BP605T · B.Pharm Semester VI · Theory Notes

# Pharmaceutical Analysis

## Unit V — Gas Chromatography, HPLC & HPTLC

**Unit V (10 hours)**

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From-zero teaching standard: every technical term is defined before use.

Syllabus coverage: Unit V (10 hours).

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## UNIT V — GAS CHROMATOGRAPHY, HPLC AND HPTLC (10 hours)

# CHAPTER 5: GAS CHROMATOGRAPHY, HPLC AND HPTLC

**(BP605T — Pharmaceutical Analysis, Unit V)**

## 5.1 Learning Objectives

After studying this chapter, you will be able to:

1 Define chromatography, gas chromatography (GC), HPLC and HPTLC in simple words.

2 Explain the theory of GC — how substances separate between a gaseous mobile phase and a stationary phase — and the meaning of retention time.

3 Name and explain each part of a GC instrument: carrier gas, injection port, columns, column oven, detectors (FID, TCD, ECD) and recorder.

4 Explain derivatization — why it is needed, and what silylation and methylation do.

5 Explain temperature programming — what it is and why it is used.

6 List the advantages, disadvantages and applications of GC (residual solvents, essential oils, blood alcohol).

7 Explain the theory of HPLC — high-pressure liquid mobile phase, normal phase vs reversed phase.

8 Name and explain each part of an HPLC instrument: solvent reservoirs, degasser, pump (isocratic/gradient), injector, column (C18), detectors (UV-Vis, PDA, fluorescence, RI), data system.

9 List the advantages and applications of HPLC (tablet assay, impurity profiling, stability studies, herbal standardization).

10 Explain the principle of HPTLC, its instrumentation and its applications (herbal fingerprinting, multi-sample screening).

11 Compare GC, HPLC and HPTLC in a table.

12 Solve numerical problems on capacity factor (k), selectivity (α), theoretical plates (N) and resolution (Rs), and interpret what the numbers mean.

## 5.2 Definitions

- **Chromatography.** A separation technique in which a mixture is carried by a moving fluid (the **mobile phase**) through or over a fixed material (the **stationary phase**). Different substances in the mixture interact differently with the stationary phase, so they travel at different speeds and come out separated.

- **Mobile phase.** The moving fluid (gas or liquid) that carries the sample mixture forward through the instrument. Think of it as the "river" in which sample substances float.
- **Stationary phase.** The fixed material (a solid or a liquid coated on a solid) that does not move. Think of it as the "riverbank" — substances that stick to it more move slower.
- **Gas chromatography (GC).** A type of chromatography in which the mobile phase is a **gas** (such as helium or nitrogen) and the stationary phase is a liquid or solid coated inside a long tube called a **column**. Only substances that can turn into vapour (gases or liquids that boil easily) can be analysed by GC.
- **Retention time (tR).** The time taken by a substance to travel from the injection point to the detector — in other words, how long it stays inside the column. Each substance has its own retention time under fixed conditions, so retention time helps **identify** the substance. (A substance that does not stick to the stationary phase at all comes out with the mobile phase; that time is called the **dead time, tM**.)
- **High performance liquid chromatography (HPLC).** A type of chromatography in which the mobile phase is a **liquid** pushed at **high pressure** through a column packed with very fine particles. It is used for substances that dissolve in liquids, especially medicines that are heat-sensitive and cannot be boiled.
- **HPTLC (high performance thin layer chromatography).** An improved, automated version of thin layer chromatography (TLC). It uses plates coated with very fine particles, applies samples as neat bands with a machine, develops them in a controlled chamber, and measures the spots with a scanner. It is used for quick screening of many samples together, especially herbal products.

## 5.3 Key Terminology (Mini-Glossary)

| Term | Simple meaning |
|---|---|
| **Analyte** | The substance you want to analyse (find or measure). |
| **Column** | A long tube (GC) or steel tube (HPLC) filled with or coated with the stationary phase, where separation happens. |
| **Carrier gas** | The mobile phase gas in GC (usually helium He or nitrogen N2) that carries the sample through the column. |
| **Eluent** | The mobile phase liquid in HPLC; the liquid coming out of the column. |
| **Injection port** | The heated entry point of GC where the liquid sample is injected and instantly turned into vapour. |
| **Split / splitless injection** | Two ways of injecting sample into a GC: *split* sends only a small part of the vapour to the column (for concentrated samples); *splitless* sends almost all of it (for very dilute, trace samples). |
| **Packed column** | A GC column filled with solid particles coated with stationary-phase liquid — short, wide, cheap, lower separation power. |

| Term | Simple meaning |
|---|---|
| **Capillary column** | A GC column that is very long and very thin (like a hair), with stationary phase coated on its inner wall — high separation power, the standard choice today. |
| **Column oven** | A temperature-controlled box that holds the GC column and keeps its temperature steady or ramps it up (temperature programming). |
| **Detector** | The part of the instrument that "sees" each separated substance as it leaves the column and converts it into an electrical signal. |
| **Chromatogram** | The graph (paper or screen output) showing peaks — each peak is one separated substance; peak position identifies it, peak size measures how much is present. |
| **Derivatization** | Chemically converting an analyte into a new compound (a *derivative*) that is more volatile (for GC) or easier to detect. |
| **Silylation** | A derivatization reaction that replaces the hydrogen of –OH, –COOH or –NH groups with a silicon-containing group, making the compound more volatile. |
| **Methylation** | A derivatization reaction that converts acids (–COOH) into methyl esters (–COOCH3), which boil more easily. |
| **Temperature programming** | Gradually increasing the column oven temperature during a GC run, so that low-boiling and high-boiling substances all separate well in one run. |
| **Normal phase (HPLC)** | Stationary phase is **polar** (silica), mobile phase is **non-polar** (e.g., hexane) — polar substances stick more. |
| **Reversed phase (HPLC)** | The opposite and most common mode: stationary phase is **non-polar** (C18 chains), mobile phase is **polar** (water mixed with methanol or acetonitrile) — non-polar substances stick more. |
| **C18 column** | The most widely used HPLC column: silica particles with 18-carbon chains attached, giving a non-polar surface (used in reversed phase). |
| **Isocratic elution** | HPLC mode where the mobile phase composition stays **the same** during the whole run. |
| **Gradient elution** | HPLC mode where the mobile phase composition **changes gradually** (e.g., from more water to more acetonitrile) during the run — like temperature programming, but for liquids. |
| **Degasser** | A part of the HPLC that removes dissolved air bubbles from the solvents, because bubbles disturb the pump and detector. |
| **PDA detector** | Photodiode Array detector — records UV absorption at many wavelengths at the same time, giving the full spectrum of each peak. |
| **Rf value** | In TLC/HPTLC, the ratio: distance moved by the spot ÷ distance moved by the solvent front. Used to identify substances. |
| **Capacity factor (k)** | A number showing how strongly a substance is held back by the stationary phase: k = (tR − tM) / tM. |
| **Selectivity (α)** | A number showing how well the column separates two substances: α = k2 / k1. α > 1 means the two substances separate. |

| Term | Simple meaning |
|---|---|
| **Theoretical plates (N)** | A number describing column efficiency — higher N means sharper, better-separated peaks. $N = 16 \times (tR / w)^2$, where w is the peak width at the base. |
| **Resolution (Rs)** | A number describing how completely two neighbouring peaks are separated: $Rs = 2 \times (tR2 - tR1) / (w1 + w2)$. $Rs \geq 1.5$ means the two peaks are fully (baseline) separated. |

## 5.4 Detailed Explanations (Concepts with Analogies)

### 5.4.1 Gas Chromatography — Introduction

**Technical concept.** Gas chromatography separates the components of a mixture that can be vaporised (turned into gas) without breaking down. A tiny amount of sample is injected into a heated port, instantly becomes vapour, and is pushed by a stream of carrier gas through a long heated column. Inside the column, each component divides ("partitions") itself between the moving gas and the stationary phase. Components that dissolve more in the stationary phase move slowly; components that prefer the gas move fast. They reach the detector one by one at different times.

