BIO & PHARMA ANALYTICAL TECHNIQUES

Chapter 2
PAT: Pre-formulation Development

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Chapter Description

- **Aims**
  - Explain Process Analytical Techniques (PAT) and the benefits to pharmaceutical industry

- **Expected Outcomes**
  - Discuss the history and background of pre-formulation studies in pharmaceutical industry
  - Describe the importance of pre-formulation studies in pharmaceutical
  - Analyze physicochemical properties related to pre-formulation study
  - Categorize analytical techniques and instruments involve in pre-formulation study

- **References**

  - Gunzler H. & Williams A. (2002). Handbook of Analytical Techniques. Wiley-VCH, Weinheim, Germany.
  - Mullertz, A., Perrie, Y. and Rades, T. (2016) Analytical Techniques in the Pharmaceutical Sciences (Advances in Delivery Science and Technology). Springer, United States.

PRE-FORMULATION STUDIES

DEFINITION:

Process of optimizing the delivery of drug through determinations of physicochemical properties of the new compound that could effect drug performance and development of an efficacious, stable and safe dosage form.

Involves:

- Investigation of physical and chemical property of a drug substance alone and when combined with excipients
- Prior to the development of any dosage form new drug, it is essential that certain fundamental physical & chemical properties of drug powder are determined.

PURPOSES

To understand the **nature and characteristics** of each component and **to optimize conditions** of the dosage form manufacture.

Physiochemical properties are those that can be determined from in vitro experiments

Need for Pre-formulation Study

- Establishment of drug specifications intended for toxicological evaluation and clinical supply preparations
- Providing scientific data to support dosage form development and evaluation of product efficacy, quality, stability and bioavailability
- Establish the identity and physico-chemical parameters of a new drug substance.
- Establish its compatibility with common excipients
- Evaluation of the stability of early developed dosage forms
- Fulfillment of the requirement of the CMC section of the IND and subsequent NDA or ANDA.

PHYSICOCHEMICAL PROPERTIES

BULK PROPERTIES

- Bulk properties of the solid form such as **crystallinity, polymorphism, particle size, powder flow property and surface characteristics**

CRYSTALLINITY:

- Degree of structural order in solid (arrangement of atom and molecule)
- It influences density, hardness, transparency, diffusion
- **How to check??**

  Differential scanning calorimetry (DSC), X-ray diffraction (XRD), FTIR, microscopy

Polymorphism

- ✓ ability of solid material to exist in many forms
- ✓ Formation of different polymorphs depends on solvents, T, P, rate of cooling etc.
- ✓ Polymorphic transitions can also occur during milling, granulating, drying and compressing operations.
- ✓ Different polymorphs can effect the dissolution, solid-state stability, compatibility.
- ✓ **How to check?**
  - → **X-ray diffraction (XRD)**
  - → **IR/Raman spectroscopy**
  - → **DSC/TGA.**

Powder Flow Property

POWDER FLOW PROPERTY

- Ability of the powder to flow/flowability
- Flow properties: bulk density, permeability, cohesive strength and wall friction
- The flow properties of a powder will determine the nature and quantity of excipients needed to prepare a compressed or a powder dosage form
- This refers mainly to factors such as the ability to process the powder through machines.
- **How to check?**

  → **Rheometry**

Hygroscopicity

- The tendency of a solid to take up and release water from the atmosphere

- **How to check?**

→ Plot change in water vs relative humidity at constant T

→ Without fancy equipment, water content can be measured by weighing the wet material, removing the water using a dessicator and weighing the dried material. Doing this for multiple humidities will give you a similar curve.

Purity

- Free from other elements/contamination.
- Clean, clear, unmixed.
- An impurity can affect stability.

  E.g: metal contamination, other trace elements
- **How to check**

  → ICP-MS, AAS, DSC/TGA, chromatography

ORGANOLEPTIC PROPERTIES

COLOR	ODOUR	TASTE
Off-white	Pungent	Acidic
Cream-yellow	Sulfurous	Bitter
Shiny	Fruity	Sweet
	Aromatic	Tasteless
	Odourless	Tasteless

Organoleptic Properties

COLOUR:

- Color intensity relates to the **presence of chromophores**

ODOUR

- The substance may exhibit an **inherent odor characteristic** of **major functional group present**
- Odor greatly affects the flavor of a preparation or food stuff

TASTE

- If taste is considered as unpalatable, consideration is to be given to the use of a less soluble chemical form of the drug.
- The odour and taste may be suppressed by using appropriate flavors and excipients or by coating the final product.

