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M.Pharm Practical Notes

Pharmaceutical Chemistry Practicals I

Semester 1

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UV-Visible Assay of a Pharmaceutical Formulation

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

Preparation	Concentration or mass	Absorbance	Replicate / comment
Independent check	Record	Record	Accuracy check
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.

UV Estimation of DNA and RNA

Practical 2 - Nucleic-acid measurement practical

Aim

To estimate nucleic-acid concentration and assess spectral quality.

Principle

At a 1 cm path length, an absorbance of 1 at 260 nm conventionally corresponds to about 50 micrograms/mL double-stranded DNA or 40 micrograms/mL RNA. These factors depend on nucleic-acid type; absorbance ratios alone do not prove purity or integrity.

Materials and Requirements

- Approved nonpathogenic teaching system or supplied anonymised dataset
- Laboratory risk assessment, controls and raw-data 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. Record nucleic-acid type, path length or instrument normalisation and the matching solvent blank.
3. Measure the full relevant spectrum and dilution within the qualified instrument range.
4. Calculate concentration with the correct factor and dilution; inspect 260/280 and 260/230 ratios with pH and blank context.
5. Use a gel or other orthogonal integrity and specificity check when required.
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.

Preparation / control	Condition / method	Raw observation	Calculated / interpreted result
Negative / vehicle control	Specify	Record	Evaluate
Positive / reference control	Specify	Record	Evaluate
Test replicates	Specify	Record	Calculate

Calculations

- Concentration = A260 x nucleic-acid factor x dilution factor, for absorbance normalised to 1 cm
- Yield = concentration x recovered volume

Worked Example

Illustrative double-stranded DNA: A260 = 0.20 and dilution 10 give 100 micrograms/mL. In 0.50 mL the yield is 50 micrograms.

Using the RNA factor would instead give 80 micrograms/mL. A DNA 260/280 ratio near 1.8 or RNA ratio near 2.0 is only a contextual indicator.

Result and Interpretation

- State nucleic-acid type, concentration, yield, spectral quality and independent integrity evidence.
- Report biological and technical replication separately and explain control performance.

Precautions

- Use only the approved biological system, containment and decontamination procedure.
- A teaching dataset does not authorise live animal, human-tissue or unknown-organism work.
- Keep original images and observations; avoid selecting only favourable fields or bands.

Viva Questions and Answers

Why use the correct factor?

Different nucleic-acid forms have different absorbance relationships.

Does a good ratio prove intact DNA?

Integrity needs another suitable measurement.

Why match the blank?

Buffer and pH can influence spectral measurements.

Simultaneous UV Estimation of Two Components

Practical 3 - Bench practical

Aim

To estimate two absorbing components from their combined absorbances at two suitable wavelengths.

Principle

At each selected wavelength, the total absorbance is the sum of the individual component contributions when the solutions obey linear response and the components do not interact spectrally.

Two independent equations can therefore resolve two concentrations. The coefficients must be established for the actual solvent, pH, path length and concentration units; a nearly singular coefficient matrix gives unstable estimates.

Materials and Requirements

- UV-visible spectrophotometer and matched cells
- Separate reference materials for both components
- Solvent blank and formulation placebo
- Volumetric glassware and a prepared mixture

Procedure

1. Scan the pure components and select wavelengths with sufficiently different relative responses.
2. Prepare separate calibration series for each component at both wavelengths. Record slopes and intercept treatment.
3. Verify that the working range is linear and that a prepared mixture behaves approximately additively.
4. Prepare and clarify the formulation extract. Dilute so both components lie in their established ranges.
5. Measure the blank-corrected mixture absorbances at both wavelengths, using the same cells and settings as calibration.
6. Solve the simultaneous equations and reverse the extraction and dilution scheme for each component.
7. Confirm performance using known mixed standards, recoveries and independently prepared test solutions. Review any negative or implausible concentration before reporting.

Observations and Record

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

Preparation	A at wavelength 1	A at wavelength 2	Calculated X and Y
Pure X levels	Record	Record	Coefficient estimation
Pure Y levels	Record	Record	Coefficient estimation
Known mixture	Record	Record	Recovery
Test mixture	Record	Record	Final calculation

Calculations

- A1 = aX1 x CX + aY1 x CY; A2 = aX2 x CX + aY2 x CY
- D = aX1 x aY2 - aY1 x aX2
- CX = (A1 x aY2 - A2 x aY1) / D

• CY = (aX1 x A2 - aX2 x A1) / D

Worked Example

Illustrative coefficients in absorbance per microgram/mL: aX1 = 0.08, aY1 = 0.02, aX2 = 0.01 and aY2 = 0.05. Mixture responses A1 = 0.46 and A2 = 0.20 give D = 0.0038.
CX = (0.46 x 0.05 - 0.20 x 0.02) / 0.0038 = 5.00 micrograms/mL.
CY = (0.08 x 0.20 - 0.01 x 0.46) / 0.0038 = 3.00 micrograms/mL. These are concentrations in the measured dilution.

Result and Interpretation

• Report both contents with the units used for the coefficients and the individual dilution corrections.
• Assess mixture recovery and matrix interference before accepting the mathematical solution.
• Similar spectra may require another wavelength strategy, derivative methods or chromatographic separation.