**Simple explanation.** Imagine a race on a road with sticky patches. All runners start together, but runners wearing sticky shoes slow down on the sticky patches while others run past. At the finish line, they arrive one by one — separated. In GC, the "runners" are the chemicals, the "road" is the column, and the "sticky patches" are the stationary phase.

**Example.** A cough syrup contains two volatile flavours, A and B. In GC, flavour A (less sticky) comes out at 3 minutes and flavour B (more sticky) at 5 minutes. Two separate peaks appear on the chromatogram, and the factory can check that both flavours are present in the right amounts.

**Key takeaway.** GC separates volatile substances by how strongly each one sticks to the stationary phase; each substance has its own retention time.

### 5.4.2 Theory of GC — Partition and Retention Time

**Technical concept.** The heart of GC theory is **partition** (division) of each analyte between two phases:

- **Mobile phase:** a gas (He or N2) that flows continuously.
- **Stationary phase:** a liquid (e.g., polysiloxane oil) coated as a thin film inside the column, or a solid adsorbent.

Each analyte has a **partition (distribution) coefficient, K** = concentration in stationary phase ÷ concentration in mobile phase. A large K means the substance spends most of its time dissolved in the stationary phase and moves slowly → **long retention time (tR)**. A small K means it stays mostly in the gas and rushes through → **short retention time**.

**Simple explanation.** Think of bees (analyte molecules) flying along a corridor where one wall is coated with honey (stationary phase). Some bees keep landing on the honey wall and take long to cross; others barely touch it and cross quickly. "How honey-loving each bee is" is the partition coefficient K; "time to cross the corridor" is the retention time tR.

**Example.** In a residual-solvent test, methanol (low boiling, small K) comes out of the GC column in 2 minutes, while toluene (higher boiling, larger K) comes out in 8 minutes.

**Key takeaway.** Bigger partition coefficient → more time in stationary phase → longer retention time. Retention time identifies the substance; peak area tells the amount.

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### 5.4.3 Derivatization — Why and How

**Technical concept.** GC needs the analyte to be **volatile** (able to become vapour) and **thermally stable** (not destroyed by heat). Many drug molecules are **polar** (they have –OH, –COOH, –NH2 groups) and form strong hydrogen bonds, so they boil at very high temperatures or stick badly to the column, giving ugly, tailing peaks. **Derivatization** solves this: a simple chemical reaction converts the polar groups into less polar, more volatile groups *before* injection.

**Simple explanation.** Some guests (polar drugs) refuse to leave the party (column) because they keep hugging everyone (hydrogen bonding). Derivatization gives each guest a smooth coat (a chemical change) so they stop hugging and walk out easily.

**Two common types, in simple words:**

1 **Silylation.** The reagent replaces the hydrogen atom of –OH, –COOH or –NH groups with a group containing silicon, –Si(CH3)3 (trimethylsilyl). It is like putting a "silicon cap" on the sticky hydrogen. Result: the molecule becomes much more volatile and gives sharp peaks. Used for alcohols, phenols, acids, amines, sugars.

1 **Methylation.** The reagent converts a carboxylic acid (–COOH) into a methyl ester (–COOCH3) — replacing the –H with a –CH3 group. The ester boils at a much lower temperature than the acid. Used for fatty acids and acidic drugs.

**Example.** Amino acids cannot be analysed directly by GC (they are polar and decompose on heating). After silylation, they become volatile and separate beautifully.

**Key takeaway.** Derivatization = chemical makeover that makes non-volatile, polar analytes volatile enough for GC.

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### 5.4.4 Temperature Programming — What and Why

**Technical concept.** In GC, column temperature controls retention times: low temperature separates low-boiling compounds well but high-boiling compounds take forever; high temperature rushes high-boiling compounds out but low-boiling ones all pile together. **Temperature programming** starts the oven cool and raises the temperature steadily (a "ramp", e.g., 50 °C → 250 °C at 10 °C per minute) during the run.

**Simple explanation.** It is like slowly turning up the heat under a pan of mixed nuts — small light pieces pop early at low heat, heavy pieces need more heat and pop later. Everyone gets their turn, nobody burns, and the whole batch finishes in reasonable time.

**Example.** An essential oil contains light monoterpenes (boil ~150 °C) and heavy sesquiterpenes (boil ~250 °C). With a fixed oven at 100 °C, the heavy ones never come out; fixed at 250 °C, the light ones all merge into one peak. A temperature ramp from 60 °C to 260 °C separates all of them in one run.

**Key takeaway.** Temperature programming lets one GC run separate mixtures with a **wide boiling range** — cool start for light compounds, hot finish for heavy ones.

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### 5.4.5 HPLC — Introduction and Theory

**Technical concept.** HPLC is the liquid-phase cousin of GC. Because the mobile phase is a **liquid** (not a gas), the analyte does **not** need to be volatile — it only needs to **dissolve** in the solvent. A pump pushes the solvent at very high pressure (up to ~400 times atmospheric pressure) through a short steel column packed with tiny particles (3–5 micrometres) coated with stationary phase. High pressure is needed because the tiny particles resist flow. Separation happens by partition or adsorption between the liquid mobile phase and the stationary phase on the particles.

**Simple explanation.** If GC is a race in air, HPLC is a race in water pushed by a powerful pump through a tube full of fine sand. Substances that cling to the sand grains move slowly; those that prefer the water move fast. No boiling needed — so heat-sensitive medicines can be analysed safely.

**Two modes:**

- **Normal phase:** polar stationary phase (bare silica), non-polar mobile phase (hexane). Polar analytes stick more and come out later. (Older, less common today.)
- **Reversed phase:** non-polar stationary phase (C18 chains on silica), polar mobile phase (water + methanol or acetonitrile). Non-polar analytes stick more and come out later. This is the **workhorse** of the pharmaceutical industry — most drug assays use reversed-phase HPLC with a C18 column.

**Example.** A paracetamol tablet assay: the tablet solution is injected into a C18 column with a water–methanol mobile phase. Paracetamol and its impurity 4-aminophenol separate; the detector measures each peak and the lab calculates the exact drug content.

**Key takeaway.** HPLC separates dissolved, non-volatile, heat-sensitive substances using a high-pressure liquid mobile phase; reversed-phase (C18) is the standard pharma mode.

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### 5.4.6 HPTLC — Principle

**Technical concept.** HPTLC is TLC upgraded to an instrumental technique. The plate is coated with **finer, uniform silica particles** (5–7 µm instead of 10–12 µm), samples are applied as **narrow bands** (not round spots) by an automated applicator, development happens in a saturated, controlled chamber, and spots are measured by a **densitometric scanner** (it shines light on each band and measures absorption/reflection). Software records Rf values, peak areas and even full spectra. Result: sharper separations, better repeatability and quantitative results — unlike classical TLC which is mostly qualitative.

**Simple explanation.** Classical TLC is like handwriting a letter; HPTLC is like printing it on a laser printer — same idea, but neater, faster, reproducible and measurable.

**Example.** Ten different batches of Ashwagandha extract are applied as ten bands on one HPTLC plate. After development and scanning, the software overlays the ten "fingerprints" — any adulterated batch shows a visibly different pattern.

**Key takeaway.** HPTLC = automated, quantitative, high-resolution TLC — ideal for fingerprinting herbs and screening many samples side by side.