Particle Size

Particle size is characterized using these terms:

1. Very coarse
2. Coarse
3. Moderately coarse
4. Fine
5. Very fine

Particle size can influence variety of important **factors:**

- **Dissolution rate**
- **Suspendability**
- **Uniform distribution**
- **Penetrability**

How to check?

1. Sieving
2. Microscopy
3. Sedimentation rate method
4. Light energy diffraction
5. Laser holography

1. Sieving method:

Range; 50 – 150 μm

Simple, inexpensive

If powder is not dry, the apertures get clogged

2. Microscopy:

Range 0.2 – 100 μm

Particle size can be determined by the use of calibrated grid background

Most direct method

Slow and tedious method

3. Sedimentation method:

Range 1 – 200 μm

Andreasen pipette is used

Particle size is calculated by stroke's law:

$$d_{st} = \sqrt{\frac{18\,\eta_0\,h}{(\rho_s - \rho_0)\,g\,t}}$$

Where,

h = distance of fall in time, t
n_o = viscosity of the medium
ρ_s = density of the particles
ρ_0 = density of the dispersion medium
g = acceleration due to gravity

4. Light energy diffraction

- Range 0.5 – 500 µm
- Particle size is determined by the reduction in light reaching the sensor as the particle
- Quick and fast

5. Laser holography:

- Range 1.4 – 100 µm
- A pulsed laser is fired through a particle and photographed three dimensional with holographic camera, allowing the particles to be individually imaged and sized.

PARTICLE SHAPE – identity

SURFACE AREA – identity

} Microscopy

SOLUBILITY

- The **amount of substance that passes into solution** in order to establish equilibrium at constant T and P to produce a saturated solution.
- Pre-formulation solubility studies **focus on drug solvent system** that could occur during the delivery of drug candidate.
- **For ex**: A drug for oral administration should be examined for solubility in media having isotonic chloride ion concentration and acidic pH.
- **How to check?**

  → **HPLC, chromatography**

Analytical techniques and instruments

- A **pre-formulation study** is performed **to gain insight from physicochemical and biological data** into the design and development of dosage forms.
- Samples are taken in each study and analysed **qualitatively and/or quantitatively**, according to the needs.

IDENTITY

- Nuclear magnetic resonance (NMR)
- Infra red spectroscopy (IR)
- Ultraviolet spectroscopy (UV)
- Thin layer chromatography (TLC)
- Differential scanning calorimetry (DSC)
- Microscopy

PURITY

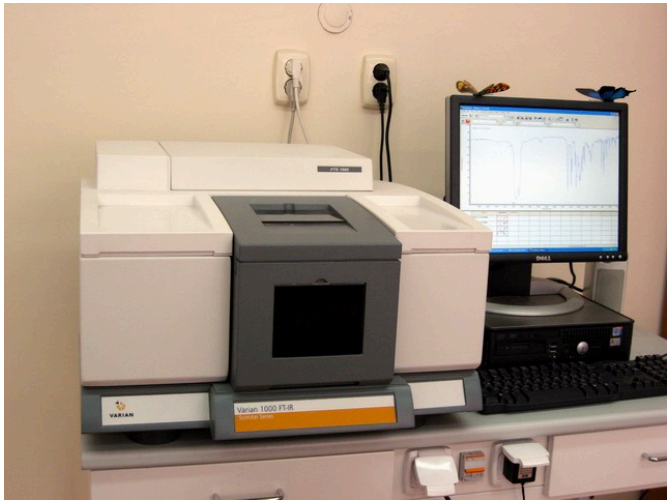
- Moisture (water and solvents)
- Inorganic elements
- Heavy metals
- Organic impurities
- Differential scanning calorimetry
- Melting point

Assay & separation

- Ultraviolet spectroscopy (UV)
- High performance liquid chromatography (HPLC)
- Thin-layer chromatography (TLC)

IR spectroscopy

- Used for fingerprint identification of a drug molecule and the proof of its structure
- IR absorption bands are characteristic of the functional group of a molecule as well as the structure configuration.
- An example of modern IR equipment is FTIR, which gives better quality determination.