Precautions

• Use consistent concentration units for both coefficient sets.
• Maintain solvent and pH conditions between standards and mixtures.
• Do not force negative concentrations to zero without investigating the model.
• Confirm the determinant and coefficient order before calculation.

Viva Questions and Answers

What does a small determinant mean?

The two equations contain little independent information, so small measurement errors can greatly affect the solution.

Why test a known mixture?

It checks spectral additivity and the complete calculation under realistic conditions.

Where is dilution applied?

After resolving the concentrations in the measured solution.

Column Chromatographic Separation

Practical 4 - Practical study

Aim

To separate a model mixture by column chromatography and assess fraction identity and recovery.

Principle

Differential interactions with the stationary and mobile phases cause compounds to migrate at different rates. Loading, solvent strength and flow influence band width and separation.

Materials and Requirements

- Approved model mixture and chromatographic materials
- TLC method for fraction monitoring
- Fraction containers, balance and identity checks
- 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. Screen the mixture by TLC and select a justified stationary phase and eluent programme.
3. Prepare and equilibrate the column through the authorised procedure, avoiding cracks or dry channels.
4. Load the mixture in a narrow compatible band and collect labelled fractions at recorded volumes.
5. Compare fraction TLC patterns with references, pool only justified fractions and measure recovery and purity.
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 / volume	Eluent composition	TLC / identity	Recovered mass
Early fractions	Record	Compare	Measure
Target fractions	Record	Compare	Measure
Late fractions	Record	Compare	Measure

Calculations

- Component recovery (%) = recovered component mass / known input component mass x 100

Worked Example

Illustrative input contains 100 mg of target component; isolated target of suitable purity is 80 mg: recovery = 80%.

Total recovered mass must be reconciled separately from component purity.

Result and Interpretation

• Present the fraction map and justify pooling using analytical evidence.

Precautions

• Avoid column drying and channel formation.
• Keep solvent and sample loading compatible.
• Do not equate one TLC zone with absolute purity.

Viva Questions and Answers

Why use a narrow loading band?

It limits initial band broadening.

Why analyse fractions?

It identifies where components elute and supports pooling decisions.

Why can stronger eluent reduce resolution?

Components may move too quickly and overlap.

Quantitative Analysis by HPLC

Practical 5 - Bench practical

Aim

To separate and quantify an analyte by a suitable HPLC method while evaluating chromatographic performance.

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

Injection	Retention time (min)	Area / area ratio	Resolution or comment
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.

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.

Analysis by Gas Chromatography

Practical 6 - 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.

Fluorimetric Estimation of Riboflavin or Quinine

Practical 7 - Bench practical

Aim

To estimate riboflavin or quinine using a calibrated fluorescence response under controlled solution conditions.

Principle

Certain molecules emit light after excitation. Emission depends on analyte concentration, excitation and emission settings, solvent, pH and the chemical environment.

At suitable low concentrations, a calibration can relate fluorescence response to analyte concentration. Quenching, scattering and inner-filter effects can invalidate this simple relationship at higher concentrations.

Materials and Requirements

- Spectrofluorimeter and suitable clean cells
- Riboflavin or quinine reference material
- Method-specified solvent and blank
- Light-protective containers and volumetric glassware

Procedure

1. Record the selected analyte, solvent, pH, excitation and emission settings, slit widths and detector conditions from the adopted method.
2. Prepare a reference stock and dilute to a working series that is sufficiently dilute for a linear response. Protect light-sensitive preparations as required.
3. Scan or verify the reference excitation and emission behaviour before the quantitative run.
4. Measure the blank, calibrators, independent check and test solutions with consistent geometry and timing.
5. Inspect calibration residuals and assess whether dilution restores proportional response in a concentrated test preparation.
6. Calculate the test concentration from the blank-corrected calibration and apply all dilution factors.
7. Record solution age, light exposure and instrument settings so that another analyst can reproduce the measurement.

Observations and Record

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

Preparation	Concentration	Fluorescence response	Blank-corrected response
Blank	0	Record	0 reference
Reference levels	Record	Record	Record
Check preparation	Record	Record	Record
Test dilution	Record	Record	Record

Calculations

- $F_{corrected} = F_{measured} - F_{blank}$

• If F_corrected = mC + b, then C = (F_corrected - b) / m

Worked Example

Illustrative data: blank response 10 units, test response 90 units and calibration F_corrected = 40C.

C = (90 - 10)/40 = 2.00 micrograms/mL in the measured dilution.

A 25-fold dilution gives 50.0 micrograms/mL in the original preparation. Verify that the response lies within the calibrated range.

Result and Interpretation

• Report the concentration and the conditions under which the calibration was established.
• A decrease in response at high concentration can reflect optical or chemical interference rather than less analyte.
• Compare recovery in the relevant matrix before treating the method as suitable for a formulation.

Precautions

• Keep pH, solvent and temperature consistent.
• Avoid fluorescent contamination from detergents or damaged cells.
• Use the selected light-protection conditions.
• Dilute preparations when required instead of extrapolating beyond the working range.

Viva Questions and Answers

What is quenching?

A reduction in fluorescence caused by the molecular environment or interacting species.

Why protect riboflavin solutions?