### 5.4.7 Advantages, Disadvantages and Applications

**GC — Advantages:** very high separation power (long capillary columns give thousands of plates); fast; very sensitive detectors (FID, ECD); small sample needed; excellent for volatile compounds. **GC — Disadvantages:** only for volatile, heat-stable substances (others need derivatization); cannot analyse salts, proteins, polymers or most drugs directly. **GC — Applications:** residual solvents in drugs (checking leftover methanol, toluene, etc. in tablets); essential oil analysis (perfume and ayurvedic oils); blood alcohol measurement (forensic labs); pesticide residues.

**HPLC — Advantages:** works for non-volatile, heat-sensitive, polar and ionic compounds — i.e., most drugs; highly accurate and quantitative; easily automated; wide detector choice. **HPLC — Disadvantages:** expensive instrument and columns; uses large volumes of costly, sometimes toxic solvents; slower sample throughput than HPTLC for many samples. **HPLC — Applications:** assay of tablets/capsules (how much drug is present); impurity profiling (finding tiny unwanted chemicals in a drug); stability studies (checking how a drug degrades over time); herbal standardization (measuring marker compounds in herbal products).

**HPTLC — Advantages:** many samples on one plate (cheap per sample); little solvent; simple sample preparation; the whole plate is a permanent visual record. **HPTLC — Disadvantages:** lower resolution than HPLC; less sensitive; more manual steps. **HPTLC — Applications:** herbal fingerprinting (identity check of medicinal plants); multi-sample screening (quickly comparing many batches); stability-indicating checks.

## 5.5 Classifications and Tables

### Table 5.1 — Comparison: GC vs HPLC vs HPTLC

| Feature | Gas Chromatography (GC) | HPLC | HPTLC |
|---|---|---|---|
| **Sample type** | Volatile, heat-stable gases/liquids only | Non-volatile, heat-sensitive, polar or ionic compounds dissolved in liquid | Dissolved samples applied on a plate; many samples together |
| **Mobile phase** | Gas (He or N2) | Liquid (water/methanol/acetonitrile), pushed at high pressure | Liquid solvent rising by capillary action on a plate |
| **Stationary phase** | Liquid film (e.g., polysiloxane) coated inside a long column, or solid adsorbent | Fine particles (e.g., C18 silica) packed in a steel column | Fine silica layer coated on a glass/aluminium plate |
| **Temperature** | High (oven 50–350 °C); temperature programming common | Room temperature (usually); no heating needed | Room temperature |
| **Sample introduction** | Injection port (split/splitless), sample vaporised | Loop injector, liquid injected directly | Automated applicator applies bands on plate |
| **Detection** | FID, TCD, ECD | UV-Vis, PDA, fluorescence, RI | Densitometric scanner (UV/visible) |
| **Resolution** | Very high (capillary columns) | High | Moderate |
| **Cost per sample** | Low (cheap gases) | High (costly solvents, columns) | Very low (many samples per plate) |

| Feature | Gas Chromatography (GC) | HPLC | HPTLC |
|---|---|---|---|
| **Typical applications** | Residual solvents in drugs, essential oils, blood alcohol, pesticides | Tablet assay, impurity profiling, stability studies, herbal standardization | Herbal fingerprinting, multi-sample screening |

## Table 5.2 — GC Detectors Compared

| Detector | Full name | How it works (simple words) | Best for | Notes |
|---|---|---|---|---|
| **FID** | Flame Ionization Detector | Sample burns in a hydrogen flame; burning makes ions (charged particles); the ion current is measured — more sample, more current | Organic compounds (almost all) | Very sensitive; destroys the sample; does not respond to water or inorganic gases |
| **TCD** | Thermal Conductivity Detector | A hot wire loses heat to the passing gas; when sample molecules pass, the gas conducts heat differently and the wire temperature changes — the change is measured | Universal — responds to all compounds | Non-destructive (sample survives); less sensitive than FID |
| **ECD** | Electron Capture Detector | A radioactive source releases electrons; sample molecules that "capture" electrons (especially those with Cl, Br, F atoms) reduce the electron current — the drop is measured | Halogen-containing compounds (pesticides, PCBs) | Extremely sensitive for its targets; blind to most other compounds |

## Table 5.3 — GC Columns Compared

| Feature | Packed column | Capillary column |
|---|---|---|
| **Size** | Short (1–3 m), wide (2–4 mm inner diameter) | Very long (15–100 m), very thin (0.1–0.5 mm, like a hair) |
| **Stationary phase** | Liquid coated on solid support particles packed inside | Thin film coated on the inner wall |
| **Separation power** | Lower | Much higher (more theoretical plates) |
| **Sample capacity** | Higher (takes larger samples) | Lower (needs split injection for concentrated samples) |
| **Use today** | Older; some routine gas analyses | Standard choice for almost all modern GC work |

## Table 5.4 — HPLC Detectors Compared

| Detector | What it measures | Simple working idea | Best for |
|---|---|---|---|
| **UV-Vis** | Light absorbed at one chosen wavelength | Many drugs absorb UV light; more drug → more light absorbed | Routine drug assays (most common detector) |

| Detector | What it measures | Simple working idea | Best for |
|---|---|---|---|
| **PDA** | UV spectra at all wavelengths at once | Like UV-Vis but records the full spectrum of every peak — confirms peak purity/identity | Impurity profiling, method development |
| **Fluorescence** | Light emitted after excitation | Sample is excited with light and glows; glow intensity is measured — extremely sensitive | Trace analysis, vitamins, some antibiotics |
| **RI (Refractive Index)** | Change in bending of light by the liquid | Universal detector — responds to any dissolved substance, since every substance bends light slightly | Sugars, polymers; compounds with no UV absorption (but less sensitive) |

### Table 5.5 — Isocratic vs Gradient Elution (HPLC)

| Feature | Isocratic | Gradient |
|---|---|---|
| **Mobile phase** | Same composition throughout the run | Composition changes gradually (e.g., 20% → 80% acetonitrile) |
| **Simple idea** | Like running at one fixed speed | Like temperature programming in GC, but for liquids |
| **Best for** | Simple mixtures with similar retention | Complex mixtures with widely different polarities |
| **Pump** | Simple, one pump enough | Needs programmable pump/mixing system |

## 5.6 Mechanisms and Processes (Step-by-Step)

### 5.6.1 How GC Instrumentation Works — Part by Part

1 **Carrier gas supply.** A cylinder of high-purity helium (He) or nitrogen (N2) provides a steady, inert gas flow. "Inert" means it does not react with the sample. Flow is controlled precisely (like a tap set to a fixed drip rate).

1 **Sample injection port (heated, ~200–300 °C).** A microsyringe injects 1 µL of liquid sample through a rubber septum. The heat instantly vaporises the sample. In **split mode**, most vapour is vented out and only a small fraction enters the column (for concentrated samples, to avoid overloading). In **splitless mode**, nearly all vapour goes to the column (for trace-level samples).

1 **Column (inside the column oven).** The vapour is swept into the long column. Each component partitions between the carrier gas and the stationary phase (a thin film of liquid such as **polysiloxane** — a silicon-based oil that stays stable at high temperature — coated inside a capillary column). Less-sticky components race ahead; more-sticky ones lag behind. Separation happens here.

1 **Column oven.** Keeps the column at the set temperature, or ramps it up during the run (**temperature programming**) so that both light and heavy components separate well.

1 **Detector (FID / TCD / ECD).** As each separated component leaves the column, the detector senses it and produces an electrical signal (see Table 5.2 for how each detector works).

1 **Recorder / data system.** The signal is drawn as a chromatogram — a series of peaks. Peak position (retention time) identifies each substance; peak area measures its quantity.

### 5.6.2 How HPLC Instrumentation Works — Part by Part

1 **Solvent reservoirs.** Bottles holding the mobile-phase liquids (e.g., water, methanol, acetonitrile, buffers). They feed the pump.

