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M.Pharm Practical Notes

Pharmacognosy Practicals I

Semester 1

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UV-Visible Assay of Natural-Origin Formulations

Practical 1 - Bench practical

Aim

To determine drug content in a pharmaceutical preparation using a calibrated UV-visible response and a documented extraction and dilution scheme.

Principle

For a suitably dilute absorbing solution, absorbance is proportional to concentration and optical path length within the established working range. A calibration relates measured response to known concentrations in the selected solvent.

A formulation assay also depends on extraction recovery, specificity against excipients and correct dilution arithmetic. The wavelength and extraction conditions are selected from the adopted monograph or validated laboratory method; they are not universal for all drugs.

Materials and Requirements

- UV-visible spectrophotometer with suitable matched cells
- Certified drug reference material with stated potency
- Calibrated balance and volumetric glassware
- Method-specified solvent, filters and placebo where available

Procedure

1. Record analyte, product strength, batch, reference potency, solvent, wavelength, cell path length and method version.
2. Prepare a reference stock by accurate weighing and quantitative transfer. Correct its concentration for the stated reference potency once.
3. Prepare at least five calibration levels spanning the expected test concentration and a solvent blank. Use independently prepared check material when available.
4. Determine average dosage-unit mass when the method calls for a composite. Weigh a portion corresponding to the target analyte mass and extract using the adopted method.
5. Clarify the extract by a qualified process and make the final volume. Record every aliquot and dilution; assess filtration recovery where required.
6. Blank the instrument, measure calibration and test preparations, and inspect the spectrum for unexpected absorption or baseline effects.
7. Calculate the concentration from the fitted response, reverse the dilutions, and express content per unit and percentage of label claim. Retain raw spectra and replicate results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation	Concentration or mass	Absorbance	Replicate / comment
Blank	0	Record	Solvent baseline
Standard levels	Record each level	Record	Calibration
Independent check	Record	Record	Accuracy check

Preparation	Concentration or mass	Absorbance	Replicate / comment
Test preparations	Record portion and dilution	Record	Prepare independently

Calculations

- A = mC + b; C = (A - b) / m
- Drug mass in extract (mg) = C_working (micrograms/mL) x dilution factor x extract volume (mL) / 1000
- Content per tablet = drug mass in portion x average tablet mass / powder portion mass

Worked Example

Illustrative data: a tablet weighs 500 mg and contains a labelled 100 mg drug. A 250 mg powder portion represents 50 mg nominal drug.

The portion is extracted to 100 mL. Diluting 1 mL to 100 mL gives a dilution factor of 100.

Calibration: A = 0.100C + 0.002, with C in micrograms/mL. Test absorbance 0.492 gives C = 4.90 micrograms/mL.

Extracted drug = 4.90 x 100 x 100 / 1000 = 49.0 mg. Content per tablet = 49.0 x 500 / 250 = 98.0 mg, or 98.0% of label claim.

Result and Interpretation

- Enter the actual assay with replicate variability and the applicable product specification.
- A good linear fit alone does not establish specificity, extraction completeness or suitability for release testing.
- Document any dilution outside the calibrated range and prepare an appropriate fresh dilution.

Precautions

- Use the solvent blank and cell type required for the spectral region.
- Avoid fingerprints, bubbles and suspended particles in the optical path.
- Confirm solution stability and extraction recovery before interpreting low recovery as low product content.
- Apply reference potency and dilution factors once, with units at each step.

Viva Questions and Answers

Why is placebo comparison useful?

It reveals absorption or extraction effects from ingredients other than the analyte.

Why prepare separate test solutions?

Independent preparations include weighing and extraction variability, unlike repeated readings of one solution.

Can any wavelength give the same assay?

The wavelength must provide suitable sensitivity and selectivity under the validated conditions.

Recorded Spectra of Simple Phytoconstituents

Practical 2 - Spectral interpretation practical

Aim

To interpret recorded spectra of simple phytoconstituents.

Principle

Each technique constrains different structural features. A defensible assignment must satisfy formula, unsaturation, functional-group, proton and fragment evidence together.