Light exposure can change the analyte and its response.

Why record slit widths?

They influence signal intensity and spectral resolution.

Sodium and Potassium Estimation by Flame Photometry

Practical 8 - 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.

Solvent Purification and Chromatographic Work-Up

Practical 9 - Practical study

Aim

To choose and document appropriate solvent purification, drying and chromatographic work-up.

Principle

Extraction exploits partitioning; drying removes residual water; distillation separates volatile components under specified conditions. Solvent purity and water content can influence both reaction performance and chromatography.

Materials and Requirements

- Approved solvent and impurity specification
- Authorised extraction and drying setup
- Solvent-quality data and chromatography references
- 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. Identify the solvent, expected impurities, hazards and required quality rather than purifying by habit.
3. Assess a suitable extraction, wash and phase-separation sequence with pH and distribution rationale.
4. Use only the approved drying or purification method and verify the required endpoint by a suitable test.
5. Record recovered quantities, quality, residual contamination and the material/waste balance.
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.

Operation	Input / phase identity	Quality evidence	Recovery
Extraction	Specify phases	Record	Measure
Drying / purification	Specify method	Test endpoint	Measure
Final solvent	Identify container	Quality check	Calculate

Calculations

- Recovery (%) = recovered acceptable solvent amount / input amount x 100

Worked Example

Illustrative input 100 mL and recovered acceptable solvent 85 mL give 85% volume recovery at the stated temperature.

For a rigorous material balance use a consistent mass basis when volume contraction or density matters.

Result and Interpretation

• Explain the chosen sequence and verify suitability for the next operation.

Precautions

• Do not distil peroxide-forming solvents to dryness.
• Use a hood and eliminate ignition sources.
• Do not improvise reactive drying agents or recycle unidentified solvent.

Viva Questions and Answers

Why can extraction fail?

Wrong phase choice, ionisation, emulsions or incomplete equilibration can reduce recovery.

What does drying remove?

Residual water by the chosen compatible method.

Does clear appearance prove purity?

A suitable analytical check is still needed.

Claisen-Schmidt Condensation

Practical 10 - Supervised synthesis and reaction-record practical

Aim

To prepare or assess dibenzalacetone by Claisen-Schmidt condensation and explain its reaction, isolation and characterisation.

Principle

Two benzaldehyde molecules and one acetone molecule form dibenzalacetone and two water molecules.

Base forms an acetone enolate; addition to the aldehyde gives a beta-hydroxy intermediate followed by dehydration. Repetition at the second methyl group extends conjugation.

Materials and Requirements

- Approved microscale reaction SOP and reagent specifications
- Supervised hood, temperature control, glassware and method-specific work-up equipment
- TLC references, balance and appropriate melting-point or spectral methods
- 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. Draw the reaction and mechanism, identify stoichiometry, limiting reagent and possible side products. Enter the authorised quantities, addition order and temperature/time limits in the record.
3. Use the approved base-catalysed condensation procedure, controlling addition and mixing to limit competing reactions. Record precipitation and the TLC time course.
4. Monitor progress by the prescribed TLC or other analytical method, retaining the starting-material comparison.
5. Isolate and purify by the authorised work-up, record dry mass and examine identity and purity by complementary methods.
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.

Stage / preparation	Conditions / observation	Analytical evidence	Mass / yield
Starting materials	Identity / quantities	Reference evidence	Calculate mol
Reaction progress	Actual time / temperature	TLC or assay	Record
Purified product	Appearance	Identity / purity	Dry mass

Calculations

- Theoretical product mass = limiting-reactant moles x stoichiometric product ratio x product molar mass
- Isolated yield (%) = dry isolated product mass / theoretical product mass x 100
- Rf = zone migration / solvent-front migration

Worked Example

Illustrative 1:1 conversion from 10.0 mmol limiting reagent to a product of molar mass 234.29 g/mol gives 2.3429 g theoretical product. A dry isolated mass of 1.7572 g corresponds to 75.0% yield.

Use the actual reaction stoichiometry, salt or solvate form and purity basis; this illustration is not an expected experimental result.

Mechanism and identity record

• Base forms an acetone enolate; addition to the aldehyde gives a beta-hydroxy intermediate followed by dehydration. Repetition at the second methyl group extends conjugation.

• Compare the conjugated carbonyl and alkene evidence with the selected reference; note possible geometric isomers and residual benzaldehyde.

Result and Interpretation

• Report isolated yield and the independent identity/purity observations.

• Compare the conjugated carbonyl and alkene evidence with the selected reference; note possible geometric isomers and residual benzaldehyde.

Precautions

• Control caustic reagent and flammable solvent exposure.

• Use the institution-approved SOP and trained supervision for reactive additions, heating and work-up.

• Do not infer purity from yield or a single TLC zone.

Viva Questions and Answers

What determines theoretical yield?

The limiting reagent and balanced reaction stoichiometry.

How is conversion different from isolated yield?

Conversion measures reactant consumption; isolated yield also reflects selectivity and recovery losses.

Why use complementary identity tests?

A single physical or chromatographic observation may not distinguish impurities or isomers.