1 **Degasser.** Removes dissolved air bubbles from the solvents (bubbles would make the pump stutter and create noise in the detector). Works by vacuum or by bubbling helium through the solvent.

1 **Pump.** Pushes the mobile phase through the column at high pressure and a very steady flow rate (e.g., 1 mL/min). In **isocratic** mode it delivers a fixed solvent mixture; in **gradient** mode it continuously changes the mixture ratio during the run.

1 **Injector (sample loop).** A valve with a fixed-volume loop (e.g., 20 µL). The sample is loaded into the loop, then the valve switches and the mobile phase sweeps the exact sample volume onto the column. This gives highly repeatable injections.

1 **Column (e.g., C18 reversed-phase).** A short steel tube (e.g., 15–25 cm) packed with tiny silica particles coated with 18-carbon chains (non-polar surface). Components partition between the polar mobile phase and the non-polar stationary phase and separate.

1 **Detector (UV-Vis / PDA / fluorescence / RI).** Senses each component as it exits the column (see Table 5.4) and sends an electrical signal.

1 **Data system (computer + software).** Records the chromatogram, measures retention times and peak areas, calculates results (e.g., % drug content), and prints the report.

### 5.6.3 HPTLC Workflow — Step by Step

1 **Plate preparation.** Take a plate coated with fine silica gel; mark the baseline.

2 **Sample application.** The automated applicator sprays samples as narrow, even **bands** (not spots) — many samples side by side on one plate.

3 **Development.** The plate stands in a saturated developing chamber; mobile phase rises by capillary action, carrying components upward at different speeds.

4 **Drying and visualization.** Dry the plate; view under UV light or spray a reagent to make spots visible.

5 **Scanning and documentation.** The densitometric scanner measures each band (position = Rf value for identity, intensity = quantity); software stores the fingerprint.

### 5.6.4 Derivatization Workflow (for GC)

1 Identify the problem: analyte is polar (has –OH/–COOH/–NH2), non-volatile, or gives tailing peaks.

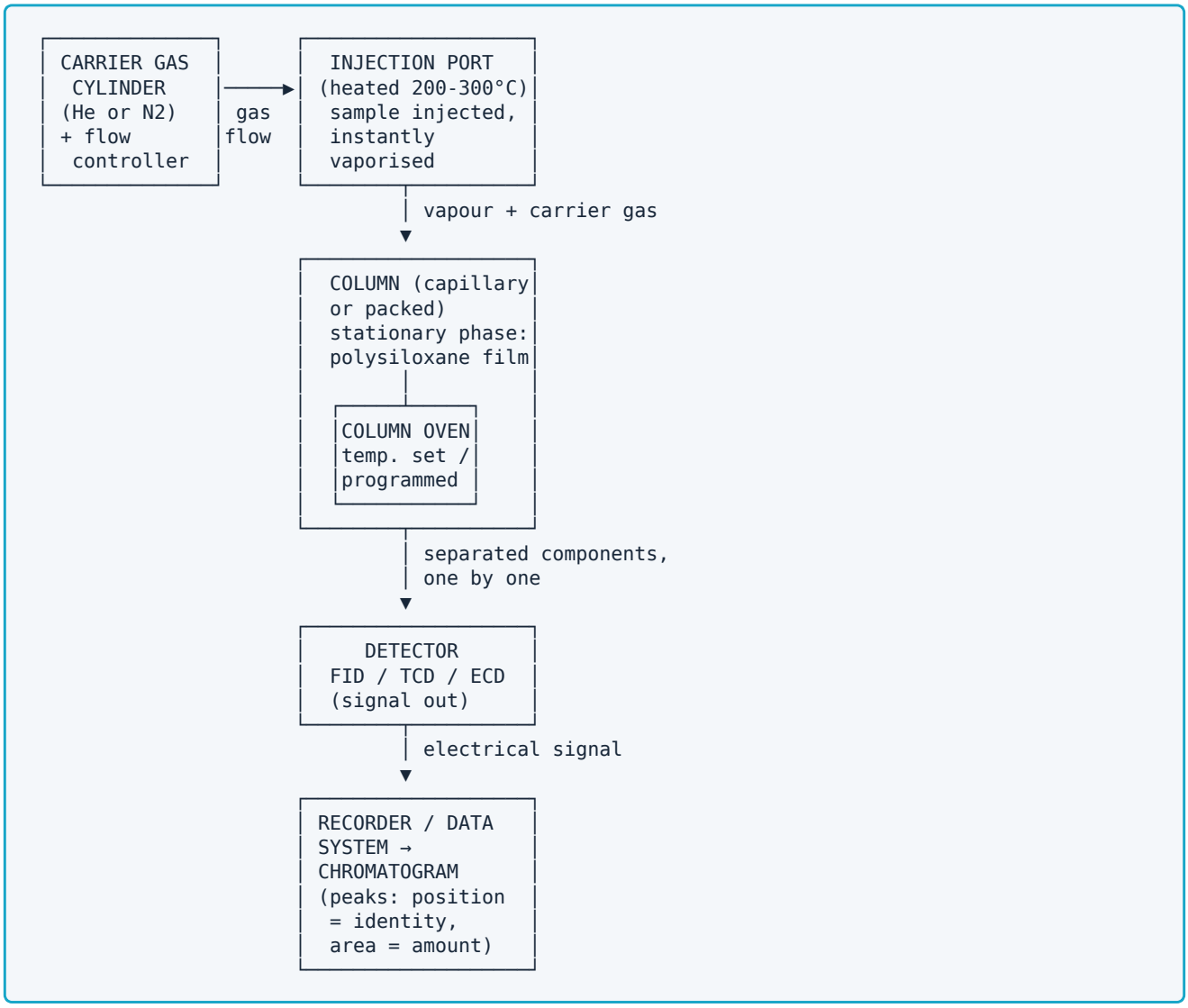
2 Choose the reaction: **silylation** for –OH/–COOH/–NH groups; **methylation** for carboxylic acids.