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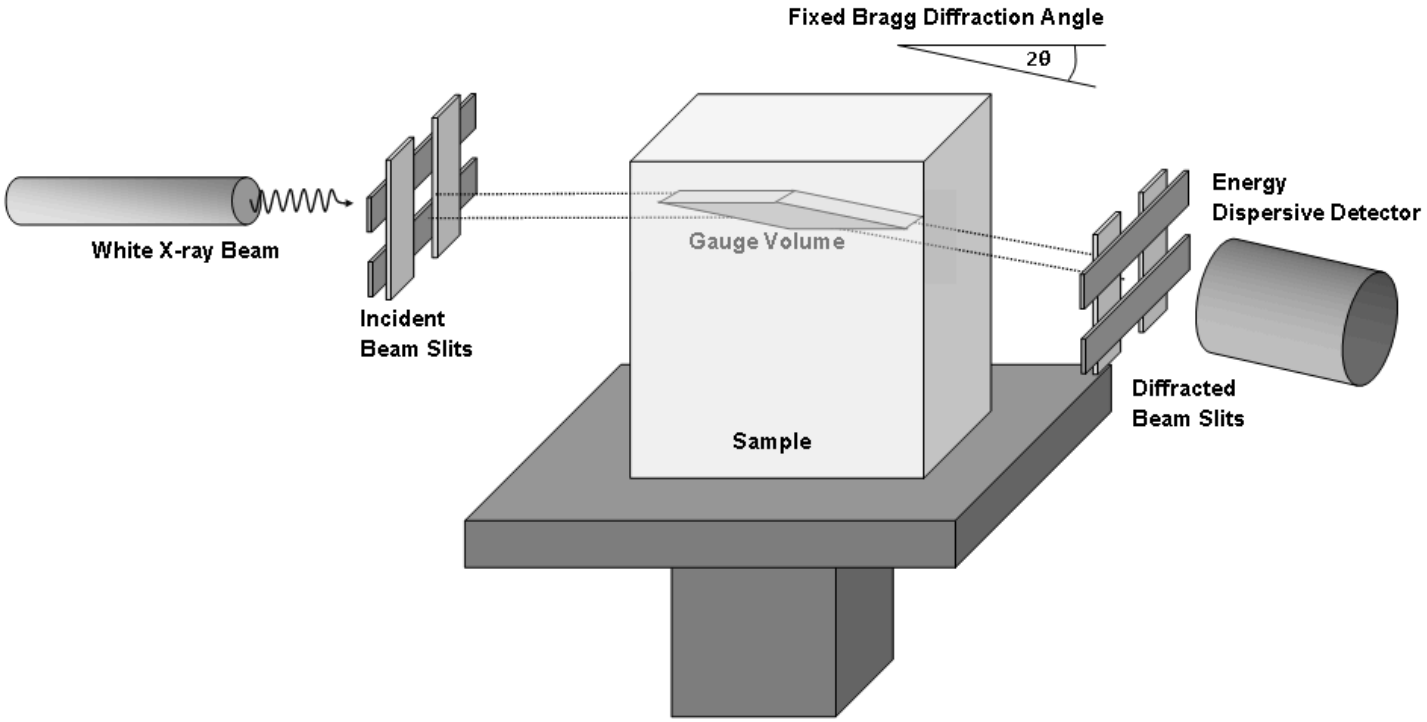
**FTIR: Fourier Transform Infra Red*

Raman spectroscopy

- A nondestruction tool and requires little or no sample preparation.
- A sample may be analysed in solid or powder form or in aqueous solution and placed in glass containers such as an NMR tube, GC vial, test tube, light-path cell, or glass bottle.
- Aside from **structure elucidation** and **functional group analysis**, FT-Raman may be used for quantitative determination of polymorphs in a pre-formulation study.

X-ray Diffraction (XRD)

- Obtains information on **substance structure** at the **atomic level**
- This technique allows measurement of both **crystalline** and **noncrystalline** materials
- The analysis is non-destructive in nature and handles samples in the form of powders, solids and liquids.
- The X-ray diffraction of a single crystal is employed for the **determination of the absolute chemical structure.**



NMR spectroscopy

- NMR involves the absorption of **electromagnetic radiation** in the radiofrequency of a longer wavelength spectrum.
- NMR spectra gives information about **structure** and **atomic environment of molecule**

Source: https://en.wikipedia.org

Mass spectrometry

- Detection of charged particles or ions **separated according to their mass to charge (*m/z*) ratio** after ionisation and acceleration through magnetic field
- Mass spectra gives information about **molecular weight** of substance and what its degraded or metabolic product will be.