Benzilic Acid Rearrangement

Practical 11 - Supervised synthesis and reaction-record practical

Aim

To prepare or assess benzilic acid by rearrangement of benzil and explain its reaction, isolation and characterisation.

Principle

Benzil undergoes hydroxide-promoted rearrangement to benzilate; the authorised acid work-up gives benzilic acid.

Hydroxide addition to an alpha-diketone is followed by phenyl migration and proton transfer, producing an alpha-hydroxy carboxylate.

Materials and Requirements

- Approved microscale reaction SOP and reagent specifications
- Supervised hood, temperature control, glassware and method-specific work-up equipment
- TLC references, balance and appropriate melting-point or spectral methods
- 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. Draw the reaction and mechanism, identify stoichiometry, limiting reagent and possible side products. Enter the authorised quantities, addition order and temperature/time limits in the record.
3. Carry out the approved alkaline transformation, then isolate the acid through the controlled work-up specified by the instructor.
4. Monitor progress by the prescribed TLC or other analytical method, retaining the starting-material comparison.
5. Isolate and purify by the authorised work-up, record dry mass and examine identity and purity by complementary methods.
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.

Stage / preparation	Conditions / observation	Analytical evidence	Mass / yield
Starting materials	Identity / quantities	Reference evidence	Calculate mol
Reaction progress	Actual time / temperature	TLC or assay	Record
Purified product	Appearance	Identity / purity	Dry mass

Calculations

- Theoretical product mass = limiting-reactant moles x stoichiometric product ratio x product molar mass
- Isolated yield (%) = dry isolated product mass / theoretical product mass x 100
- Rf = zone migration / solvent-front migration

Worked Example

Illustrative 1:1 conversion from 10.0 mmol limiting reagent to a product of molar mass 228.24 g/mol gives 2.2824 g theoretical product. A dry isolated mass of 1.7118 g corresponds to 75.0% yield.

Use the actual reaction stoichiometry, salt or solvate form and purity basis; this illustration is not an expected experimental result.

Mechanism and identity record

• Hydroxide addition to an alpha-diketone is followed by phenyl migration and proton transfer, producing an alpha-hydroxy carboxylate.

• Compare the loss of diketone character with carboxylic-acid and hydroxyl evidence; distinguish benzilic acid from unchanged benzil.

Result and Interpretation

• Report isolated yield and the independent identity/purity observations.

• Compare the loss of diketone character with carboxylic-acid and hydroxyl evidence; distinguish benzilic acid from unchanged benzil.

Precautions

• Use controlled alkaline and acid handling; account for heat during neutralisation.

• Use the institution-approved SOP and trained supervision for reactive additions, heating and work-up.

• Do not infer purity from yield or a single TLC zone.

Viva Questions and Answers

What determines theoretical yield?

The limiting reagent and balanced reaction stoichiometry.

How is conversion different from isolated yield?

Conversion measures reactant consumption; isolated yield also reflects selectivity and recovery losses.

Why use complementary identity tests?

A single physical or chromatographic observation may not distinguish impurities or isomers.

Beckmann Rearrangement

Practical 12 - Supervised synthesis and reaction-record practical

Aim

To prepare or assess caprolactam by Beckmann rearrangement and explain its reaction, isolation and characterisation.

Principle

Cyclohexanone oxime rearranges to epsilon-caprolactam under the selected activated-oxime conditions. Activation of the oxime leaving group enables migration of the group anti to it, giving a nitrilium intermediate that leads to an amide. A cyclic ketoxime gives ring expansion.

Materials and Requirements

- Approved microscale reaction SOP and reagent specifications
- Supervised hood, temperature control, glassware and method-specific work-up equipment
- TLC references, balance and appropriate melting-point or spectral methods
- 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. Draw the reaction and mechanism, identify stoichiometry, limiting reagent and possible side products. Enter the authorised quantities, addition order and temperature/time limits in the record.
3. Review the authorised acid-activation process and its temperature controls with the supervisor. Execute only the approved scale and quench; a supplied reaction record may be used.
4. Monitor progress by the prescribed TLC or other analytical method, retaining the starting-material comparison.
5. Isolate and purify by the authorised work-up, record dry mass and examine identity and purity by complementary methods.
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.

Stage / preparation	Conditions / observation	Analytical evidence	Mass / yield
Starting materials	Identity / quantities	Reference evidence	Calculate mol
Reaction progress	Actual time / temperature	TLC or assay	Record
Purified product	Appearance	Identity / purity	Dry mass

Calculations

- Theoretical product mass = limiting-reactant moles x stoichiometric product ratio x product molar mass
- Isolated yield (%) = dry isolated product mass / theoretical product mass x 100
- Rf = zone migration / solvent-front migration

Worked Example

Illustrative 1:1 conversion from 10.0 mmol limiting reagent to a product of molar mass 113.16 g/mol gives 1.1316 g theoretical product. A dry isolated mass of 0.8487 g corresponds to 75.0% yield.

Use the actual reaction stoichiometry, salt or solvate form and purity basis; this illustration is not an expected experimental result.

Mechanism and identity record

• Activation of the oxime leaving group enables migration of the group anti to it, giving a nitrilium intermediate that leads to an amide. A cyclic ketoxime gives ring expansion.

• Differentiate oxime and lactam using the selected IR and NMR evidence. Identical molecular formulae do not distinguish the isomers.