Materials and Requirements

- Recorded qualified spectra with acquisition settings
- Candidate formulae and structures
- Peak-assignment worksheet
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Check axis, units, calibration, solvent or reference and acquisition quality.
3. Derive candidate formulae and unsaturation from mass and composition evidence.
4. Create a joint assignment table for UV chromophore, IR groups, proton/carbon NMR environments and mass fragments; reject candidates that fail essential observations.
5. Assign features with multiplicity, intensity or fragmentation context and list alternative explanations.
6. Compare all available evidence with the candidate structures, distinguishing a provisional assignment from a confirmed identity.
7. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Feature / region	Observed value	Proposed assignment	Supporting / conflicting evidence
Major feature	Enter	Assign	Assess
Diagnostic feature	Enter	Assign	Assess
Missing feature	State	Explain	Assess

Calculations

- Double-bond equivalents for an ordinary closed-shell CHNX formula = (2C + 2 + N - H - X)/2; oxygen and sulfur do not enter this simple expression.

Worked Example

Illustrative C8H9NO2 has DBE = (16 + 2 + 1 - 9)/2 = 5.

An aromatic ring plus one carbonyl accounts for five, but further evidence is needed to identify the substitution pattern.

Natural-constituent worksheet

• Select authenticated examples such as a phenolic acid, alkaloid and terpenoid. Record their actual formulae and spectra.

• Assign UV chromophores, IR functionality, proton and carbon NMR features and mass evidence; do not infer biological activity from structural identity alone.

Carbon NMR evidence

• Record the carbon NMR solvent, reference, acquisition and decoupling conditions. Compare carbonyl, aromatic/alkene and aliphatic carbon environments with the proposed structure.

• A broadband proton-decoupled carbon spectrum generally does not show ordinary proton n+1 splitting, and peak areas are not automatically quantitative carbon counts.

• Use symmetry and overlap when comparing the number of observed environments with the molecular formula; DEPT or two-dimensional correlations can add constraints when available.

Result and Interpretation

• Provide an annotated spectrum and a reasoned structural conclusion with limitations.

Precautions

• Do not read isolated peaks without context.

• Retain unprocessed data and processing choices.

• Use orthogonal evidence when an assignment is ambiguous.

Viva Questions and Answers

Why combine techniques?

They constrain different aspects of a structure.

What is DBE?

A formula-based count of rings and pi-bond equivalents under stated valence assumptions.

What if evidence conflicts?

Recheck data and candidates rather than ignoring the conflict.

Gas Chromatography of Natural Constituents

Practical 3 - Bench practical

Aim

To quantify a suitable volatile analyte using gas chromatography and evaluate separation and detector response.

Principle

A vaporised preparation is carried through a temperature-controlled column by a gas stream. Compounds separate according to volatility and interactions with the stationary phase.

Quantification may use an external standard or an internal-standard area ratio. The analyte must be sufficiently volatile and stable for the selected method; derivatisation, where needed, is a separate validated operation.

Materials and Requirements

- Gas chromatograph with the selected detector and column
- Qualified carrier-gas supply and gas-specific leak-check equipment
- Reference analyte and internal standard where specified
- Suitable diluent, crimped vials and calibrated glassware

Procedure

1. Record detector, column, gas identities, flow or pressure mode, inlet mode, temperature program and acquisition settings from the selected method.
2. Confirm instrument readiness and gas-supply checks with the trained operator. Inspect blanks before introducing test material.
3. Prepare standards and test solutions in closed containers, controlling evaporation and using the same internal-standard concentration where applicable.
4. Establish calibration using the intended response or area ratio and verify a separate check preparation.
5. Inject the analytical sequence with blanks and suitable reference checks. Assess retention repeatability, separation and carryover.
6. Review chromatograms for interference and integration consistency. Retain the temperature program and raw data with the report.
7. Calculate the measured concentration, apply dilutions and assess suitability using the method-specific limits.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation	Analyte area	Internal-standard area	Ratio / concentration
Blank	Record	Record	Check interference
Calibrators	Record	Record	Fit relationship
Independent check	Record	Record	Recovery
Test solutions	Record	Record	Final result

Calculations

- Response ratio R = analyte area / internal-standard area

- If R = mC + b, then C = (R - b) / m
- Original concentration = measured concentration x documented dilution factor

Worked Example

Illustrative calibration: R = 2.50C with C in mg/mL. A test ratio of 1.20 gives C = 1.20/2.50 = 0.480 mg/mL. With a 20-fold dilution, the original concentration is 9.60 mg/mL.
A consistent internal-standard amount and acceptable recovery are assumed; the numerical example does not specify a universal GC program.

