Super critical fluid chromatography

Principles of Supercritical Fluid Chromatography (SFC)

Definition

- **Supercritical Fluid** Chromatography is a technique that uses supercritical **fluid as the mobile phase** for separation of compounds.

Supercritical Fluid

- A substance above its critical temperature and critical pressure.
- **Shows properties of both:**
  - Liquid (good solvating power)
  - Gas (low viscosity, high diffusion)
- **Common fluid used:**
  - Carbon dioxide (CO_2)

Principle

- **Separation** is based on distribution of **analytes between:**
  - Supercritical mobile phase
  - Stationary phase
- **Compounds differ in:**
  - Solubility in supercritical fluid
  - Interaction with stationary phase

Modifiers

- **Organic solvents (e.g., methanol) are added to:**
  - Increase polarity
  - Improve solubility of analytes

Mechanism of Separation

- Similar to HPLC (partitioning)
- Can also show gas chromatography-like behavior

Properties of Supercritical Fluids

- Low viscosity → faster flow
- High diffusivity → better separation
- Adjustable density → tunable solvent strength

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Advantages

- Fast analysis
- High efficiency
- Low solvent consumption
- Environment friendly (uses CO_2)

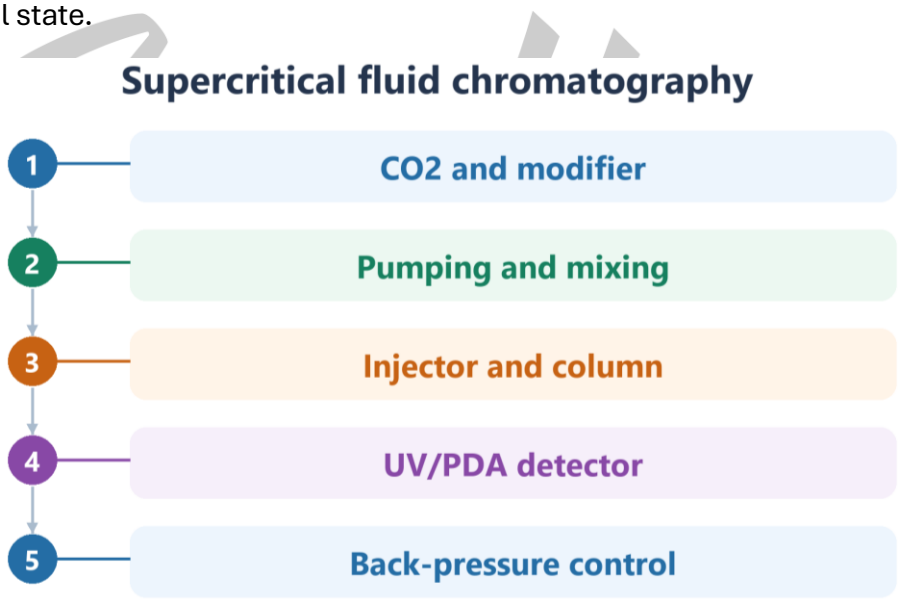
Limitations

- Limited to moderate polar compounds
- Requires specialized equipment

Instrumentation of Supercritical Fluid Chromatography

Overview

- **SFC** uses a supercritical fluid (commonly CO_2) as the mobile phase.
- The **System operates under high** pressure and temperature to maintain the fluid in a supercritical state.



An SFC system controls the mobile-phase flow, column conditions and back pressure during separation.

Components

A. Mobile Phase System

- CO_2 cylinder with high pressure control
- Pump used to deliver supercritical fluid
- Modifier pump for organic solvents

B. Injector

- Introducing sample into system

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- Must handle high pressure

C. Column

- Similar to HPLC columns
- Packed with stationary phase (e.g., silica, C18)

D. Column Oven

- Maintains temperature above critical temperature
- Ensures supercritical conditions

E. Back Pressure Regulator (BPR)

- Maintains constant pressure in system
- Essential to keep fluid in supercritical state

F. Detector

- **Common detectors:**
  - UV detector
  - FID (after decompression)
  - MS detector

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Working Summary

- CO_2 is pressurized and heated → becomes supercritical
- Sample injected → separation occurs in column
- Pressure released → detection

Importance of BPR

- Controls pressure
- Prevents phase change

Pharmaceutical Applications of Supercritical Fluid Chromatography

1. Drug Analysis

- Separation and analysis of pharmaceutical compounds
- Suitable for non-volatile and thermally unstable drugs
- Works well for non-polar and moderately polar compounds

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2. **Chiral Separation**
   - Widely used for enantiomeric separation
   - Often faster than HPLC
   - Provides high resolution
3. **Impurity Profiling**
   - Detection and analysis of impurities in drugs
   - Important for quality control and safety
4. **Natural Product Analysis**
   - Separation of plant constituents
   - Used in herbal and natural medicine research
5. **Lipid and Steroid Analysis**
   - **Analysis of:**
     - Fats and oils
     - Lipophilic compounds
     - Steroids

Advantages in Pharmaceuticals

- Faster analysis compared to HPLC
- High resolution and efficiency
- Reduced solvent consumption
- Suitable for thermally sensitive compounds
- Uses CO_2 (non-toxic, recyclable) → environmentally friendly

Limitations

- Limited solubility for highly polar compounds
- Requires specialized and expensive equipment

Environmental Benefit

- Uses CO_2 as mobile phase
- **CO_2 is:**
  - Non-toxic
  - Non-flammable
  - Recyclable

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Electrophoresis

Capillary Electrophoresis (CE): Overview in Pharmaceutical Analysis

Definition

- **Capillary** Electrophoresis is a separation technique based on movement of charged particles in an electric field through a narrow capillary tube.

Basic Concept

- **When an electric field is applied:**
  - Charged particles move toward opposite electrodes
  - **Separation occurs based on:**
    - Charge
    - Size (and charge-to-size ratio)

Role in Pharmaceutical Analysis

- **Widely used for:**
  - Drug analysis
  - Impurity profiling
  - Chiral separations
  - Protein and peptide analysis

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Why CE is Important

- Requires very small sample volume
- High efficiency and resolution
- Fast analysis

Applications in Pharmaceuticals

- Analysis of ionic drugs
- Biological samples (proteins, DNA)
- Quality control
- Stability studies

Advantages

- High separation efficiency
- Low solvent consumption

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