Industrial Pharmacognosy

Unit III

Modern Methods of Analysis of Plants and Plant-Based Products

Pills & Python Notes

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

Syllabus coverage: Unit III (12 hours).

Pills & Python · Pharmacy • Data • AI • Healthcare · Instagram @pillsandpython · pillsandpython.com · YouTube @PillsAndPython

CHAPTER 5

Spectroscopic Methods

5.1 Learning Objectives

1. State the basic principle of each spectroscopic method: UV-Visible, IR, NMR, Mass, AAS.
2. Explain what each method tells us about a botanical sample.
3. Give one application of each in herbal analysis.

DEFINITIONS

- **Spectroscopy:** Studying how matter interacts with light/energy — each molecule absorbs or emits energy in its own unique pattern, like a fingerprint.
- **UV-Visible spectroscopy:** Measures absorption of ultraviolet and visible light; tells us about conjugated systems (colour-causing parts) and is used for quantitative assay (how much is present).
- **IR (Infrared) spectroscopy:** Measures absorption of infrared light by vibrating bonds; identifies functional groups (OH, C=O, NH).
- **NMR (Nuclear Magnetic Resonance) spectroscopy:** Uses magnets and radio waves to map hydrogen/carbon atoms; reveals the full structure of a molecule.
- **Mass spectroscopy (MS):** Breaks molecules into charged fragments and weighs them; gives molecular weight and fragmentation pattern.
- **AAS (Atomic Absorption Spectroscopy):** Measures metals by absorbing light from a flame/furnace; used for heavy-metal testing (lead, arsenic, mercury, cadmium).

5.3 Terminology

Term	Simple meaning
Chromophore	The part of a molecule that absorbs UV-Vis light (e.g., C=C–C=O)
Functional group	A reactive atom-group (OH, COOH) identified by IR
Chemical shift (δ)	Position of an NMR signal, in ppm — tells the atom's environment
m/z	Mass-to-charge ratio in mass spectra
Fingerprint region	IR region 400–1500 cm^{-1}, unique for each molecule

5.4 Explanation — Principles in simple words

UV-Visible — "How dark is the colour?" Technical: Beer–Lambert law — absorbance is proportional to concentration. Simple: Shine light through the solution; the more the absorbing compound, the less light comes out. From the "darkness" we calculate how much is present. Used to assay curcumin in turmeric extract, total phenolics, etc. Example: A turmeric extract's curcumin content is measured at its λmax.

IR — "Which bonds are dancing?" Technical: Bonds vibrate at specific frequencies and absorb IR there. Simple: Every bond type (O–H, C=O) absorbs a specific IR "note". The spectrum is like a piano chord — from the notes, we name the functional groups. Used to identify powders and detect adulteration. Example: An OH stretch band confirms a phenolic compound like curcumin.

NMR — "The full address of every atom." Technical: Nuclei in a magnetic field resonate at frequencies depending on their chemical environment. Simple: NMR tells exactly which hydrogen is next to which carbon — it draws the molecule's map. Used to confirm the structure of an isolated compound (e.g., proving the isolate is really artemisinin). Example: ^{1}H-NMR and ^{13}C-NMR of withanolides confirm their steroidal skeleton.

Mass spectroscopy — "Weighing broken pieces." Technical: Molecules are ionized, fragmented, and fragments separated by m/z. Simple: Smash the molecule, weigh the pieces — from the total weight (molecular ion) and piece pattern, identify the compound. Often joined with chromatography (LC-MS, GC-MS). Example: Confirming the molecular weight of boswellic acid in an extract.

AAS — "Metal detector for medicines." Technical: Atoms in a flame absorb light of a characteristic wavelength; absorption ∝ metal concentration. Simple: Herbal products must be free of toxic heavy metals (lead, mercury, arsenic, cadmium). AAS measures exactly how much metal is present, in ppm. Example: Every exported herbal batch is tested for lead by AAS.

5.5 Master comparison table

Method	What it measures	Main use in herbal analysis	Simple memory hook
UV-Visible	Light absorption (concentration)	Quantitative assay of markers	"How much?"
IR	Bond vibrations	Functional groups, identity	"Which bonds?"
NMR	Atomic environment	Full structure confirmation	"Full address"
MS	Fragment masses	Molecular weight, identity	"Weigh the pieces"
AAS	Metal atom absorption	Heavy-metal testing	"Metal detector"

5.6 Flowchart — Choosing the method

Question → Which method?