Result and Interpretation

• Report isolated yield and the independent identity/purity observations.

• Differentiate oxime and lactam using the selected IR and NMR evidence. Identical molecular formulae do not distinguish the isomers.

Precautions

• Strong-acid activation and quenching require specific supervised controls; do not substitute reagents or scale.

• Use the institution-approved SOP and trained supervision for reactive additions, heating and work-up.

• Do not infer purity from yield or a single TLC zone.

Viva Questions and Answers

What determines theoretical yield?

The limiting reagent and balanced reaction stoichiometry.

How is conversion different from isolated yield?

Conversion measures reactant consumption; isolated yield also reflects selectivity and recovery losses.

Why use complementary identity tests?

A single physical or chromatographic observation may not distinguish impurities or isomers.

Hofmann Rearrangement

Practical 13 - Supervised synthesis and reaction-record practical

Aim

To prepare or assess aniline by Hofmann rearrangement of benzamide and explain its reaction, isolation and characterisation.

Principle

Benzamide gives aniline with loss of the original carbonyl carbon through the selected Hofmann rearrangement.

N-halogenation and base-promoted rearrangement give an isocyanate; hydrolysis and decarboxylation produce a primary amine with one fewer carbon atom.

Materials and Requirements

- Approved microscale reaction SOP and reagent specifications
- Supervised hood, temperature control, glassware and method-specific work-up equipment
- TLC references, balance and appropriate melting-point or spectral methods
- 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. Draw the reaction and mechanism, identify stoichiometry, limiting reagent and possible side products. Enter the authorised quantities, addition order and temperature/time limits in the record.
3. Use the authorised microscale or supplied-data protocol for the halogenating and alkaline stages. Track the reaction and review the controlled hydrolysis and isolation record.
4. Monitor progress by the prescribed TLC or other analytical method, retaining the starting-material comparison.
5. Isolate and purify by the authorised work-up, record dry mass and examine identity and purity by complementary methods.
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.

Stage / preparation	Conditions / observation	Analytical evidence	Mass / yield
Starting materials	Identity / quantities	Reference evidence	Calculate mol
Reaction progress	Actual time / temperature	TLC or assay	Record
Purified product	Appearance	Identity / purity	Dry mass

Calculations

- Theoretical product mass = limiting-reactant moles x stoichiometric product ratio x product molar mass
- Isolated yield (%) = dry isolated product mass / theoretical product mass x 100
- Rf = zone migration / solvent-front migration

Worked Example

Illustrative 1:1 conversion from 10.0 mmol limiting reagent to a product of molar mass 93.13 g/mol gives 0.9313 g theoretical product. A dry isolated mass of 0.6985 g corresponds to 75.0% yield.

Use the actual reaction stoichiometry, salt or solvate form and purity basis; this illustration is not an expected experimental result.

Mechanism and identity record

• N-halogenation and base-promoted rearrangement give an isocyanate; hydrolysis and decarboxylation produce a primary amine with one fewer carbon atom.

• Confirm amide disappearance and amine evidence by an appropriate analytical comparison; account for the one-carbon change.

Result and Interpretation

• Report isolated yield and the independent identity/purity observations.

• Confirm amide disappearance and amine evidence by an appropriate analytical comparison; account for the one-carbon change.

Precautions

• Reactive halogenating reagents and toxic aniline require a hood, approved handling and segregated waste.

• Use the institution-approved SOP and trained supervision for reactive additions, heating and work-up.

• Do not infer purity from yield or a single TLC zone.

Viva Questions and Answers

What determines theoretical yield?

The limiting reagent and balanced reaction stoichiometry.

How is conversion different from isolated yield?

Conversion measures reactant consumption; isolated yield also reflects selectivity and recovery losses.

Why use complementary identity tests?

A single physical or chromatographic observation may not distinguish impurities or isomers.

Mannich Reaction

Practical 14 - Supervised synthesis and reaction-record practical

Aim

To prepare or assess 3-(dimethylamino)-1-phenylpropan-1-one by a Mannich reaction and explain its reaction, isolation and characterisation.

Principle

Acetophenone, formaldehyde and dimethylamine form the beta-aminoketone 3-(dimethylamino)-1-phenylpropan-1-one in the selected authorised model.

The amine and formaldehyde generate an iminium electrophile; the enol or enolate forms a carbon-carbon bond at the alpha position of the carbonyl compound.

Materials and Requirements

- Approved microscale reaction SOP and reagent specifications
- Supervised hood, temperature control, glassware and method-specific work-up equipment
- TLC references, balance and appropriate melting-point or spectral methods
- 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. Draw the reaction and mechanism, identify stoichiometry, limiting reagent and possible side products. Enter the authorised quantities, addition order and temperature/time limits in the record.
3. Specify the exact authorised model and salt form before calculating quantities. Follow its controlled condensation and product isolation, retaining pH and analytical records.
4. Monitor progress by the prescribed TLC or other analytical method, retaining the starting-material comparison.
5. Isolate and purify by the authorised work-up, record dry mass and examine identity and purity by complementary methods.
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.