Result and Interpretation

- Report concentration with the calibration model, dilution and sequence checks.
- Consider evaporative losses and inlet discrimination when recoveries differ between preparations.
- Interpret identity with the specificity available from the detector and method.

Precautions

- Observe instrument-specific precautions for hot inlets, detectors and pressurised gases.
- Hydrogen-containing systems require the prescribed leak checks and shutdown procedure.
- Keep volatile preparations tightly closed.
- Use the appropriate gas regulator and approved exhaust arrangements.

Viva Questions and Answers

Why use an internal standard?

A suitable standard can compensate for some preparation and injection variability.

Can every drug be analysed directly by GC?

No; volatility and thermal stability may limit direct analysis.

Why include blanks after concentrated injections?

They help assess carryover and contamination.

Sodium and Potassium Estimation by Flame Photometry

Practical 4 - Bench practical

Aim

To determine sodium and potassium concentrations by comparison with suitable flame-emission calibration standards.

Principle

In flame photometry, dissolved species are introduced into a flame, and emitted light at a selected elemental channel is measured. The response is calibrated against known concentrations.

The result depends on aspiration, flame stability, background, ionisation and matrix effects. Sodium and potassium channels require their own calibration; a response in one channel is not the concentration of the other ion.

Materials and Requirements

- Flame photometer with the selected channels
- Certified sodium and potassium standards
- Method-specified blank and matrix-matched diluent
- Calibrated pipettes and volumetric glassware

Procedure

1. Record the instrument channel, gas and aspiration settings, standard concentrations and intended concentration units.
2. Prepare separate or approved mixed calibration standards and an independent check. Match the diluent and relevant matrix conditions.
3. Have the operator complete the prescribed gas, flame and waste checks and establish a stable blank signal.
4. Measure the calibrators from the selected range, then the check and diluted test preparations. Rinse as prescribed between preparations.
5. Repeat checks at intervals and inspect readings for drift, memory effects or unstable aspiration.
6. Calculate concentrations using each channel calibration, reverse dilution and convert units only after checking the atomic-mass basis.
7. Record observed replicate variation and review the result against the applicable material specification.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation	Na response	K response	Dilution / result
Blank	Record	Record	Baseline
Calibration levels	Record	Record	Concentration known
Independent check	Record	Record	Recovery
Test preparations	Record	Record	Calculate each ion

Calculations

- Original ion concentration = concentration from channel calibration x dilution factor
- For Na: mmol/L = mg/L / 22.99; for K: mmol/L = mg/L / 39.10

Worked Example

Illustrative sodium calibration gives 2.30 mg/L in a ten-fold dilution.
Original sodium concentration = 23.0 mg/L; amount concentration = 23.0/22.99 = 1.00 mmol/L approximately.
If potassium is 3.91 mg/L in the same dilution, its original level is 39.1 mg/L, or 1.00 mmol/L. These are analytical teaching values, not patient reference intervals.

Result and Interpretation

- Report sodium and potassium separately with the dilution and units.
- Acceptability depends on check-standard recovery, repeatability and the relevant product specification.
- Unexpected concentrations require review of matrix effects and preparation steps.

Precautions

- Gas ignition and shutdown follow the instrument procedure.
- Prevent high-salt deposits and aspirator blockage.
- Use suitable low-contamination water and containers.
- Do not apply biological reference intervals to a pharmaceutical or food preparation.

Viva Questions and Answers

Why use matrix matching?

Matrix differences can alter aspiration and emission response.

Is mg/L the same as mmol/L?

No; conversion depends on the atomic or molecular mass.

Why recheck a standard during the run?

It can reveal drift or changing aspiration efficiency.

TLC and HPTLC Fingerprints of Nine Medicinal Plants

Practical 5 - Practical study

Aim

To construct and assess TLC/HPTLC records for nine authenticated medicinal-plant materials.

Principle

A fingerprint compares a multizone pattern with an authenticated reference under controlled conditions. Marker choice depends on species, plant part and preparation; common names and one matching Rf do not establish authenticity.

