase:</u> no gas nor liquid, but a supercritical fluid.

V phase diagram of carbon dioxide



Supercritical Fluids

- At temperatures and pressures above its critical temperature and pressure (**critical point**), a substance is called a **supercritical fluid**. The **critical temperature** is the temperature above which a distinct liquid phase cannot exist. The vapor pressure at its critical temperature is its **critical pressure**.

- Where supercritical fluids exist: The forces from the kinetic energy of the molecules exceeds the forces from condensing influence of the intermolecular forces, so no distinct liquid phase exists

SFC Mobile Phases

- Mobile phases should have critical parameters that are easily reached using chromatographic pumps and ovens common to currently used instrumentation.
- Advantages of supercritical fluids over carrier gasses and liquid mobile phases are in its solubility properties, physical properties, and detector compatibility.

Use of Supercritical Fluids

Properties (density) **between gas and liquid**

Speed <u>and</u> **resolution** better compared to liquid chromatography (faster diffusion inside the column cavity, thus faster equilibration)

- **Supercritical fluids**
  - ...possess **lower surface tension** *than liquids* (i.e. they spread faster over stat. phase).
  - ...**dissolve non-volatile substances** *unlike gases*
  - ...evaporate upon pressure reduction after passing the column: **analytes** are in gaseous phase and thus easily detectable
- **mostly used: CO_2**
  - compatible with common detectors (FID, UV).
  - low critical temperature.
  - non-toxic.

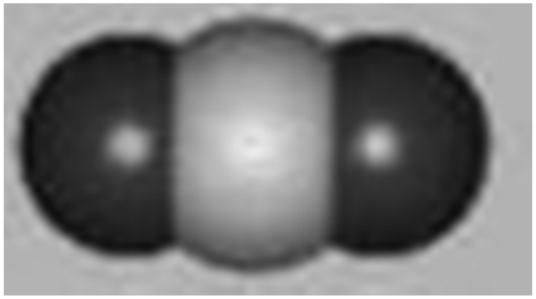


TABLE 29-1 Comparison of Properties of Supercritical Fluids with Liquids and Gases

Property	Gas (STP)	Supercritical Fluid	Liquid
Density, g/cm^3	$(0.6–2) \times 10^{-3}$	0.2–0.5	0.6–2
Diffusion coefficient, cm^2/s	$(1–4) \times 10^{-1}$	$10^{-3}–10^{-4}$	$(0.2–2) \times 10^{-5}$
Viscosity, $g\ cm^{-1}\ s^{-1}$	$(1–3) \times 10^{-4}$	$(1–3) \times 10^{-4}$	$(0.2–3) \times 10^{-2}$

Note: All data are to an order of magnitude only.

TABLE 29-2 Properties of Some Supercritical Fluids

Fluid	Critical Temperature, °C	Critical Pressure, atm	Critical Point Density, g/mL	Density at 400 atm, g/mL
CO_2	31.3	72.9	0.47	0.96
N_2O	36.5	71.7	0.45	0.94
NH_3	132.5	112.5	0.24	0.40
n-Butane	152.0	37.5	0.23	0.50