3 React the sample with the derivatizing reagent under dry, controlled conditions.

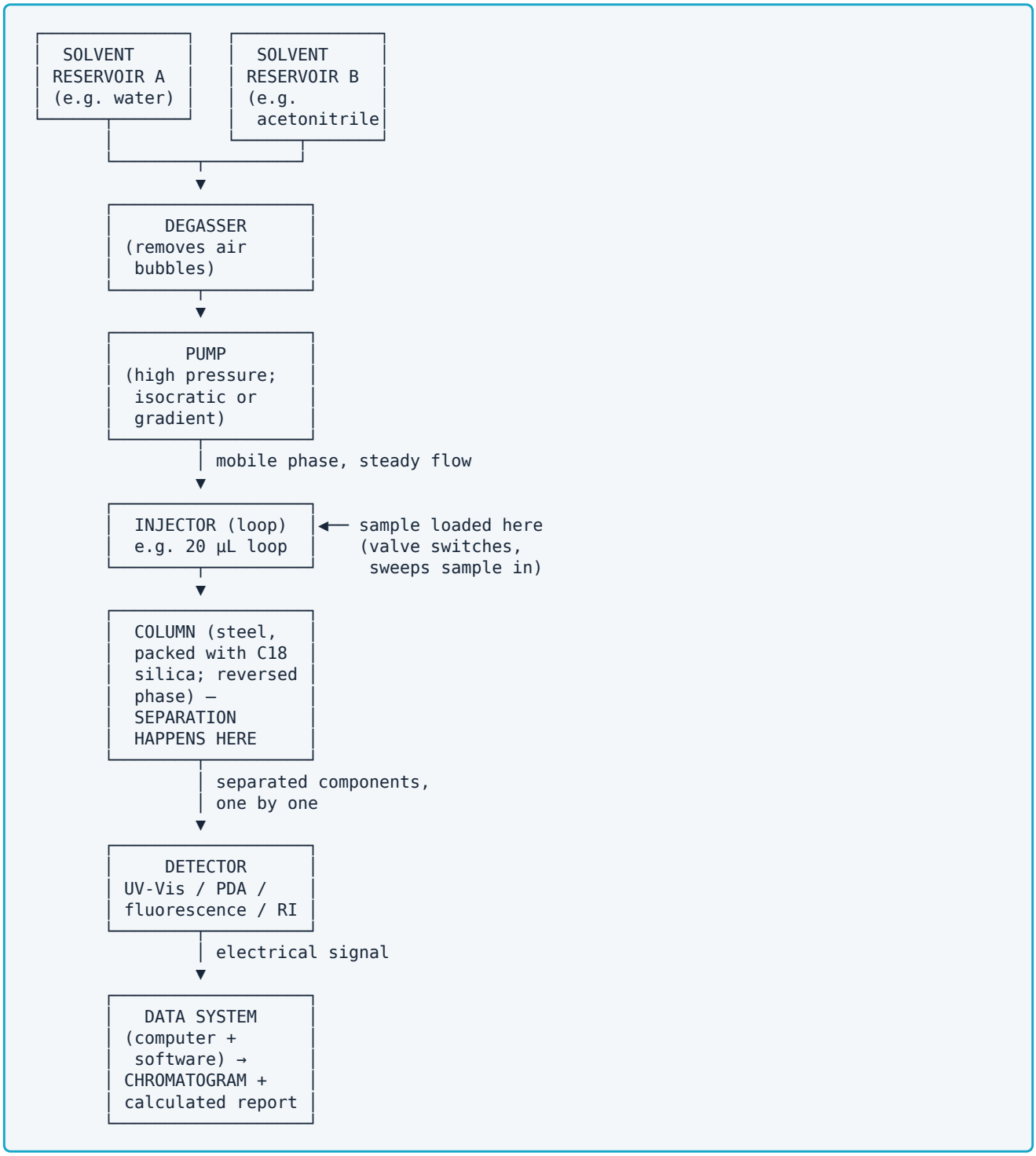
4 Inject the volatile derivative into the GC — sharp, symmetrical peaks appear.

## 5.7 Diagrams (Textual Block Diagrams, Labeled)

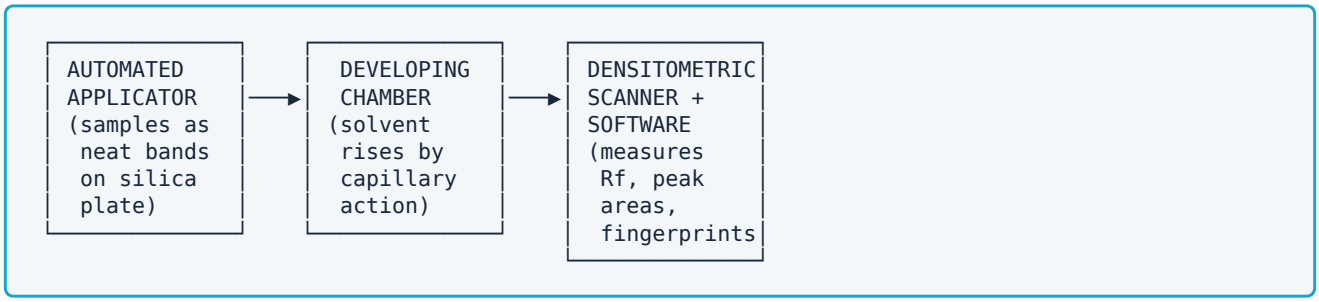
### Diagram 5.1 — Gas Chromatograph (Full Instrument Block Diagram)