- "How much curcumin is in this extract?" → UV-Visible (quantitative)
- "Is this powder really ashwagandha?" → IR / TLC fingerprint (identity)
- "What is the structure of this new isolate?" → NMR + MS (structure)
- "Is this batch safe from lead?" → AAS (heavy metals)

5.7 Diagrams (describe for drawing)

- **Diagram 1 — UV-Vis:** light source → sample cuvette → detector → absorbance peak graph with λmax marked.
- **Diagram 2 — AAS:** flame with sample spray → hollow-cathode lamp beam through flame → detector → ppm readout.

INFOGRAPHIC CONCEPT

Five Lights, Five Answers UV-Vis → HOW MUCH (quantity) IR → WHICH BONDS (identity) NMR → FULL STRUCTURE (map of atoms) MS → HOW HEAVY (molecular weight) AAS → HOW SAFE (heavy metals) **Together they prove a herbal product is real, strong and safe.**

EXAMPLES

1. Curcumin assay of a turmeric extract by UV-Visible spectrophotometry.
2. IR spectrum of a sennoside sample matched with a reference to confirm identity.
3. AAS shows lead at 8 ppm — batch rejected (limit as per pharmacopoeia).

ANALOGIES

- **Spectroscopy = detective's toolkit:** UV counts the suspects, IR checks fingerprints, NMR draws the face, MS weighs the evidence, AAS checks for poison.
- **Beer–Lambert law = tea strength:** darker tea = more tea leaves; darker solution = more compound.

PHARMACY RELEVANCE

QC pharmacists run these instruments daily; you must know which method answers which question and how to read a basic spectrum/report.

INDIA CONTEXT

Indian Pharmacopoeia monographs prescribe UV assays and AAS heavy-metal limits for herbal drugs; export consignments are routinely AAS-tested.

KEY POINTS

- UV-Vis: quantitative (Beer–Lambert).
- IR: functional groups + fingerprint region.
- NMR: complete structure (^{1}H, ^{13}C, chemical shift).
- MS: molecular weight + fragmentation (LC-MS/GC-MS).
- AAS: heavy metals (Pb, As, Hg, Cd).

COMMON CONFUSIONS

- **UV vs IR:** UV measures concentration; IR identifies bonds.
- **NMR vs MS:** NMR maps atom positions; MS gives weight.
- **AAS vs flame photometry:** AAS measures absorption by ground-state atoms (more sensitive for heavy metals).

COMMON MISTAKES

- Saying "NMR measures molecular weight" — that is MS.
- Forgetting that UV needs a chromophore — compounds without one need derivatization or another method.
- Writing "AAS tests purity" — it specifically tests metals.

MNEMONIC

- **"U I N M A"** — **U**V (mUch?), **I**R (bonds), **N**MR (map), **M**S (mass), **A**AS (metAls). Or: "**U**nsafe **I**ngredients **N**eed **M**ethods of **A**nalysis".

EXAM SUMMARY

Modern spectroscopic analysis of botanicals: UV-Visible for quantitative assay (Beer–Lambert), IR for functional-group identity, NMR for full structure elucidation, Mass spectroscopy for molecular weight and fragmentation, AAS for heavy-metal safety testing.

ONE-MINUTE REVISION

- UV = how much; IR = which bonds; NMR = full map; MS = weight; AAS = metals.
- Beer–Lambert: $A \propto c$.
- Fingerprint region: 400–1500 cm^{-1}.
- Chemical shift in ppm; m/z in MS.

5.19 Exam questions

Short answer:

1. State the Beer–Lambert law and its use in herbal analysis.
2. What is the fingerprint region in IR?
3. Why is AAS used in herbal QC?
4. Differentiate NMR and Mass spectroscopy.

Long answer:

5. Describe the basic principles and applications of UV-Visible, IR, NMR, Mass and AAS in the analysis of botanicals.
6. A new compound is isolated from a plant. Which spectroscopic methods would you use, in what order, and why?