Materials and Requirements

- Nine authenticated materials and plant-part records
- Current material-specific monograph or validated fingerprint method
- Suitable references, plates, chamber, application and imaging system
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Create separate extraction and plate records for every listed plant; verify the selected plant part and method.
3. Record plate, application amount, mobile phase, chamber saturation, development distance and detection conditions from the adopted material-specific procedure.
4. Run reference and test preparations together with extraction blanks and acquire images under the specified visible or UV conditions.
5. Compare zone order, relative migration, intensity pattern and marker evidence and investigate missing or additional zones.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Plant	Authenticated part	Candidate reference focus	Pattern / conclusion
Ashwagandha	Root	Withanolide reference / pattern	Record
Tulsi	Defined aerial part	Eugenol or selected phenolic pattern	Record
Bael	Specified fruit or leaf	Marmelosin or part-specific reference	Record
Amla	Fruit	Gallic-acid-related pattern	Record

Plant	Authenticated part	Candidate reference focus	Pattern / conclusion
Ginger	Rhizome	6-Gingerol-related pattern	Record
Aloe	Defined gel or latex-derived material	Aloin-related reference only for the appropriate material	Record
Vidang	Fruit	Embelin reference	Record
Senna	Leaf or pod as specified	Sennoside-related pattern	Record
Henna	Leaf	Lawsone reference	Record

Calculations

• Rf = zone-centre migration / solvent-front migration
• Compare complete patterns under the same method; do not transfer Rf values between unrelated methods.

Worked Example

Illustrative reference zone travels 28 mm and solvent front 70 mm: Rf = 0.40. A test zone at 0.40 is supporting evidence only when the rest of the material-specific pattern and controls agree.

Botanical identity register

• Ashwagandha: Withania somnifera; Tulsi: Ocimum tenuiflorum / authenticated synonym; Bael: Aegle marmelos.
• Amla: Phyllanthus emblica; Ginger: Zingiber officinale; Aloe: Authenticated Aloe species.
• Vidang: Embelia ribes; Senna: Authenticated Senna species; Henna: Lawsonia inermis.

Result and Interpretation

• Present nine labelled plate records and a separate conclusion for each material. The candidate references shown are not universal official monograph requirements.

Precautions

• Use material-specific extraction and detection.
• Distinguish aloe gel from latex-related preparations.
• Verify synonyms and plant parts; do not substitute a shared common name.

Viva Questions and Answers

Why are fingerprints multizone?

The combined pattern is more discriminating than one zone.

Is Rf a universal constant?

It depends on stationary phase and development conditions.

Why identify the plant part?

Constituent profiles can differ markedly between organs.

Extraction Methods for Medicinal Plants

Practical 6 - Practical study

Aim

To compare extraction methods for an authenticated medicinal-plant material.

Principle

Extraction depends on solvent selectivity, particle size, solid-to-solvent ratio, temperature, time and mass transfer. A high extract yield may reflect inactive or interfering matrix material rather than a higher marker recovery.

Materials and Requirements

- Authenticated material, plant-part and voucher record
- Approved maceration, percolation or supervised hot-extraction method
- Solvent-quality, moisture, dry-extract and marker assays
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Confirm botanical identity and plant part and determine the input moisture basis.
3. Compare selected methods on equal dry-material and solvent-accounting bases; record actual conditions and extraction repetitions.
4. Separate solids, recover extract through the authorised solvent-removal procedure and record dry residue.
5. Measure marker or fingerprint quality and reconcile extraction yield with marker recovery and solvent use.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Method	Dry plant input	Dry extract / marker	Yield / recovery
Maceration	Record g	Record g / mg	Calculate
Percolation	Record g	Record g / mg	Calculate
Hot extraction	Record g	Record g / mg	Calculate

Calculations

- Extract yield (%) = dry extract mass / dry plant mass x 100
- Marker recovery (%) = recovered marker mass / independently established input marker mass x 100

Worked Example

Illustrative 20 g dry plant gives 3 g dry extract: yield = 15%. If 100 mg input marker yields 70 mg recovered marker, marker recovery = 70%.

The two percentages measure different things.

Result and Interpretation

• Compare extraction, marker evidence, solvent use and degradation risk on consistent bases.

Precautions

• Use authenticated material and approved solvent handling.
• Avoid uncontrolled heating of thermolabile compounds.
• Do not equate dry residue with active-compound purity.

Viva Questions and Answers

Why correct moisture?

It makes dry-material comparisons meaningful.

Does higher extract yield prove better extraction?

Selectivity and marker recovery must also be considered.

Why keep a voucher?

It supports traceable botanical identity.