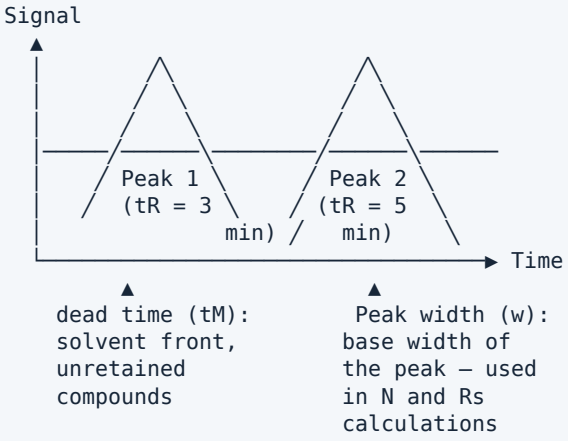

### Diagram 5.2 — HPLC System (Full Instrument Block Diagram)




## Diagram 5.3 — HPTLC System (Block Diagram)

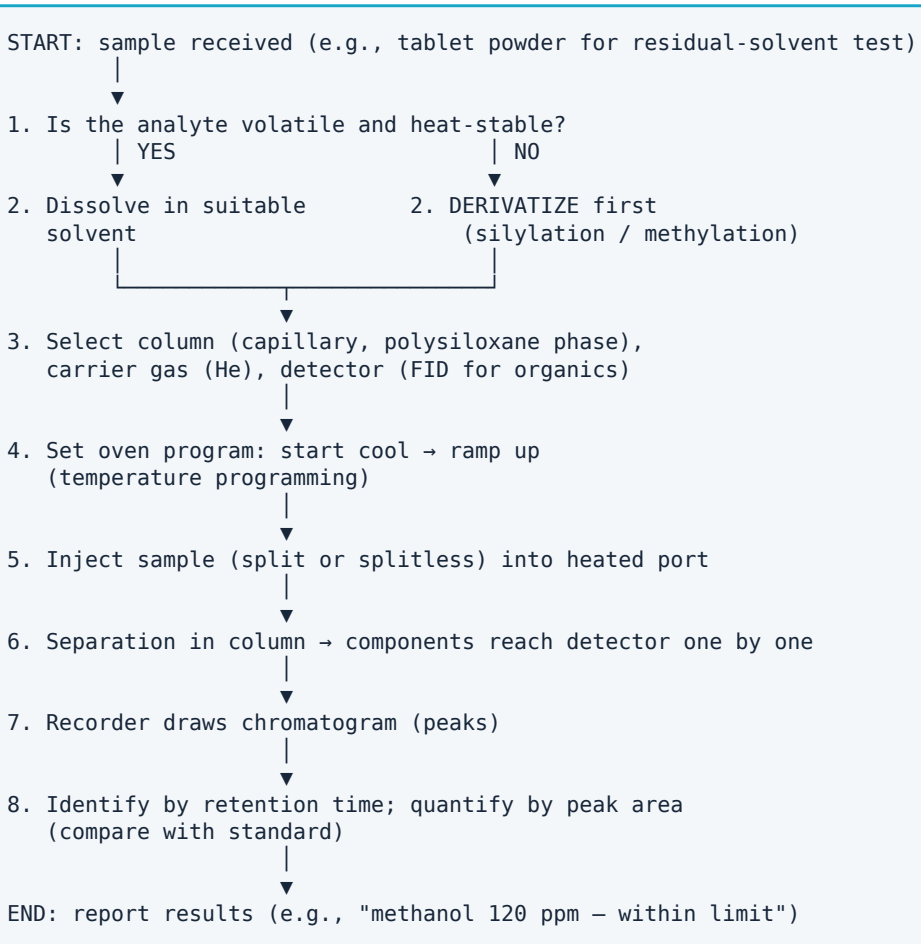


### Diagram 5.4 — What a Chromatogram Looks Like (GC or HPLC)




## 5.8 Flowcharts (Textual)

### Flowchart 5.1 — GC Analysis Workflow




### Flowchart 5.2 — HPLC Method Development Steps

```
START: new drug sample to analyse
        |
        ▼
Step 1. Define the goal
        (assay? impurity profiling? stability study?)
        |
        ▼
Step 2. Study the analyte
        (solubility, UV absorption, pKa, stability)
        |
        ▼
Step 3. Choose the mode
        → Reversed phase (C18) for most drugs
        → Normal phase only for very non-polar samples
        |
        ▼
Step 4. Choose mobile phase
        (water + methanol/acetonitrile; add buffer if drug is ionizable)
        |
        ▼
Step 5. Choose detector
        (UV-Vis if drug absorbs UV → most common;
         PDA for impurity work; fluorescence for trace levels)
        |
        ▼
Step 6. Trial injections — isocratic first
        → Peaks overlapping? → try gradient elution
        → Peaks tailing? → adjust pH/buffer
        |
        ▼
Step 7. Optimize: retention (k between 2–10),
        resolution (Rs ≥ 1.5), run time as short as possible
        |
        ▼
Step 8. Validate the method
        (accuracy, precision, linearity, robustness —
         as per ICH guidelines)
        |
        ▼
END: approved method used for routine QC analysis
```

## 5.9 Infographic Concept Box

**What a one-page infographic for this chapter would show:**

- **Title banner:** "GC • HPLC • HPTLC — Three Chromatography Workhorses of Pharma QC".
- **Three vertical panels, one per technique**, each with a simple icon (GC: gas cylinder + flame; HPLC: pump + steel column; HPTLC: plate with bands), the mobile phase (gas / pressurised liquid / rising solvent), the sample type (volatile / dissolved drug / many bands), and one star application (residual solvents / tablet assay / herbal fingerprint).
- **Centre strip:** a single timeline-arrow showing "mixture in → separation → peaks out", with retention time (tR) marked on a sample chromatogram.
- **Bottom row:** three detector badges — FID flame, UV-Vis eye, densitometer scanner — with one-line "how it works".
- **Side box:** "Numbers that matter" — k, α, N, Rs with their formulae and the golden rules (k = 2–10 ideal; Rs ≥ 1.5 = baseline separation).

- **Footer strip:** the memory hook "Gas Goes up in GC (heat); Liquids stay Low-temp in HPLC; Plates Pile many samples in HPTLC".

## 5.10 Examples (Pharmacy Examples)

1 **Residual solvents by GC.** After manufacturing, a paracetamol tablet batch may contain traces of methanol used in processing. Pharmacopoeias set strict limits. A GC with FID measures the methanol peak; if it is below the limit, the batch passes.

1 **Essential oil analysis by GC.** Eucalyptus oil must contain a fixed percentage of cineole. GC separates all oil components; the cineole peak area gives its percentage — an adulterated oil is caught.

1 **Blood alcohol by GC.** In forensic labs, a blood sample is heated in a sealed vial (headspace) and the vapour is injected into a GC. The ethanol peak area tells the blood alcohol concentration.

1 **Tablet assay by HPLC.** Twenty tablets are powdered, dissolved, filtered and injected into a C18 HPLC with UV detection. The drug peak area is compared with a standard of known concentration to calculate the exact mg per tablet.

1 **Impurity profiling by HPLC-PDA.** A new drug batch is run on HPLC with a PDA detector. Besides the main drug peak, two tiny extra peaks appear — impurities at 0.1%. The PDA spectra help identify them; the batch is rejected if impurities exceed limits.

1 **Stability study by HPLC.** Tablets stored at 40 °C/75% humidity for 6 months are analysed monthly. A growing degradation-product peak shows the drug is breaking down — this data fixes the expiry date.

1 **Herbal standardization by HPLC.** A guggul extract must contain a fixed amount of guggulsterone (marker compound). HPLC quantifies it so every bottle has uniform strength.

1 **Herbal fingerprinting by HPTLC.** Ten supplier batches of senna leaves are run on one HPTLC plate. Nine show identical band patterns; one shows extra bands — it is adulterated and rejected.

1 **Derivatization example.** Fatty acids in an oil sample are methylated to methyl esters before GC, giving sharp, well-separated peaks instead of broad, tailing ones.

## 5.11 Analogies

1 **Chromatography = a school race with hurdles of different heights.** All students (molecules) start together; those who jump hurdles easily (weak interaction with stationary phase) finish early; those who struggle (strong interaction) finish late. Finish times = retention times.

1 **Partition coefficient = love for honey.** Bees flying down a corridor with one honey-coated wall: honey-loving bees (large K) keep landing and arrive late; others (small K) fly straight through and arrive early.

1 **GC column oven = slowly heated pan of mixed nuts.** Light pieces pop early at low heat; heavy pieces need high heat and pop later — temperature programming gives every piece its turn.

1 **HPLC pump = a heart pushing blood.** Just as the heart pushes blood through narrow vessels at pressure, the HPLC pump pushes solvent through the tightly packed column at high pressure.

1 **Derivatization = giving guests smooth coats.** Polar molecules "hug" the column (hydrogen bonding) and won't leave; derivatization puts a smooth chemical coat on them so they walk out easily.

1 **HPTLC vs TLC = laser printer vs handwriting.** Same principle, but HPTLC is automated, neat, reproducible and measurable.

1 **Resolution Rs = two trains on parallel tracks.** Rs < 1 means the trains overlap (peaks merged); Rs ≥ 1.5 means they run on fully separate tracks with clear ground between them (baseline separation).

## 5.12 Pharmacy Relevance

- **Every tablet you dispense was checked by HPLC.** The assay (exact drug content), impurity profile and dissolution results printed on a batch certificate come from HPLC runs. Without HPLC, quality control of modern medicines would be impossible.
- **GC protects patients from toxic solvents.** Methanol, benzene and toluene residues from manufacturing can poison patients; GC is the pharmacopoeial method that keeps them below safe limits.
- **Expiry dates come from HPLC stability studies.** The "use before" date on a strip is fixed by measuring drug breakdown over months with HPLC.
- **Herbal medicines need HPTLC/HPLC fingerprints.** Since plants vary by season and region, fingerprinting proves that what is in the bottle matches the approved plant — protecting patients from adulteration.
- **Forensic pharmacy uses GC.** Blood alcohol and drug-abuse testing in medico-legal cases rely on GC.
- As a pharmacist, you may never operate these instruments yourself, but you will read their reports (COA — Certificate of Analysis), answer drug-quality questions, and trust these techniques every working day.

## 5.13 India Context

- The **Indian Pharmacopoeia (IP)** prescribes GC for residual-solvent testing and HPLC assays for most official drugs — these are legal quality standards in India, not optional extras.
- India is the **"pharmacy of the world"** — a huge exporter of generic medicines. Foreign buyers (US FDA, European regulators) accept Indian drugs largely because Indian QC labs run validated HPLC/GC methods.
- **Ayurvedic and herbal industry:** AYUSH regulations increasingly require HPTLC fingerprints and HPLC marker assays for classical formulations (e.g., ashwagandha, guggul, senna) to prove identity and uniform strength — HPTLC is popular because it is cheap per sample.

- **Essential oil trade:** Indian sandalwood, lemongrass and eucalyptus oils are certified for export using GC analysis of their characteristic components.
- **Forensic labs** in every Indian state use GC for blood-alcohol (drink-and-drive) cases under the Motor Vehicles Act.

## 5.14 Key Points

1 GC: gaseous mobile phase + liquid/solid stationary phase; only for volatile, heat-stable substances.

2 Retention time (tR) identifies a substance; peak area measures its amount.

3 Partition coefficient K decides retention: bigger K → longer tR.

4 GC parts in order: carrier gas (He/N2) → heated injection port (split/splitless) → column in oven → detector (FID/TCD/ECD) → recorder.