Metal analysis

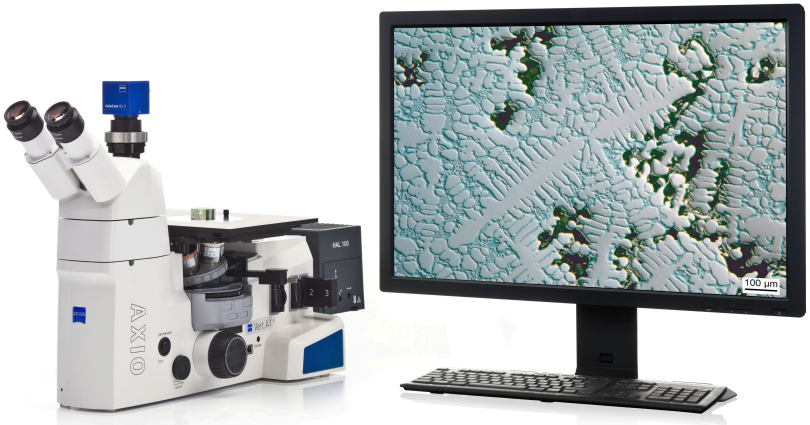
- Pharmaceutical compounds such as ferrous sulfate, ferros gluconate, zinc undecylenate and magnesium stearate (a commonly used excipient)
- Sodium, potasium, zinc detection for certain preparations like protamine zinc insulin etc.
- Presence of metal in pharmaceuticals, even in trace amounts, is a form of contaminant.
- For ex., metallic ions may act as a catalyst in oxidation that may be detected in drug products.
- **ICP-MS**

ICP-MS

Source: https://en.wikipedia.org

Microscopy

- In this technique, substances are examined under the **microscope.**
- It gives information about **shape, thickness , particle size** of drug molecules.
- By this method we can study **crystal morphology,** difference between polymorphic character of molecule.



Thermal Analytical Methods

1. Differential scanning calorimetry, DSC
2. Thermal gravimetric analysis, TGA

- **DSC**: measuring the endothermic and exothermic behaviours of sample materials
- **TGA**: measures the weight change (gains and losses) as a function of temperature or time is recorded which provides information about the material's thermal stability and compositional analysis (e.g., moisture content of the materials, melting points).
- DSC and differential thermal analysis (DTA) are particularly useful in **the investigation of polymorphism.**

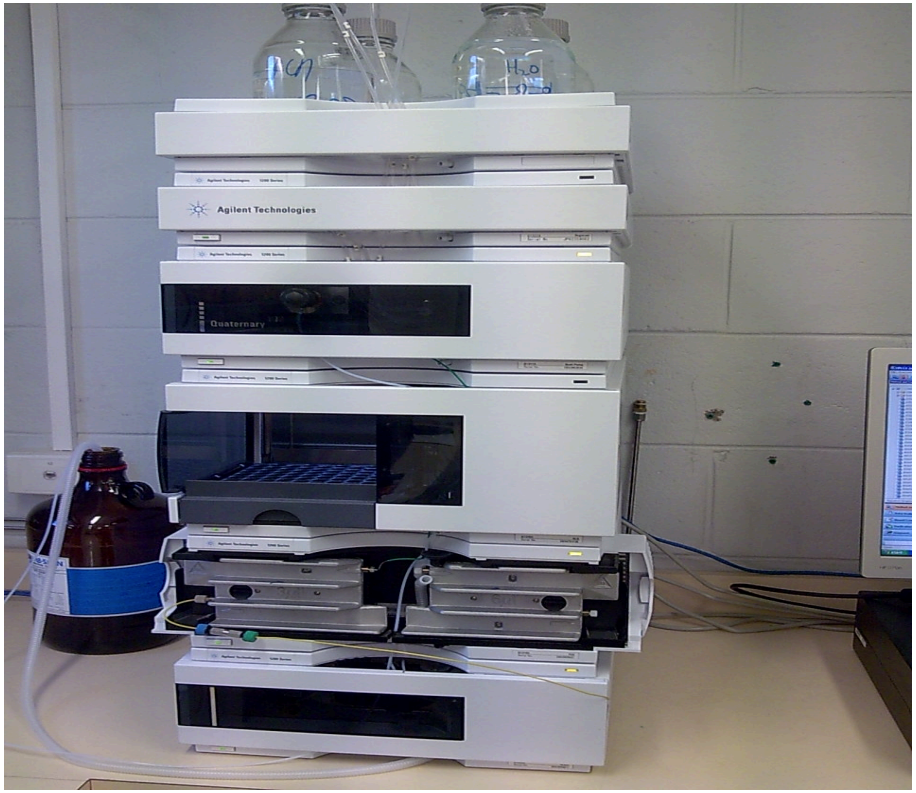
Thin Layer Chromatography (TLC)