Phytochemical Screening

Practical 7 - Practical study

Aim

To perform and interpret a controlled preliminary phytochemical screen.

Principle

Class-selective tests provide presumptive evidence and can be affected by colour, concentration and cross-reactivity. A negative test may reflect inadequate extraction or sensitivity rather than absence of every class member.

Materials and Requirements

- Authenticated plant extract and extraction blank
- Approved microscale class tests with positive and negative controls
- TLC or instrumental follow-up evidence
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Record the plant part, extraction solvent, concentration and blank appearance.
3. Select approved tests for alkaloids, flavonoids/phenolics, saponins and relevant glycoside classes; do not use highly hazardous classical tests without an authorised system.
4. Run the same reagent and observation conditions for extract, positive reference and negative control.
5. Describe actual precipitate, colour or foam observations and confirm important findings with a suitable chromatographic or spectroscopic method.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Class / selected test	Extract observation	Control observations	Presumptive interpretation
Alkaloid screen	Describe	Positive / negative	Assess
Phenolic / flavonoid screen	Describe	Positive / negative	Assess
Saponin / glycoside screen	Describe	Positive / negative	Assess

Calculations

- Record the extract concentration and observation time; no universal colour-to-content calculation is justified.

Worked Example

Illustrative a precipitate forms in both the extract and its matrix blank. The result is uninterpretable as an alkaloid-positive finding until the interference is resolved.

Result and Interpretation

• Deliver a screening matrix distinguishing presumptive class evidence from confirmed constituent identity.

Precautions

• Include matrix and reagent blanks.
• Do not taste or administer plant preparations.
• Avoid assigning identity from one colour reaction.

Viva Questions and Answers

Why use positive controls?

They demonstrate that the selected reaction can respond as intended.

Does a positive class test identify a molecule?

No; selective confirmation is needed.

Why can a negative result be misleading?

Extraction and detection limits may be inadequate.

HPLC Estimation of Glycyrrhizin

Practical 8 - Bench practical

Aim

To estimate glycyrrhizin in an authenticated liquorice preparation by a validated HPLC method.

Principle

An analyte distributes between a stationary phase and flowing mobile phase. Retention and separation depend on the column chemistry, mobile-phase composition, flow, temperature and analyte ionisation.

A detector response is related to concentration using a qualified calibration or comparison with a reference preparation. Retention agreement supports identification but needs the selectivity evidence required for the intended use.

Materials and Requirements

- HPLC system with suitable column and detector
- Method-specified mobile phase and diluent
- Reference material, blank and placebo
- Qualified filters, vials and volumetric glassware

Procedure

1. Record the column identity, mobile-phase composition, flow, temperature, detector settings, injection volume and method version.
2. Prepare mobile phase and solutions using compatible grades and the adopted filtration and degassing process. Record solution stability and reference potency.
3. Check the system for leaks and equilibrate using the instrument procedure. Establish a stable baseline before the analytical sequence.
4. Run a blank, reference preparations and system-suitability injections. Evaluate retention, resolution, peak shape and repeatability against predefined method criteria.
5. Inject independently prepared test solutions bracketed by appropriate check standards. Include a blank after high concentrations when carryover is a concern.
6. Review peak identification and integration consistently. Retain the original chromatograms and document any justified integration changes.
7. Calculate concentration or assay, reverse dilutions and review the complete sequence before reporting. Preserve audit-trail and processing information.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Injection	Retention time (min)	Area / area ratio	Resolution or comment
Blank	Record	Record	Interference / carryover
Reference replicates	Record	Record	Suitability
Test preparation 1	Record	Record	Assay
Test preparation 2	Record	Record	Preparation precision

Calculations

• Resolution, Rs = 2(tR2 - tR1) / (w1 + w2), using baseline peak widths
• Theoretical plates, N = 16(tR / w)^2, using baseline width
• For matched dilutions in a valid external-standard comparison: assay (%) = test area / standard area x reference concentration / nominal test concentration x reference potency (%)

Worked Example

Illustrative adjacent peaks: retention times 5.00 and 5.40 min; baseline widths 0.20 and 0.24 min.
Rs = 2(0.40) / 0.44 = 1.82. The first peak gives N = 16(5.00/0.20)^2 = 10000.
For equal nominal concentrations, test and standard areas of 49000 and 50000 with reference potency 99.7% give assay = 49000/50000 x 99.7 = 97.706%.
These values illustrate calculations; use the adopted method criteria to decide whether a sequence is acceptable.