Stage / preparation	Conditions / observation	Analytical evidence	Mass / yield
Starting materials	Identity / quantities	Reference evidence	Calculate mol
Reaction progress	Actual time / temperature	TLC or assay	Record
Purified product	Appearance	Identity / purity	Dry mass

Calculations

- Theoretical product mass = limiting-reactant moles x stoichiometric product ratio x product molar mass
- Isolated yield (%) = dry isolated product mass / theoretical product mass x 100
- Rf = zone migration / solvent-front migration

Worked Example

Illustrative 1:1 conversion from 10.0 mmol limiting reagent to a product of molar mass 177.24 g/mol gives 1.7724 g theoretical product. A dry isolated mass of 1.3293 g corresponds to 75.0% yield.

Use the actual reaction stoichiometry, salt or solvate form and purity basis; this illustration is not an expected experimental result.

Mechanism and identity record

• The amine and formaldehyde generate an iminium electrophile; the enol or enolate forms a carbon-carbon bond at the alpha position of the carbonyl compound.

• Assign carbonyl, aminomethyl and dimethylamino features. The molar mass below is for the neutral base; an isolated salt needs its own mass basis.

Result and Interpretation

• Report isolated yield and the independent identity/purity observations.

• Assign carbonyl, aminomethyl and dimethylamino features. The molar mass below is for the neutral base; an isolated salt needs its own mass basis.

Precautions

• Formaldehyde and amine exposure require approved hood controls; use only the selected validated reaction model.

• Use the institution-approved SOP and trained supervision for reactive additions, heating and work-up.

• Do not infer purity from yield or a single TLC zone.

Viva Questions and Answers

What determines theoretical yield?

The limiting reagent and balanced reaction stoichiometry.

How is conversion different from isolated yield?

Conversion measures reactant consumption; isolated yield also reflects selectivity and recovery losses.

Why use complementary identity tests?

A single physical or chromatographic observation may not distinguish impurities or isomers.

Multi-Step Preparation and Characterisation of Benzocaine

Practical 15 - Multistep synthesis practical

Aim

To plan and document a two-step synthesis with intermediate and final-product characterisation.

Principle

A multistep sequence connects the identity, amount and purity of an isolated intermediate to the next reaction. Overall isolated yield is the product of the fractional stage yields when based on consistent stoichiometry.

Materials and Requirements

- Approved two-step synthesis and supervised reaction records
- Intermediate and product references
- TLC, melting-point or other physical tests and appropriate spectra
- 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. Draw the named sequence, atom changes, balanced stoichiometry and expected intermediates.
3. Prepare a stage-by-stage table of actual amounts, limiting reagents, authorised conditions and analytical checkpoints.
4. For each stage use the approved reaction and work-up, retain progress evidence and isolate a characterised intermediate before advancing.
5. Characterise the final product, reconcile masses and calculate both individual and overall yields with their purity basis.
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.

Stage	Starting amount / limiting reagent	Product identity / purity	Dry yield
Step 1	Record mol	Characterise intermediate	Calculate
Step 2	Record mol	Characterise final product	Calculate
Overall	Original basis	Traceability	Calculate

Calculations

- Overall fractional yield = stage-1 fractional yield x stage-2 fractional yield for a consistent sequence

Worked Example

Illustrative stage yields 80.0% and 75.0% give an overall yield of 0.80 x 0.75 = 60.0%.

A two-step 60% yield is not obtained by averaging the stage percentages.

Selected sequence

- p-Nitrobenzoic acid to ethyl p-nitrobenzoate to benzocaine
- Esterification followed by selective nitro-to-amino conversion. Confirm that the ethyl ester is retained.
- Final neutral-product molar mass for the model: 165.19 g/mol. Confirm the actual salt or solvate form.

Result and Interpretation

- Present the reaction scheme, mechanisms, raw observations, identity evidence and stage/overall yield calculations.

Precautions

- Apply the specific authorised hazards and quench procedure at each stage.
- Do not carry an unidentified intermediate into the next reaction.
- Do not label an untested teaching product for administration.

Viva Questions and Answers

Why isolate an intermediate?

It supports identity, purity and accurate input to the next step.

How are overall yields combined?

Multiply fractional yields on consistent stoichiometric bases.

Why can high conversion give low final yield?

Selectivity, purification and transfer losses may reduce recovery.

Multi-Step Preparation and Characterisation of Paracetamol

Practical 16 - Multistep synthesis practical

Aim

To plan and document a two-step synthesis with intermediate and final-product characterisation.

Principle

A multistep sequence connects the identity, amount and purity of an isolated intermediate to the next reaction. Overall isolated yield is the product of the fractional stage yields when based on consistent stoichiometry.

Materials and Requirements

- Approved two-step synthesis and supervised reaction records
- Intermediate and product references
- TLC, melting-point or other physical tests and appropriate spectra
- 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. Draw the named sequence, atom changes, balanced stoichiometry and expected intermediates.
3. Prepare a stage-by-stage table of actual amounts, limiting reagents, authorised conditions and analytical checkpoints.
4. For each stage use the approved reaction and work-up, retain progress evidence and isolate a characterised intermediate before advancing.
5. Characterise the final product, reconcile masses and calculate both individual and overall yields with their purity basis.
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.