5 Capillary columns (long, thin, wall-coated) beat packed columns in separation power.

6 FID: burns sample in flame, measures ions — best for organics. TCD: measures heat-conduction change — universal, non-destructive. ECD: captures electrons — ultra-sensitive for halogen compounds.

7 Derivatization (silylation, methylation) makes polar/non-volatile analytes GC-ready.

8 Temperature programming = oven ramp during the run; separates wide-boiling-range mixtures.

9 HPLC: high-pressure liquid mobile phase; for non-volatile, heat-sensitive drugs; reversed-phase C18 is the standard pharma mode.

10 HPLC parts in order: solvent reservoirs → degasser → pump (isocratic/gradient) → loop injector → C18 column → detector (UV-Vis/PDA/fluorescence/RI) → data system.

11 HPTLC = automated, quantitative TLC: fine particles, band applicator, controlled chamber, densitometric scanner; best for herbal fingerprinting and multi-sample screening.

12 GC vs HPLC vs HPTLC differ in sample type, mobile phase, temperature and cost (see Table 5.1).

13 $k = (tR - tM)/tM$; $\alpha = k2/k1$ (>1 means separation); $N = 16(tR/w)^2$ (higher = sharper peaks); $Rs = 2(tR2 - tR1)/(w1 + w2)$ (≥1.5 = baseline separation).

## 5.15 Common Confusions

1 **Normal phase vs reversed phase.** "Reversed" does not mean the instrument runs backwards! Normal = polar stationary + non-polar mobile (old style). Reversed = non-polar stationary (C18) + polar mobile (the modern standard). Remember: the *phases* are reversed relative to each other.

2 **Retention time vs dead time.** Dead time (tM) is how long an *unretained* substance takes (it just rides the mobile phase). Retention time (tR) is how long *your* analyte takes. tR is always ≥ tM.

3 **FID vs TCD.** FID is more sensitive but *destroys* the sample (burns it); TCD is *non-destructive* but less sensitive. For trace organics choose FID; if you must recover the sample, choose TCD.

4 **Split vs splitless injection.** Split = throws away most of the sample (for concentrated samples). Splitless = sends almost all (for trace samples). Do not mix them up: "splitless" is the *sensitive* one.

5 **Isocratic vs gradient.** Isocratic = mobile phase composition *constant*; gradient = composition *changes* during the run. Gradient is the HPLC cousin of GC temperature programming.

6 **TLC vs HPTLC.** Not just "better TLC" in words — HPTLC uses finer particles, automated band application and a scanner, giving quantitative, reproducible results that ordinary TLC cannot.

7 **k vs α.** Capacity factor (k) describes *one* peak's retention; selectivity (α) compares *two* peaks (k2/k1). You need both peaks' k values to get α.

## 5.16 Common Mistakes

1 Writing that GC can analyse "all drugs" — most drugs are non-volatile; GC needs volatility or derivatization.

2 Forgetting the degasser in the HPLC block diagram — it is a favourite exam trap.

3 Saying FID responds to water — it does not (water gives no FID signal).

4 Using peak *height* instead of peak *area* for quantification in numericals — area is the standard measure.

5 Calculating k with tR alone: k = (tR − tM)/tM — you must subtract the dead time first.

6 In the Rs formula, using only one peak's width — the denominator is (w1 + w2), the *sum* of both widths.

7 Claiming Rs = 1.0 means complete separation — baseline separation needs Rs ≥ 1.5.

8 Mixing up which phase is polar in reversed-phase HPLC — stationary is NON-polar (C18), mobile is polar.

9 Writing "temperature programming" for HPLC — it is called *gradient elution* in HPLC; temperature programming belongs to GC.

10 In HPTLC answers, omitting the scanner/software — without densitometric scanning it is just ordinary TLC.

## 5.17 Mnemonics

- **GC instrument order:** "**C**arrier **I**njects **C**old-to-hot **D**etects **R**ecords" — **C-I-C-D-R**: Carrier gas → Injection port → Column (in oven) → Detector → Recorder.
- **HPLC instrument order:** "**S**olvents **D**egassed, **P**umped, **I**njected, **C**olumned, **D**etected, **D**ocumented" — **S-D-P-I-C-D-D**.
- **GC detectors:** "**F**ire **T**ests **E**verything **C**hlorinated" — **FID** (fire/flame, organics), **TCD** (tests everything — universal), **ECD** (chlorinated/halogen compounds).
- **Reversed phase:** "**R**eversed = **R**oomy non-polar **R**ug" — in reversed phase the stationary phase is the non-polar "rug" (C18) that non-polar analytes sit on.
- **Rs ≥ 1.5:** "**One-point-five keeps peaks alive (apart)**" — baseline separation needs Rs of at least 1.5.

- **Derivatization types:** "**S**ilylation **S**ilences **S**ticky **H**ydrogens; **M**ethylation **M**akes **E**sters" — silylation caps –OH/–COOH/–NH hydrogens; methylation makes methyl esters from acids.

## 5.18 Exam Summary

- Chromatography = separation by a moving mobile phase over a fixed stationary phase; components travel at different speeds.
- **GC:** gas mobile phase (He/N2), volatile + heat-stable samples only. Theory: partition between gas and stationary phase (K); retention time tR identifies. Parts: carrier gas → injection port (split/splitless, heated) → column (packed vs capillary; polysiloxane phases) in column oven → detector → recorder. Detectors: FID (flame, ions, organics), TCD (heat conduction, universal, non-destructive), ECD (electron capture, halogens). Derivatization: silylation (caps –OH/–COOH/–NH with silicon group), methylation (acids → methyl esters) — for non-volatile/polar analytes. Temperature programming: oven ramp for wide-boiling-range mixtures. Applications: residual solvents, essential oils, blood alcohol.
- **HPLC:** high-pressure liquid mobile phase; for non-volatile, heat-sensitive drugs. Normal phase (polar silica/non-polar mobile) vs reversed phase (non-polar C18/polar mobile — the standard). Parts: reservoirs → degasser → pump (isocratic/gradient) → loop injector → C18 column → detector (UV-Vis, PDA, fluorescence, RI) → data system. Applications: tablet assay, impurity profiling, stability studies, herbal standardization.
- **HPTLC:** high-performance TLC — fine particles, automated band application, controlled development, densitometric scanning + software. Applications: herbal fingerprinting, multi-sample screening.
- Formulae: $k = (tR - tM)/tM$; $\alpha = k2/k1$; $N = 16(tR/w)^2$; $Rs = 2(tR2 - tR1)/(w1 + w2)$; baseline separation at $Rs \geq 1.5$.

## 5.19 One-Minute Revision

- GC = gas mobile phase, volatile samples, heated column; tR identifies, peak area quantifies.
- GC order: carrier gas → injection port → column in oven → detector → recorder.
- Detectors: FID (flame/organics), TCD (universal), ECD (halogens).
- Derivatization: silylation, methylation — make polar analytes volatile.
- Temperature programming: oven ramp for wide boiling ranges.
- HPLC = high-pressure liquid, non-volatile drugs; reversed-phase C18 is standard.
- HPLC order: solvents → degasser → pump → injector → column → detector → data system.
- HPTLC = automated quantitative TLC; herbal fingerprints, many samples per plate.
- $k = (tR-tM)/tM$; $\alpha = k2/k1$; $N = 16(tR/w)^2$; $Rs = 2\Delta tR/(w1+w2)$; $Rs \geq 1.5$ = baseline separated.