Glycyrrhizin-specific record

• Specify the Glycyrrhiza species, plant part, reference identity and whether content is expressed as glycyrrhizic acid, a salt or another defined equivalent.
• Use the adopted extraction, chromatographic conditions and suitability criteria, with matrix blank, spike recovery and reference-potency correction.
• Illustrative 0.20 mg/mL in a 50 mL extract from 1.00 g material gives 10.0 mg/g, or 1.00% on that stated basis, before any required moisture correction.

Result and Interpretation

• Report assay or concentration together with the observed suitability results.
• A resolved, reproducible peak must also be shown to be specific for the intended analysis.
• Investigate failed suitability or carryover before using affected test results.

Precautions

• Use compatible solvents and the column pressure and temperature limits.
• Collect solvent waste in designated containers and keep incompatible wastes separate.
• Prevent particles and bubbles entering the flow path.
• Avoid selective reintegration or reinjection to obtain a preferred result.

Viva Questions and Answers

Why equilibrate the column?

A stable stationary-phase environment improves retention and response reproducibility.

Does a single retention time prove identity?

No; coelution is possible and identity requires the intended method evidence.

Why bracket test injections?

Check standards can reveal response drift during the run.

Monograph Analysis of Clove Oil

Practical 9 - Practical study

Aim

To assess clove-oil identity and quality against the selected current monograph.

Principle

A volatile oil is assessed by a defined identity and composition profile together with physicochemical tests. Eugenol content alone cannot establish the full identity, absence of adulteration or quality of every clove-oil preparation.

Materials and Requirements

- Authenticated Syzygium aromaticum oil and monograph
- Qualified GC identity/composition data
- Density, refractive-index and other specified test records
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Record botanical source, plant part, preparation, batch, storage and the exact monograph edition.
3. Review appearance, density, refractive index and other specified physicochemical tests under their stated temperatures.
4. Assess the GC profile and eugenol-related evidence with suitable references and method suitability.
5. Compare all required results with the actual monograph limits and investigate inconsistent or missing evidence.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Attribute	Method condition	Result	Monograph criterion / decision
Identity / profile	Record	Enter	Compare
Density / refraction	Temperature	Enter	Compare
Specified composition	Reference basis	Enter	Compare

Calculations

- For a validated response-factor method, component amount follows the calibrated response; area normalisation alone is not automatically a mass percentage.

Worked Example

Illustrative eugenol peak area is 80% of total integrated area. Report it as area percentage unless the selected validated method supports a content conversion.

Result and Interpretation

• Deliver a monograph-compliance worksheet with method conditions and traceable evidence.

Precautions

• Handle concentrated volatile oils under approved exposure controls.
• Prevent evaporation and oxidation during sampling.
• Do not invent universal monograph limits.

Viva Questions and Answers

Why quote temperature for density?

Density changes with temperature.

Does area percentage always equal mass percentage?

Detector response factors and method validation determine that relationship.

Why assess the full profile?

Adulteration and source variation may not be captured by one marker.

Monograph Analysis of Castor Oil

Practical 10 - Analytical practical

Aim

To assess authenticated castor oil against a selected current monograph.

Principle

Acid value measures neutralisable acidity, saponification value measures alkali consumption under the specified saponification process, iodine value describes halogen uptake under its method, and peroxide value assesses primary oxidation products. These indices answer different quality questions.

The test preparation, blank, calibration or titrant standardisation and independent controls are part of the measurement. State the actual concentration units, test conditions and adopted method before interpreting the result.

Materials and Requirements

- Representative oil and the selected adopted methods
- Standardised alkali, acid and thiosulphate reagents as required
- Appropriate blanks and controlled reaction equipment
- Qualified balance and volumetric equipment
- Controlled method and raw-data worksheet