Stage	Starting amount / limiting reagent	Product identity / purity	Dry yield
Step 1	Record mol	Characterise intermediate	Calculate
Step 2	Record mol	Characterise final product	Calculate
Overall	Original basis	Traceability	Calculate

Calculations

- Overall fractional yield = stage-1 fractional yield x stage-2 fractional yield for a consistent sequence

Worked Example

Illustrative stage yields 80.0% and 75.0% give an overall yield of 0.80 x 0.75 = 60.0%.

A two-step 60% yield is not obtained by averaging the stage percentages.

Selected sequence

- p-Nitrophenol to p-aminophenol to paracetamol
- Selective reduction followed by controlled N-acetylation. Examine possible O-acetylation and unreacted intermediate.
- Final neutral-product molar mass for the model: 151.16 g/mol. Confirm the actual salt or solvate form.

Result and Interpretation

- Present the reaction scheme, mechanisms, raw observations, identity evidence and stage/overall yield calculations.

Precautions

- Apply the specific authorised hazards and quench procedure at each stage.
- Do not carry an unidentified intermediate into the next reaction.
- Do not label an untested teaching product for administration.

Viva Questions and Answers

Why isolate an intermediate?

It supports identity, purity and accurate input to the next step.

How are overall yields combined?

Multiply fractional yields on consistent stoichiometric bases.

Why can high conversion give low final yield?

Selectivity, purification and transfer losses may reduce recovery.

Multi-Step Preparation and Characterisation of Aspirin

Practical 17 - Multistep synthesis practical

Aim

To plan and document a two-step synthesis with intermediate and final-product characterisation.

Principle

A multistep sequence connects the identity, amount and purity of an isolated intermediate to the next reaction. Overall isolated yield is the product of the fractional stage yields when based on consistent stoichiometry.

Materials and Requirements

- Approved two-step synthesis and supervised reaction records
- Intermediate and product references
- TLC, melting-point or other physical tests and appropriate spectra
- 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. Draw the named sequence, atom changes, balanced stoichiometry and expected intermediates.
3. Prepare a stage-by-stage table of actual amounts, limiting reagents, authorised conditions and analytical checkpoints.
4. For each stage use the approved reaction and work-up, retain progress evidence and isolate a characterised intermediate before advancing.
5. Characterise the final product, reconcile masses and calculate both individual and overall yields with their purity basis.
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.

Stage	Starting amount / limiting reagent	Product identity / purity	Dry yield
Step 1	Record mol	Characterise intermediate	Calculate
Step 2	Record mol	Characterise final product	Calculate
Overall	Original basis	Traceability	Calculate

Calculations

- Overall fractional yield = stage-1 fractional yield x stage-2 fractional yield for a consistent sequence

Worked Example

Illustrative stage yields 80.0% and 75.0% give an overall yield of 0.80 x 0.75 = 60.0%.

A two-step 60% yield is not obtained by averaging the stage percentages.

Selected sequence

- Methyl salicylate to salicylic acid to aspirin
- Ester hydrolysis gives salicylic acid, followed by phenolic acetylation. Assess residual salicylic acid and hydrolytic instability.
- Final neutral-product molar mass for the model: 180.16 g/mol. Confirm the actual salt or solvate form.

Result and Interpretation

- Present the reaction scheme, mechanisms, raw observations, identity evidence and stage/overall yield calculations.

Precautions

- Apply the specific authorised hazards and quench procedure at each stage.
- Do not carry an unidentified intermediate into the next reaction.
- Do not label an untested teaching product for administration.

Viva Questions and Answers

Why isolate an intermediate?

It supports identity, purity and accurate input to the next step.

How are overall yields combined?

Multiply fractional yields on consistent stoichiometric bases.

Why can high conversion give low final yield?

Selectivity, purification and transfer losses may reduce recovery.

Multi-Step Preparation and Characterisation of Lidocaine

Practical 18 - Multistep synthesis practical

Aim

To plan and document a two-step synthesis with intermediate and final-product characterisation.

Principle

A multistep sequence connects the identity, amount and purity of an isolated intermediate to the next reaction. Overall isolated yield is the product of the fractional stage yields when based on consistent stoichiometry.

Materials and Requirements

- Approved two-step synthesis and supervised reaction records
- Intermediate and product references
- TLC, melting-point or other physical tests and appropriate spectra
- 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. Draw the named sequence, atom changes, balanced stoichiometry and expected intermediates.
3. Prepare a stage-by-stage table of actual amounts, limiting reagents, authorised conditions and analytical checkpoints.
4. For each stage use the approved reaction and work-up, retain progress evidence and isolate a characterised intermediate before advancing.
5. Characterise the final product, reconcile masses and calculate both individual and overall yields with their purity basis.
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.

Stage	Starting amount / limiting reagent	Product identity / purity	Dry yield
Step 1	Record mol	Characterise intermediate	Calculate
Step 2	Record mol	Characterise final product	Calculate
Overall	Original basis	Traceability	Calculate

Calculations

- Overall fractional yield = stage-1 fractional yield x stage-2 fractional yield for a consistent sequence

Worked Example

Illustrative stage yields 80.0% and 75.0% give an overall yield of 0.80 x 0.75 = 60.0%.

A two-step 60% yield is not obtained by averaging the stage percentages.