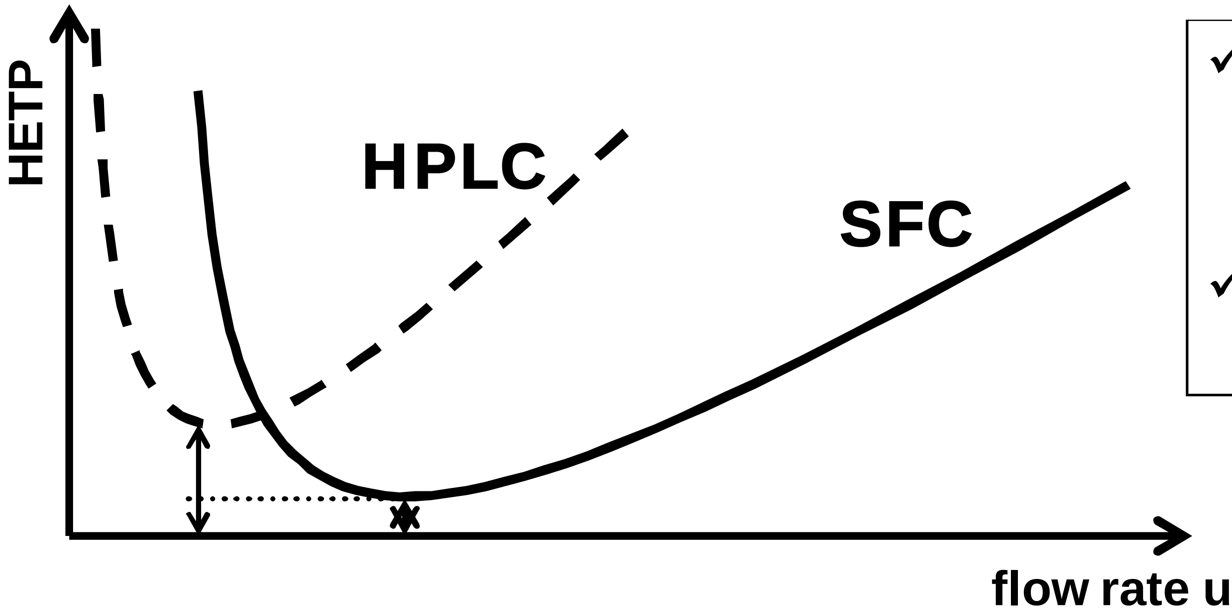
SFC Separations

- SFC is a hybrid of gas and liquid chromatography that combines some of the best features of each
- As in HPLC, variation of the mobile phase composition affects separation
- In SFC, mobile phase affinity for the analyte is a function of mobile phase density
- Density is controlled by controlling system pressure
- Highly polar samples are not easy to handle (high critical parameters & high reactivity)

SFC Advantages vs HPLC

- Supercritical fluids have low viscosities
  - faster analysis (5 to 10 X faster)
  - less pressure drop across the column
  - the use of open tubular columns is feasible
- Column lengths from 10 to 20 m are used
- Can be used with a wide range of sensitive detectors
- Resolving power is ~5X that of HPLC

Flow Rate and Gradient Elution in SFC



- **best** (smallest) **plate height** at <u>higher flow rate compared to HPLC</u>
- **min. plate height** <u>only **1/3** compared to HPLC</u>