- **PURPOSE: impurity profiling** in drug development
- Involves most convenient, inexpensive and portable equipment
- General detection technique is to spray a sample with a detecting agent, which reacts chemically with the ingredient to be detected or visual observation under short- or long-wave **UV light** is also employed.
- The **disadvantages of TLC**:
  - Irreproducibility
  - Detection inconsistency
  - Person-to-person variations (human error)

HPLC

- High performance liquid chromatography (HPLC) is used extensively in the laboratory for **quantization of drugs and related compounds.**
- Identification of a drug component can simultaneously be determined **by retention times** in the chromatogram.
- **Reliable analytical tool** for pre-formulation study because of the high-resolution capacity, accuracy and reproducibility of the equipment.
- Its primary function includes search for and detection of impurities in drug substances, stability evaluation of dosage forms in terms of detection and quantization of degradation.

HPLC

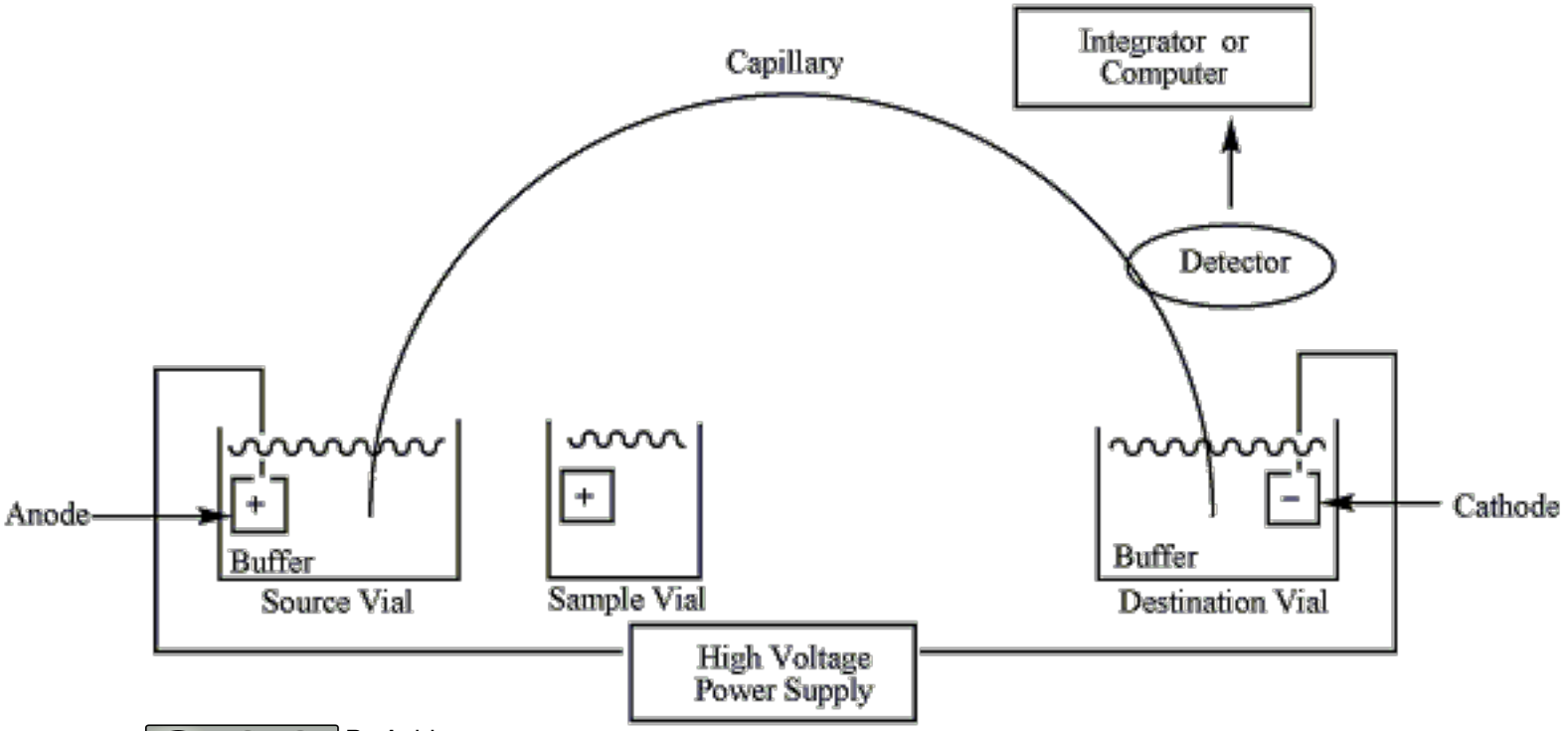


Capillary electrophoresis (CE)

- In free solution CE, the separation and migration of the molecules through the capillary are based on electrophoretic migration (based on net charge) and electroosmotic flow (the bulk flow of electrolyte buffer)

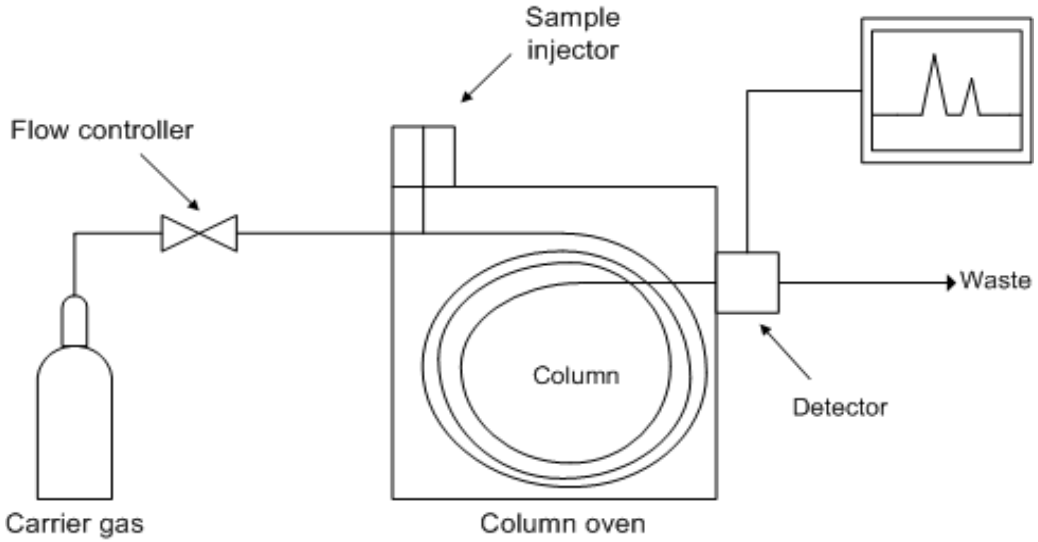
- Other mechanisms for separation depend on molecular size, isoelectric focusing and hydrophobicity.

CAPILLARY ELECTROPHORESIS



Gas Chromatography

- GC is used for speedy separation or for high resolution separation of volatile or thermal labile substances.
- GC has good sensitivity, with detection limits of 1 ppb to 100 ppm.