MCQs:

7. Heavy metals in herbal drugs are tested by: (a) NMR (b) AAS (c) UV (d) IR — **Answer: (b)**
8. The fingerprint region in IR is: (a) 4000–2500 cm^{-1} (b) 400–1500 cm^{-1} (c) 200–400 nm (d) 0–10 ppm — **Answer: (b)**
9. Chemical shift is measured in: (a) nm (b) cm^{-1} (c) ppm (d) m/z — **Answer: (c)**
10. Molecular ion peak appears in: (a) IR (b) Mass spectrum (c) UV (d) AAS — **Answer: (b)**

Viva:

11. What is a chromophore? Give an example.
12. Why is MS often coupled with LC or GC?

Application-based:

13. An export batch of ashwagandha powder is suspected of lead contamination. Which instrument, and what would you report?

CHAPTER 6

Chromatographic Methods

6.1 Learning Objectives

1. State the basic principle of chromatography.
2. Describe HPTLC, HPLC, UPLC and GC with simple working.
3. Explain applications of each in botanical analysis.

DEFINITIONS

- **Chromatography:** Separation of a mixture into its components by passing it through a stationary phase with a moving mobile phase — like a race where each compound runs at its own speed.
- **HPTLC (High-Performance Thin Layer Chromatography):** An advanced TLC on fine plates; many samples run side-by-side; used for fingerprinting herbal extracts.
- **HPLC (High-Performance Liquid Chromatography):** Separation in a high-pressure column with liquid mobile phase; the workhorse for quantitative marker assay.
- **UPLC (Ultra-Performance LC):** Faster, higher-pressure HPLC with smaller particles — quicker results, less solvent.
- **GC (Gas Chromatography):** Separation of volatile compounds in a gas stream; ideal for essential oils.

6.3 Terminology

Term	Simple meaning
Stationary phase	The fixed material compounds stick to (silica plate, column packing)
Mobile phase	The moving solvent/gas carrying compounds
Rf value	Distance travelled by spot ÷ distance travelled by solvent (TLC)
Retention time (Rt)	Time a compound takes to come out of an HPLC/GC column
Fingerprint	The characteristic pattern of peaks/spots identifying an extract

6.4 Explanation — each method in simple words

HPTLC — "Many samples, one photo." Technical: Samples applied as bands on silica plates, developed in a chamber, visualized under UV/derivatization, scanned densitometrically. Simple: You can run 15–20 samples on ONE plate next to standards — perfect for comparing many batches or detecting adulteration at a glance. The fingerprint (pattern of bands) must match the genuine herb. Example: HPTLC fingerprint of ashwagandha root extract vs leaf (leaf is a common adulterant — fingerprints differ).

HPLC — "The exact measurer." Technical: High-pressure pump pushes mobile phase through a column; compounds separate and a detector (often UV/PDA) records peaks; peak area ∝ concentration. Simple: The standard method to quantify markers — "this capsule contains X mg of sennosides." Used for assay, stability studies and impurity checks. Example: Assay of curcuminoids in turmeric extract by HPLC.

UPLC — "HPLC on fast-forward." Technical: Sub-2-micron particles, very high pressure; faster runs, sharper peaks, less solvent. Simple: Same job as HPLC but quicker and greener.

Used in modern QC labs for high sample load. Example: Quick batch-release testing of multiple markers in a polyherbal tablet.

GC — "For things that fly." Technical: Volatile sample vaporized, carried by inert gas through a column; separates by boiling point/affinity; FID/MS detector. Simple: The method for essential oils — separates menthol, cineole, linalool etc. Often as GC-MS for identification. Example: GC fingerprint of peppermint oil to check menthol/methone ratio and detect adulteration with synthetic menthol.

6.5 Master comparison table

Method	Sample type	Main use	Memory hook
HPTLC	Extracts (many at once)	Fingerprinting, adulteration check	"Group photo"
HPLC	Extracts/formulations	Quantitative marker assay	"Exact measurer"
UPLC	Same as HPLC	Fast, high-throughput assay	"Fast-forward"
GC	Volatile oils	Oil composition & purity	"For things that fly"

6.6 Flowchart — Choosing the chromatographic method

- Many batches to compare visually? → HPTLC
- Exact mg of marker needed? → HPLC (or UPLC for speed)
- Essential oil composition? → GC (GC-MS for identity)

6.7 Diagrams (describe for drawing)

- **Diagram 1 — HPTLC plate:** baseline with 6 sample bands + 1 standard band, solvent front line, developed bands under UV with Rf labels.
- **Diagram 2 — HPLC:** pump → injector → column → detector → chromatogram peaks with retention times.