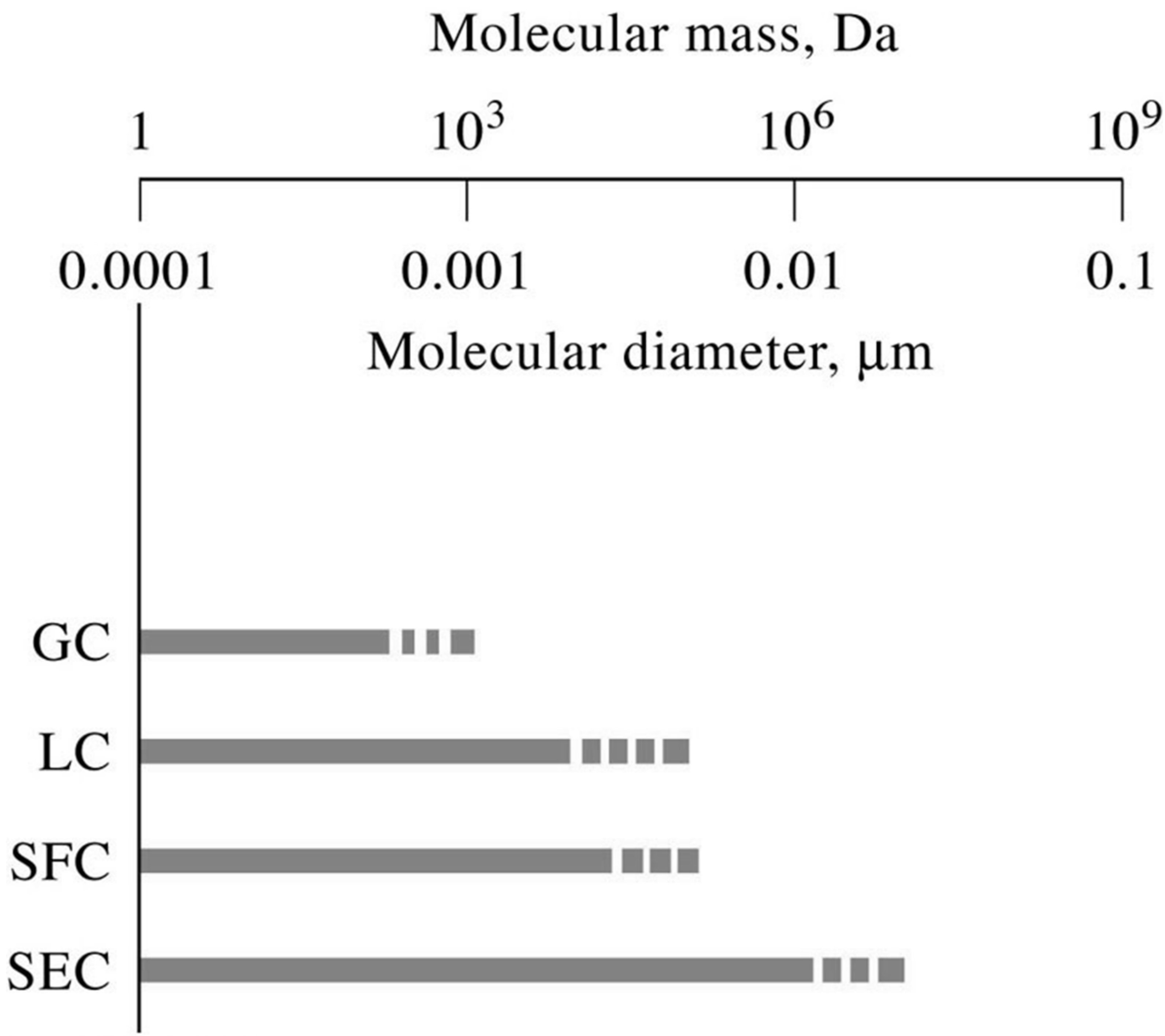
➢ ***What gradient?***

- in HPLC: usually solvent gradient
- in GC: usually temperature gradient
- In ion exchange: concentration or pH gradient
- ***in SFC: pressure gradient***

Density increases as **pressure increases**.
The **denser** the mobile phase,
the **bigger** its capacity for a solute,
the **less distribution** into stat. phase,
the **lower** the **retention.**