Procedure

1. Identify the material, intended attribute, method version and acceptance criteria. Record preparation and instrument or reagent identities.
2. Prepare the prescribed blank and reference or standardised reagent. Verify the measurement with an independent check where applicable.
3. Choose the individual test and record the oil mass, reagent identity, concentration and method conditions.
4. Perform acid-value titration with the prescribed solvent and endpoint.
5. For saponification value, treat test and blank under the same defined saponification and back-titration conditions.
6. For iodine and peroxide determinations, use their separate reagent and endpoint procedures and preserve the required light and timing controls.
7. Record individual readings for independently prepared test portions. Keep all raw observations, including unsuccessful measurements and their investigation.
8. Calculate the result on the stated mass, volume or area basis, including dilution, blank and recovery corrections as justified.
9. Compare the evidence with the applicable criteria and write a conclusion limited to the tested preparation and conditions.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation / attribute	Method condition	Raw measurement	Calculated result
Index	Record	Record	Calculate
Oil mass	Record	Record	Calculate

Preparation / attribute	Method condition	Raw measurement	Calculated result
Blank and test volumes	Record	Record	Calculate
Calculated index	Record	Record	Calculate

Calculations

- Acid value = 56.1 x N x (Vtest - Vblank) / m, in mg KOH/g
- Saponification value = 56.1 x N x (Vblank - Vtest) / m, in mg KOH/g
- Iodine value = 12.69 x N x (Vblank - Vtest) / m, in g iodine/100 g
- Peroxide value = 1000 x N x (Vtest - Vblank) / m, in milliequivalents/kg

Worked Example

Illustrative acid value: m = 2.00 g, N = 0.100 and corrected volume 1.00 mL give 2.805 mg KOH/g.
Illustrative saponification value: m = 2.00 g, N = 0.500 and blank-minus-test difference 14.00 mL give 196.35 mg KOH/g.
Illustrative iodine value: m = 0.500 g, N = 0.100 and difference 10.00 mL give 25.38 g iodine/100 g.
Illustrative peroxide value: m = 2.00 g, N = 0.0100 and corrected volume 0.200 mL give 1.00 milliequivalent/kg. Each example belongs to its own test.

Castor-oil quality record

- Record Ricinus communis oil identity, specified grade and monograph. Review the required density, refractive index, acid, saponification, hydroxyl and other identity/composition tests as applicable.
- The hydroxyl functionality of its characteristic fatty-acid composition makes the selected hydroxyl-value or other identity evidence relevant. Use the actual method and limits rather than assigning the generic worked examples as monograph values.
- Do not handle raw castor seeds or attempt seed-to-oil preparation in this exercise.

Result and Interpretation

- Report each index with its units and separate method conditions. Apply the relevant oil monograph or material specification.
- Report replicate variability and the evidence that the method can measure the intended attribute in this matrix.

Precautions

- Record normality consistently with the formula.
- Check the direction of each blank correction.
- Control oxidation during storage and handling.
- Use the designated precautions for corrosive and halogen-containing reagents.
- Use the adopted laboratory procedure, suitable controls and the designated waste arrangements.

Viva Questions and Answers

Are acid value and peroxide value interchangeable?

No; they assess different chemical changes.

Why use a saponification blank?

It establishes the reagent consumption basis for the back-titration.

Does iodine value directly measure rancidity?

It primarily characterises unsaturation under its method.

Identification of Bioactive Plant Constituents

Practical 11 - Practical study

Aim

To isolate or evaluate a natural constituent and build a justified identity record.

Principle

Isolation, purity, chemical identity and biological activity are separate claims. Chromatographic fractionation and complementary spectra support a structural assignment; an active crude fraction can contain multiple contributors.

Materials and Requirements

- Authenticated source material and approved extraction/fractionation record
- Reference constituent and suitable chromatography
- Orthogonal spectral and purity measurements
- Approved method or teaching-data pack and a dated record sheet

Procedure

1. Define the preparation or dataset, study question and method version. Record identities, units and the criteria to be used before examining the outcome.
2. Trace the botanical source, extraction and fractionation through a labelled material balance.
3. Compare fractions by an approved chromatographic method and select the candidate based on the stated objective.
4. Purify through the authorised procedure and measure recovered mass and analytical purity.
5. Combine formula, UV/IR, proton and carbon NMR and mass evidence with a suitable reference; record unresolved isomeric or stereochemical ambiguity.
6. Retain the original observations and calculations. State deviations, missing information and the limits of the conclusion; do not replace measured values with expected results.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Fraction / constituent	Mass / recovery	Chromatographic evidence	Spectral assignment
Crude extract	Record	Fingerprint	Assess
Selected fraction	Record	Purity profile	Assign
Purified candidate	Record	Orthogonal check	Conclude

Calculations

- Isolation yield (%) = isolated constituent mass / stated dry-source mass x 100

Worked Example

Illustrative 50 mg candidate isolated from 10.0 g dry material gives 0.50% yield.
If analytical purity is 90%, this is not automatically 50 mg of pure constituent; specify the reporting basis.