INFOGRAPHIC CONCEPT

Separation Squad HPTLC — one plate, 20 samples, visual fingerprint (catches adulteration) **HPLC** — exact mg of marker (the QC workhorse) **UPLC** — HPLC but faster & greener **GC** — volatile oils only (menthol? cineole? linalool?) **Rule: fingerprint first (HPTLC), quantify next (HPLC), volatilize with GC.**

EXAMPLES

1. HPTLC shows an extra band in "ashwagandha" powder → adulterated with leaf.
2. HPLC assay: label claims 100 mg curcuminoids per capsule; result 98 mg → passes.
3. GC of "pure" peppermint oil shows a synthetic-menthol spike → adulterated.

ANALOGIES

- **Chromatography = school race:** all runners start together; each finishes at its own time (retention time); photo-finish (detector) records order.
- **HPTLC = class group photo:** everyone visible at once, easy to spot the outsider.

PHARMACY RELEVANCE

QC and R&D pharmacists validate these methods (as per ICH Q2) and release batches only when fingerprints and assays match specifications.

INDIA CONTEXT

Indian Herbal Pharmacopoeia and API monographs include TLC/HPLC methods; AYUSH premium-mark products must show chromatographic fingerprints.

KEY POINTS

- HPTLC: multi-sample fingerprinting, adulteration detection.
- HPLC: quantitative marker assay (peak area ∝ concentration).
- UPLC: faster, smaller particles, less solvent.
- GC: volatile/oil analysis; GC-MS for identity.

COMMON CONFUSIONS

- **TLC vs HPTLC:** same principle; HPTLC uses finer plates, automated application and densitometry — more precise.
- **HPLC vs UPLC:** same principle; UPLC is faster with higher pressure and smaller particles.
- **Rf vs Rt:** Rf for planar (TLC/HPTLC); Rt for column (HPLC/GC).

COMMON MISTAKES

- Using HPLC for a highly volatile oil without considering GC first.
- Forgetting that HPTLC's strength is comparison of many samples, not ultra-precise quantitation.
- Saying "GC for curcumin" — curcumin is non-volatile; use HPLC.

MNEMONIC

- **"H H U G"** — **H**PTLC (fingerprint), **H**PLC (quantify), **U**PLC (fast), **G**C (gas/volatiles).

EXAM SUMMARY

Chromatographic analysis of botanicals: HPTLC for multi-sample fingerprinting and adulteration detection; HPLC for quantitative marker assay; UPLC for fast high-throughput analysis; GC (GC-MS) for volatile oils and their purity.

ONE-MINUTE REVISION

- HPTLC = group photo fingerprint; HPLC = exact mg; UPLC = fast HPLC; GC = volatiles.
- Rf (TLC) vs Rt (column).
- Peak area ∝ concentration.

6.19 Exam questions

Short answer:

1. Define Rf and retention time.
2. Why is HPTLC preferred for detecting adulteration?

3. When would you choose GC over HPLC?
4. What is a chromatographic fingerprint?

Long answer:

5. Describe the principles and applications of HPTLC, HPLC, UPLC and GC in the analysis of botanicals.
6. A company receives 50 batches of senna leaves. Design a QC plan using chromatographic methods.

MCQs:

7. Which method runs many samples on one plate? (a) HPLC (b) HPTLC (c) GC (d) UPLC — **Answer: (b)**
8. Peppermint oil composition is best analysed by: (a) AAS (b) GC (c) NMR (d) UV — **Answer: (b)**
9. In HPLC, peak area is proportional to: (a) Flow rate (b) Concentration (c) Temperature (d) pH — **Answer: (b)**
10. UPLC differs from HPLC mainly in: (a) Detector (b) Particle size & pressure (c) Sample type (d) Principle — **Answer: (b)**

Viva:

11. What does GC-MS add over GC alone?
12. Why is derivatization sometimes needed in HPTLC?

Application-based:

13. Your HPLC assay of sennoside tablets shows 80% of label claim. List three possible causes before blaming the instrument.

UNIT III — REFERENCES

1. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Government of India.
2. Indian Drug Manufacturers' Association. Indian Herbal Pharmacopoeia.
3. International Council for Harmonisation. ICH Guidelines on Quality, Safety, Efficacy and Multidisciplinary Topics.