## 5.20 Exam Questions

### A. Short-Answer Questions

1 Define gas chromatography and retention time in your own words.

2 Why is helium or nitrogen used as the carrier gas in GC?

3 Differentiate between split and splitless injection.

4 Compare packed and capillary columns (any four points).

5 Explain the working of the Flame Ionization Detector (FID) in simple words.

6 Why is the TCD called a universal but less sensitive detector?

7 What is derivatization? Why is it needed in GC?

8 Explain silylation and methylation with one example each.

9 What is temperature programming? Give one example where it is essential.

10 Differentiate between normal-phase and reversed-phase HPLC.

11 What is a C18 column? Why is it the most used column in pharma?

12 Differentiate between isocratic and gradient elution.

13 What is the function of the degasser in HPLC?

14 Compare UV-Vis, PDA, fluorescence and RI detectors (one line each).

15 What makes HPTLC "high performance" compared to ordinary TLC?

16 List any four applications of GC and four of HPLC in pharmacy.

17 Draw a labelled block diagram of a gas chromatograph.

18 Draw a labelled block diagram of an HPLC system.

19 Write the formulae for k, α, N and Rs and state what each tells you.

20 What does Rs ≥ 1.5 signify?

## B. Multiple-Choice Questions (with Answer Key)

1 In GC, the mobile phase is —

a) a liquid b) a gas c) a solid d) a supercritical fluid

1 Retention time is —

a) time the sample spends in the detector b) time from injection to the detector for an analyte c) time to prepare the sample d) column lifetime

1 Which carrier gas is commonly used in GC?

a) Oxygen b) Helium c) Chlorine d) Carbon dioxide

1 Splitless injection is preferred for —

a) concentrated samples b) trace-level samples c) gaseous samples only d) solid samples

1 The FID works by —

a) measuring heat conduction b) burning the sample and measuring ions c) capturing electrons d) measuring light absorption

1 Which detector is NON-destructive?

a) FID b) TCD c) ECD d) None of these

1 The ECD is most sensitive for compounds containing —

a) carbon b) halogens c) hydrogen d) oxygen

1 Silylation replaces the hydrogen of –OH groups with —

a) a methyl group b) a silicon-containing group c) a sodium atom d) a phenyl group

1 Methylation converts carboxylic acids into —

a) alcohols b) methyl esters c) ketones d) amides

1 Temperature programming means —

a) constant oven temperature b) gradually increasing oven temperature during the run c) cooling the detector d) heating the injector only

1 In reversed-phase HPLC, the stationary phase is —

a) polar silica b) non-polar C18 c) alumina d) water

1 A C18 column is used in —

a) normal-phase HPLC b) reversed-phase HPLC c) GC d) TLC

1 In gradient elution, the mobile phase composition —

a) stays constant b) changes gradually during the run c) is always 100% water d) is changed only between runs

1 The degasser in HPLC removes —

a) impurities b) dissolved air bubbles c) water d) buffer salts

1 A PDA detector records —

a) one wavelength b) all UV wavelengths at once c) fluorescence only d) refractive index

1 The most sensitive HPLC detector listed is —

a) RI b) UV-Vis c) fluorescence d) PDA

1 HPTLC differs from TLC mainly by —

a) using paper instead of plates b) finer particles, automated application and densitometric scanning c) using gas as mobile phase d) heating the plate to 300 °C

1 In HPTLC, Rf value =

a) distance of spot ÷ distance of solvent front b) distance of solvent front ÷ distance of spot c) peak area ÷ peak height d) tR ÷ tM

1 Blood alcohol analysis is done by —

a) HPLC b) GC c) HPTLC d) polarimetry

1 Herbal fingerprinting of many batches on one plate is best done by —

a) GC b) HPLC c) HPTLC d) titration

**Answer Key:** 1-b, 2-b, 3-b, 4-b, 5-b, 6-b, 7-b, 8-b, 9-b, 10-b, 11-b, 12-b, 13-b, 14-b, 15-b, 16-c, 17-b, 18-a, 19-b, 20-c.

## C. Viva Questions

1 What is chromatography in one sentence?

2 Name the mobile phase in GC and HPLC.

3 What does a detector do?

4 Which GC detector would you choose for pesticide analysis, and why?

5 Why can't proteins be analysed directly by GC?

6 What is the dead time (tM)?

7 Why is high pressure needed in HPLC?

8 What does "C18" mean?

9 Isocratic or gradient — which is the HPLC parallel of temperature programming?

10 What does a densitometer measure in HPTLC?

11 Interpret: two peaks have Rs = 2.1. Are they separated?

12 Interpret: α = 1.0 for two compounds. What does it mean?

## D. Application-Based Questions

1 A QC lab must check residual methanol in a tablet batch as per the Indian Pharmacopoeia. Which technique will you choose — GC, HPLC or HPTLC? Justify your choice and name the detector.

2 A stability study shows a new small peak growing next to the drug peak in HPLC month after month. What does this indicate, and which detector would best help identify the new peak?

3 An ayurvedic company receives tulsi raw material from five suppliers and wants a quick identity comparison. Which technique is most economical, and why?

4 A forensic lab receives a blood sample in a drink-and-drive case. Describe in four steps how GC would be used.

5 A drug has no UV absorption. Which HPLC detector would you select, and what is its drawback?

## E. Numericals with Worked Solutions

**CALCULATION BOX 1 — Capacity factor (k) and selectivity (α) Question.** In a GC run, the dead time tM = 1.5 min. Two compounds elute at tR(A) = 4.5 min and tR(B) = 6.0 min. Calculate the capacity factor of each compound and the selectivity factor α. Interpret the result. **Step 1 — Write the formula.** k = (tR − tM) / tM ; α = kB / kA (larger k divided by smaller k) **Step 2 — Capacity factor of A.** kA = (4.5 − 1.5) / 1.5 = 3.0 / 1.5 = **2.0 Step 3 — Capacity factor of B.** kB = (6.0 − 1.5) / 1.5 = 4.5 / 1.5 = **3.0 Step 4 — Selectivity.** α = kB / kA = 3.0 / 2.0 = **1.5 Step 5 — Interpret.** k values between 1 and 10 are ideal — both compounds are well retained (not rushing out with the solvent, not stuck forever). α = 1.5 (> 1) means the column genuinely separates the two compounds — B is held back 1.5 times more strongly than A. If α were 1.0, the two would come out together and no separation would occur.

**CALCULATION BOX 2 — Theoretical plates (N) and resolution (Rs) Question.** An HPLC chromatogram shows two peaks: Peak 1: tR = 5.20 min, base width w1 = 0.25 min. Peak 2: tR = 5.80 min, base width w2 = 0.30 min. Calculate N for each peak and the resolution Rs between them. Interpret the numbers. **Step 1 — Write the formulae.** $N = 16 \times (tR / w)^2$ ; Rs = 2 × (tR2 − tR1) / (w1 + w2) **Step 2 — Plates for Peak 1.** $N1 = 16 \times (5.20 / 0.25)^2 = 16 \times (20.8)^2 = 16 \times 432.64 =$ **6922 plates Step 3 — Plates for Peak 2.** $N2 = 16 \times (5.80 / 0.30)^2 = 16 \times (19.33)^2 = 16 \times 373.7 =$ **5979 plates Step 4 — Resolution.** Rs = 2 × (5.80 − 5.20) / (0.25 + 0.30) = 2 × 0.60 / 0.55 = 1.20 / 0.55 = **2.18 Step 5 — Interpret.** N ≈ 6000–7000 means the column is efficient — peaks are sharp and narrow (more plates = sharper peaks = better separation power). Rs = 2.18 is greater than 1.5, so the two peaks are **baseline separated** — they return fully to the baseline between them, and each can be measured accurately with no overlap. The method is suitable for quantitative analysis.

## 5.21 References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