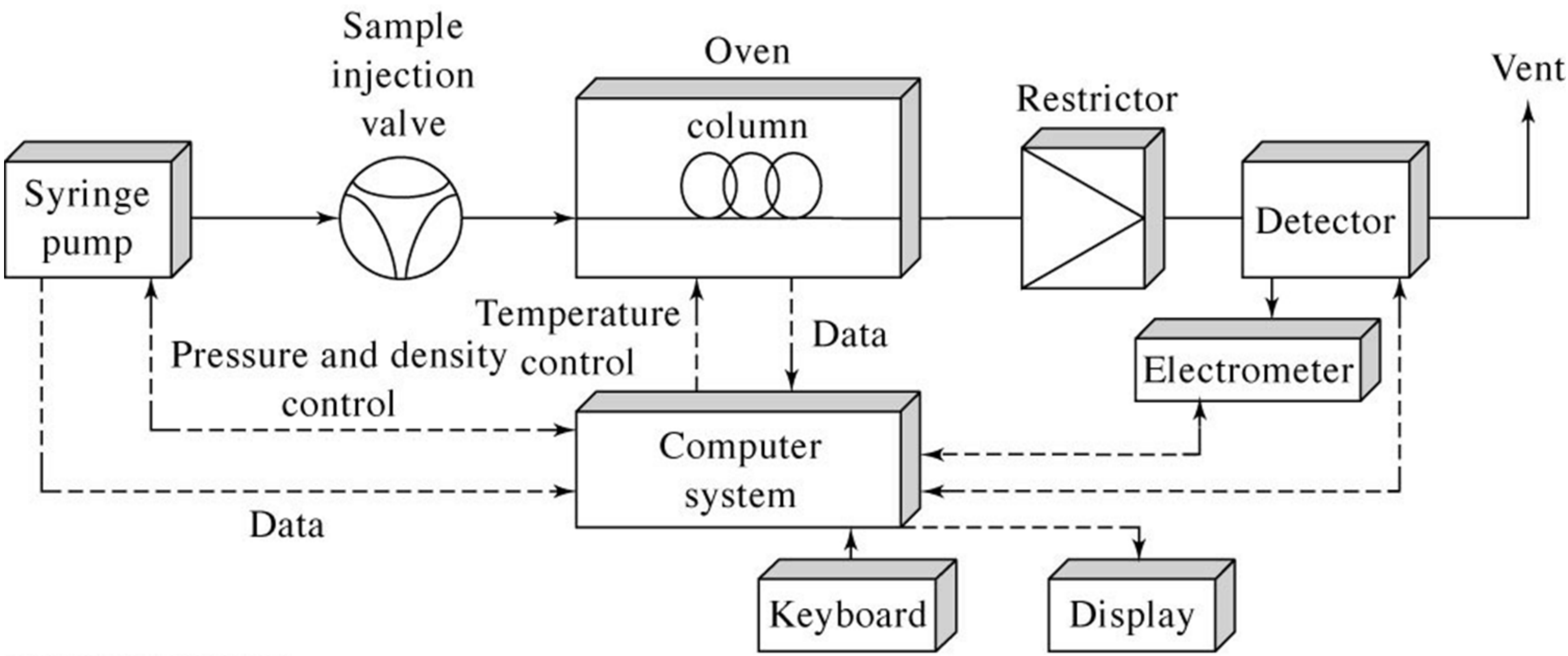
SFC Advantages vs GC

- Can analyze non-volatile, polar, or adsorptive solutes without derivatization.
- Can analyze thermally labile compounds.
- Can analyze solutes of much higher molecular weight.


Molecular mass, Da
1
10^3
10^6
10^9
0.0001
0.001
0.01
0.1
Molecular diameter, μm
GC
LC
SFC
SEC

SFC Instrumentation

- Solvent delivery system
- Injector
- Column/Column Oven
- Restrictor
- Detector
- Data System


Sample injection valve
Oven
column
Restrictor
Vent
Syringe pump
Detector
Temperature control
Data
Pressure and density control
Electrometer
Computer system
Data
Keyboard
Display

Solvent Delivery System

- Maintains precise mobile phase flow (1 to 10 μL/min {OT} or 1 to 10 mL/min {Packed}).
- Aids in the control of the system pressure (up to 60 Mpa).
- Moves mobile phase in the liquid state under pressure through the injector & into the column.

Injectors

- Typical HPLC design injectors for packed columns.
- Split/Splitless valve injector (0.01 to 0.05 μL injections) for open tubular columns.
- Timed - split injector (0.01 to 0.05 μL injections) for open tubular columns.

Detectors

- Most any detector used in GC or HPLC can be used.
- FID and UV detectors commonly used.
- Coupled Detectors
    - MS
    - FTIR

SFC Columns

- Open tubular (derived from GC)
  - Large # theoretical plates (~X500)
  - Easier to control pressure (low P drop)
- Packed (derived from HPLC)
  - Faster analysis
  - Higher flow rates
  - Higher sample capacity