Result and Interpretation

• Report the isolated fraction, yield, purity basis and strength of identity evidence; assess activity separately.

Precautions

• Use authenticated and approved materials.
• Keep fractions and solvents traceable.
• Do not administer isolated teaching constituents.

Viva Questions and Answers

Does a pure-looking crystal prove identity?

No; chemical characterisation is needed.

Why use carbon NMR?

It adds carbon-environment constraints to the structure.

Does an active fraction identify the active molecule?

Contributors need separate confirmation.

Herbal Dosage Forms and Standardisation

Practical 12 - Bench formulation practical

Aim

To prepare and standardise an approved herbal dosage form using identity, marker and product-quality controls.

Principle

Tablet quality depends on material attributes, blend uniformity, flow, granulation where used, lubrication and compaction. Different manufacturing processes need different controls; direct compression and wet granulation are not interchangeable instructions.

A formulation comparison is meaningful when the raw-material grades, batch size and evaluation conditions are recorded. Change one planned factor at a time or use a documented experimental design, and compare independently prepared batches rather than only repeated readings from one batch.

Materials and Requirements

- Approved teaching formula and identified raw materials
- Selected blending or granulation process
- Guarded tablet press operated by trained staff and qualified evaluation equipment
- Calibrated balance, suitable containers and identified evaluation equipment
- Controlled batch sheet and raw-data forms

Procedure

1. Define the purpose, formula, batch size, material grades and comparison conditions. Calculate all quantities before weighing.
2. Check equipment readiness and record material identities and actual quantities. Reconcile theoretical and actual batch mass.
3. Issue the batch sheet for the selected route and calculate material quantities on the stated potency and moisture basis.
4. Prepare the blend or granules using the approved sequence and record intermediate weights, conditioning and blend or granule observations.
5. Have the trained operator compress at the planned settings, checking unit mass and relevant in-process attributes.
6. Evaluate appearance, dimensions, mechanical properties, content and release attributes required by the intended tablet.
7. Evaluate the stated quality attributes on representative portions using the same method for all comparison batches. Record individual readings and any excluded observations with reasons.
8. Calculate the specified response, variability and material balance. Compare with predefined teaching or product criteria and discuss sources of variation.
9. Label retained preparations and dispose of remaining material through the laboratory procedure. Keep the complete preparation and evaluation record together.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Attribute or stage	Method / conditions	Individual readings	Calculation / comment
Blend / granule record	Record the adopted method	Record	Evaluate
In-process unit measurements	Record the adopted method	Record	Evaluate
Content and physical tests	Record the adopted method	Record	Evaluate
Release result	Record the adopted method	Record	Evaluate

Calculations

• Average unit mass = total mass of units / number of units
• Friability loss (%) = (initial dedusted mass - final dedusted mass) / initial dedusted mass x 100

Worked Example

Illustrative initial and final dedusted masses in the adopted friability test are 6.000 and 5.970 g.
Mass loss = 0.030 g; friability loss = 0.50%. Apply the actual product and test requirements.
If 20 units weigh 8.000 g together, average unit mass is 400.0 mg.

Herbal-material additions

• Authenticate the material and record its organ, preparation, marker or fingerprint specification and contaminant requirements.
• Assess the applicable uniformity, content, disintegration or dissolution and stability attributes of the selected dosage form.

Result and Interpretation

• Report each applicable quality attribute separately and investigate discordant results.
• State the preparation conditions and the evidence supporting the result. A satisfactory physical observation alone does not establish long-term stability, clinical performance or suitability for administration.

Precautions

• Use equipment guards and trained operators.
• Maintain material and batch identity throughout processing.
• Apply the adopted compendial or product method rather than a universal pass rule.
• Use the laboratory-approved formulation and equipment procedure. Teaching batches are identified for laboratory evaluation.

Viva Questions and Answers

Is unit mass the same as content uniformity?

No; drug distribution can vary even when unit mass is consistent.

Why remove loose dust for friability?

The method defines a consistent mass-loss basis.

Why retain in-process data?

They connect process behaviour to final quality.

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