- With the advances in HPLC, GC is utilised less often. It is still used for the analysis of retained solvents, such as the USP test for volatile organic solvents.



Ion Chromatography (IC)

- IC is a modified version of HPLC with a capacity for precise and **highly sensitive detection of inorganic ions** in a complex matrix.
- IC has instrumental configurations similar to those of HPLC, but the **stationary phase is an ion exchange column**, and the detector can be either an electrochemical detector or a colorimeter with a mixer to carry out color formation by chemical reaction with the detected ion.

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Supercritical Fluid Chromatography (SFC)

- SFC uses **highly compressed gas** above its critical temperature and pressure instead of an organic solvent as the solvent phase.
- Major advantage is allowance in the analysis **for thermal unstable components.**

Conclusion of the Chapter

1. In the field of drug analysis, the analytical investigation of bulk drug materials, the intermediates in their synthesis is a very important area of research.
2. Drug discovery requires a **solid analytical background**, with a great variety of methods to be used.
3. **Chromatography** and **spectroscopy** are orthogonal techniques, i.e. their types of information are very different.
4. Chromatography is a separation method and spectroscopy is a technique which yields a 'fingerprint' of molecules.
5. **HPLC** is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatilised, thermally unstable and have high molecular weights.

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Any Question?

Please refer to:

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