Open Tubular Columns

- Smaller than GC capillary columns, typically 50 μm i.d., 10 to 20 m in length

- MP must be more stable due to extreme conditions of supercritical fluids

Packed Columns

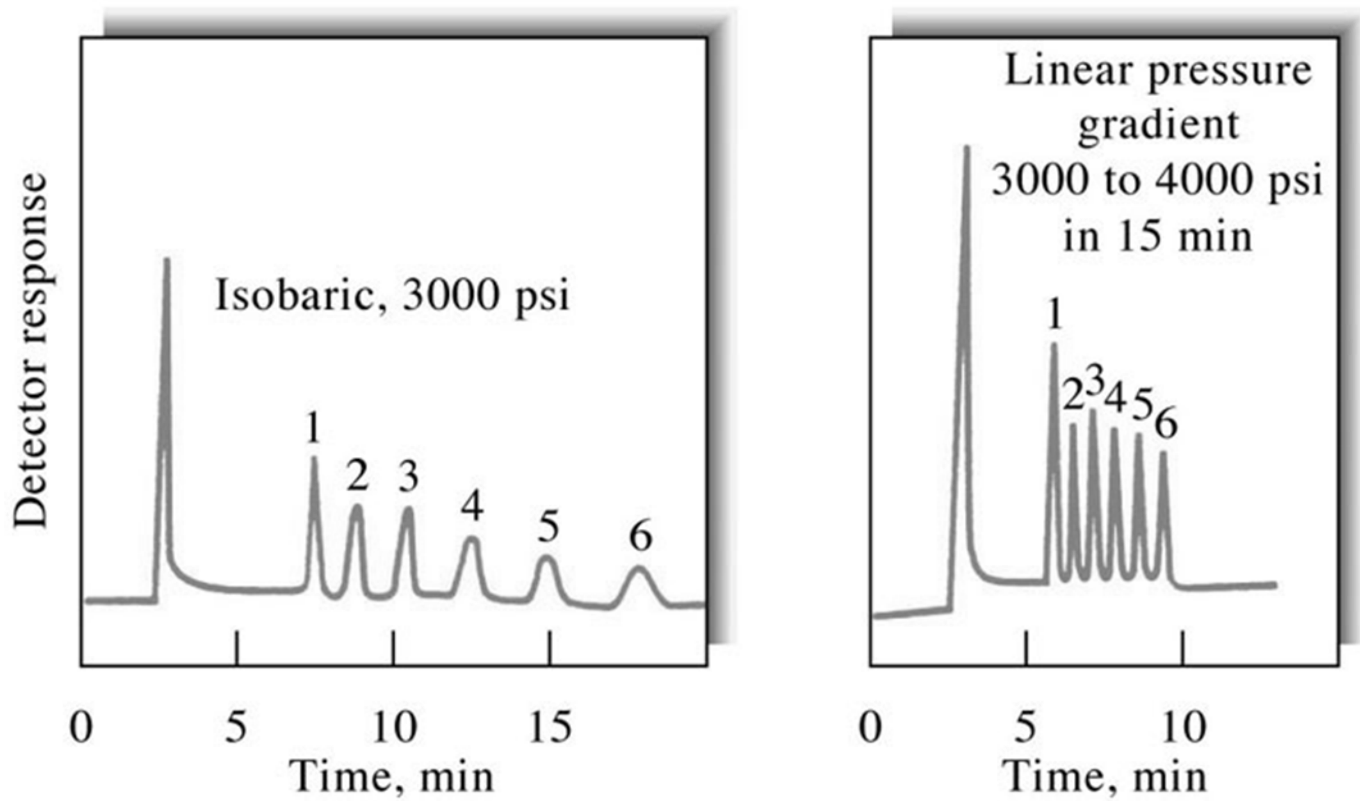
- Similar to HPLC columns (10, 5, or 3 μm porous particles)
- Silica based chemically bonded phases
- Typically 10 cm long X 4.6 mm i.d

SFC and Retention

- Retention dependent on temperature, pressure, mobile phase density, and composition of the stationary and mobile phase.
- Complex interactions and not easily predictable.
- For supercritical fluids

  - solvating properties similar to liquids - viscosity closer to gases
- Solvating power α density

Sample:	1. cholesteryl octanoate
	2. cholesteryl decylate
	3. cholesteryl laurate
	4. cholesteryl myristate
	5. cholesteryl palmitate
	6. cholesteryl stearate
Column:	DB-1
Mobile phase:	CO_2
Temperature:	90°C
Detector:	FID Flame ionization



Temperature/Pressure Effects

- At lower P, > T, < solubility
- At higher P, > T, > solubility

  -> T, P_v of solute > solute solubility

  -< fluid density < solubilizing power
- > T,   < solvent ρ
- >P,   > solvent ρ

Supercritical CO_2 Density

P (MPa)	T (^oC)	ρ (g/cm^3)
7.3	40	0.22
7.3	80	0.14
7.3	120	0.12
40	40	0.96
40	80	0.82
40	120	0.70

Solvent Programming

- Programming is very useful in controlling solvent strength.
- Variations in P (density), T, and mobile phase composition.
- Density programming is most widely used (not simple relationship, T & P).

  -> density, > solubility, < retention

  - Combined T & P programming to control ρ and thereby solubility and diffusion

SFC Mobile Phases

- Generally non-polar compounds with low to moderate critical properties

  - CO_2, N_2O, ethane, pentane

- Normal phase type separations

  - non-polar mp and low polarity sp (substrate + amino, diol, or cyano groups)

- Elution = function of molecular mass & polarity

Carbon Dioxide: SFC Solvent

- Low T_c

  - operating T as low as 40^{o}C
- Moderate P_c and ρ_c of 0.448g/cm3

  - reach high ρ with $P < 40$ MPa
- Safe to use

  - nontoxic, nonflammable, noncorrosive, inert
- Detector compatible
- Wide ρ range

Other SFC Solvents

- Nitrous Oxide - Similar in solvating and separations properties to CO_2
- Alkanes - less safe and not as detector compatible than CO_2

  - better solvent characteristics for non-polar solutes
- Halocarbons, xenon, etc. - specialty applications only
- More polar solvents for highly polar & high molecular weight compounds

Solvent Modifiers

- Add organic modifiers to > solvent strength

  - methanol

  - isopropanol

  - dichloromethane

  - THF

  - acetonitrile