Selected sequence

- 2,6-Dimethylaniline to a chloroacetamide intermediate to lidocaine
- Acylation followed by controlled amine substitution. Specify whether the final product is the base or a salt.
- Final neutral-product molar mass for the model: 234.34 g/mol. Confirm the actual salt or solvate form.

Result and Interpretation

- Present the reaction scheme, mechanisms, raw observations, identity evidence and stage/overall yield calculations.

Precautions

- Apply the specific authorised hazards and quench procedure at each stage.
- Do not carry an unidentified intermediate into the next reaction.
- Do not label an untested teaching product for administration.

Viva Questions and Answers

Why isolate an intermediate?

It supports identity, purity and accurate input to the next step.

How are overall yields combined?

Multiply fractional yields on consistent stoichiometric bases.

Why can high conversion give low final yield?

Selectivity, purification and transfer losses may reduce recovery.

Quantitative Elemental and Functional-Group Analysis

Practical 19 - Practical study

Aim

To connect elemental and functional-group measurements to a justified structural assignment.

Principle

Elemental composition constrains a molecular formula; group-selective chemical or instrumental tests provide additional evidence. A positive qualitative test alone cannot establish an entire structure or quantify all functional groups.

Materials and Requirements

- Certified or supplied elemental-analysis data
- Approved group-selective assays and blanks
- Candidate structures and spectral data
- 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. Calculate the proposed elemental percentages and compare them with measured values, accounting for salt, water and solvent form.
3. Choose selective quantitative tests for the relevant acid, hydroxyl, amino or other groups and state reaction equivalents.
4. Review positive and negative controls and identify interfering functionalities.
5. Combine equivalent content, elemental evidence and spectra; record inconsistent evidence rather than forcing a match.
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.

Evidence	Expected / model	Measured	Interpretation
Elemental content	Calculate	Enter	Compare
Group equivalents	Calculate	Enter	Compare
Orthogonal spectrum	Assign	Enter	Assess

Calculations

- Element mass (%) = number of atoms x atomic mass / molecular mass x 100
- Equivalent content follows the validated group-reaction stoichiometry.

Worked Example

Illustrative C8H9NO2 with molar mass about 151.16 g/mol has carbon percentage about 63.56%.
A close carbon result cannot distinguish constitutional isomers with the same formula.

Result and Interpretation

• Provide a supported assignment and identify evidence that remains insufficient.

Precautions

• Use validated group chemistry and suitable blanks.
• Avoid hazardous classical elemental-decomposition experiments outside an approved setup.
• Account for hydrates, salts and residual solvent.

Viva Questions and Answers

Can elemental analysis distinguish isomers?

Not when the molecular formula is identical.

Why use a blank in group analysis?

Reagents and matrix can contribute to the measurement.

Why combine methods?

Independent evidence reduces ambiguity.

Isolation and Characterisation of a Natural Constituent

Practical 20 - 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.

Degradation Studies of Plant Constituents

Practical 21 - Practical study

Aim

To assess degradation of a selected plant constituent using controlled stress and analytical evidence.

Principle

Stability assesses time-dependent chemical, physical, microbiological and performance attributes in the proposed container system. Accelerated conditions support an assessment but do not by themselves establish a universal expiry period.

Materials and Requirements

- Approved stability protocol and product-specific specifications
- Qualified chamber records and container details
- Stability-indicating assay, impurity and physical-test data
- 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. Define batches, pack orientation, storage conditions, intervals, tests and statistical plan in the protocol.
3. Verify chamber qualification and retain actual monitoring, excursions and sample-pull records.
4. Review content, degradants, physical attributes and performance at each interval using qualified methods.
5. Plot each relevant attribute against time; investigate atypical results without deleting them and assess the supported storage period.
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.

Interval / batch	Actual storage condition	Assay / degradants	Physical / performance result
Initial	Record	Record	Record
Intermediate	Record	Record	Record
Final available point	Record	Record	Record

Calculations

- For a justified first-order model: ln(Ct/C0) = -kt; t90 = -ln(0.90)/k
- Do not extrapolate a shelf life without the applicable stability and statistical justification.

Worked Example

Illustrative first-order k = 0.0020 month^-1 gives t90 = 52.7 months mathematically.

This is a model calculation, not a product expiry claim. Real assignment requires the complete attribute, packaging and stability evidence.

Natural-product degradation

• Use a named reference such as an approved hydrolysable glycoside model; define the proposed transformation and expected products.
• Review matched unstressed, stress-blank and stressed records. Separate true degradation from extraction loss, precipitation and detector-response changes.
• Measure intact constituent and relevant products with a suitable stability-indicating method; report mass balance only on a justified response and molar basis.

Result and Interpretation

• Report the actual data period, trends, excursions and limits of any extrapolation.

Precautions

• Use product- and market-appropriate conditions rather than a universal chamber schedule.
• Keep container and sampling traceability.
• Do not estimate shelf life from assay alone.

Viva Questions and Answers

What is stability indicating?

The method can distinguish the intended analyte from relevant degradation and matrix effects.

Why study the marketed pack?

The pack can influence moisture, light and gas exposure.

Can one accelerated result prove expiry?

A justified programme and complete evidence are required.

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