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M.Pharm Practical Notes

Pharmacology 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.

Simultaneous UV Estimation of Two Components

Practical 2 - 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.

Quantitative Analysis by HPLC

Practical 3 - 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 4 - 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 5 - 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 6 - 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.

Laboratory Animal Handling and Welfare Records

Practical 7 - Pharmacology simulation and data practical

Aim

To review laboratory-animal identification, handling and welfare records.

Principle

Species-appropriate care and trained handling reduce distress and improve data quality. Behaviour and welfare must be interpreted in the animal and study context, with documented humane endpoints and veterinary oversight.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Review animal source, identification, acclimatisation, housing, enrichment and health-status records.
4. Use approved demonstration images or simulation to identify species-specific handling and restraint considerations without attempting unfamiliar live restraint.
5. Complete a fictional daily welfare record with observations, escalation triggers, responsible personnel and humane-endpoint review.
6. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- Welfare observation completion (%) = documented scheduled checks / required scheduled checks x 100

Worked Example

Illustrative 18 documented checks out of 20 required checks give 90% completion. Missing checks are a welfare and documentation gap, not evidence that the animals were well.

Result and Interpretation

• Provide an identification and welfare checklist and explain when trained veterinary review is required.

Precautions

• Apply replacement, reduction and refinement to study planning.
• Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
• Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

What are the 3Rs?

Replacement, reduction and refinement.

Why record acclimatisation?

It supports welfare and interpretation of baseline behaviour.

Who determines humane endpoints?

They are defined in the approved study with appropriate ethical and veterinary oversight.

Routes of Administration - Record Simulation

Practical 8 - Pharmacology simulation and data practical

Aim

To compare administration-route records without performing live dosing.

Principle

Route affects absorption, onset and exposure; feasibility and welfare depend on species, formulation and trained technique. A computed amount does not establish a safe route, concentration or volume.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Review supplied oral, intravenous, intraperitoneal, subcutaneous and other authorised-route records, noting their scientific purpose.
4. Check formulation identity, route, timing, weight basis and record consistency against the original authorised instruction.
5. Use a simulator or diagram to map documentation requirements and identify missing training, compatibility or monitoring evidence.
6. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- For checking an existing authorised order: amount = stated amount per unit body mass x recorded body mass
- Volume = authorised amount / preparation concentration

Worked Example

Illustrative dimensional check: a fictional record requires 0.50 mg from a 2.0 mg/mL preparation, giving 0.25 mL mathematically. This is not a recommended route, animal dose or allowable volume.

Result and Interpretation

• Deliver a route-comparison and documentation table, including the limitations of extrapolating between routes.

Precautions

• Apply replacement, reduction and refinement to study planning.
• Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
• Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

Does equal dose give equal exposure across routes?

Absorption and first-pass effects can differ.

Why check concentration?

It determines the volume associated with the recorded amount.

Can a calculation establish welfare suitability?

Species-specific authorised procedures and supervision are separately required.

Blood Sampling Anaesthesia and Euthanasia Records

Practical 9 - Pharmacology simulation and data practical

Aim

To assess blood-sampling, anaesthesia and euthanasia documentation.

Principle

Invasive procedures and endpoints require a defined scientific need, trained personnel, welfare controls and confirmation criteria. Records must distinguish sedation, anaesthesia, analgesia and euthanasia.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Use supplied records or simulation only; identify the approved protocol, personnel and procedure scope.
4. Review blood-sampling rationale, cumulative-sampling account and post-procedure monitoring against the authorised plan.
5. Review anaesthetic-depth, analgesia, recovery and adverse-event monitoring records without creating an administration schedule.
6. Assess the approved euthanasia and death-confirmation records and escalation when criteria were not met.
7. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
8. 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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

• Cumulative recorded sample volume = sum of the individually documented sample volumes over the specified period

Worked Example

Illustrative supplied samples of 20, 30 and 25 microlitres total 75 microlitres. Whether this was permitted depends on the actual species, animal, interval, welfare assessment and approved protocol.

Result and Interpretation

• Prepare a completeness and welfare-gap report; no blood-collection, surgical or killing instructions are part of this exercise.

Precautions

• Apply replacement, reduction and refinement to study planning.
• Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
• Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

Are sedation and analgesia equivalent?

Reduced responsiveness does not automatically establish pain control.

Why track cumulative sampling?

Repeated collections must be assessed together.

Why confirm death?

An approved endpoint requires documented confirmation, not assumption.

Functional Observation Battery and Irwin-Test Records

Practical 10 - Pharmacology simulation and data practical

Aim

To analyse a supplied pharmacological screening dataset with appropriate controls.

Principle

Screening endpoints are operational measurements. Blinding, randomisation, baseline comparability, welfare and confounding effects determine what the results support.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Identify the named screen, species in the source study, randomisation, blinding, endpoint definitions and humane limits.
4. Review raw time or individual-unit records and baseline comparability before deriving summary scores.
5. Calculate only the screen-appropriate effects, retain missing observations and evaluate confounders such as sedation, motor changes or collection loss.
6. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- For an appropriate continuous endpoint: relative reduction (%) = (control - test)/control x 100
- Do not apply ratio arithmetic automatically to ordinal scales.

Worked Example

Illustrative score falls from 4 to 2 on a defined ordinal scale. Report the category change; do not assume a 50% biological reduction.

Functional observation / Irwin battery

- Score supplied records of appearance, posture, spontaneous activity, reflex-related observations and autonomic signs using a predefined scale.
- Distinguish a broad functional observation from a specific receptor or disease-mechanism claim.

Result and Interpretation

- Present independent-unit results, uncertainty and a conclusion limited to the model and endpoint.

Precautions

- Apply replacement, reduction and refinement to study planning.
- Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
- Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

Why blind scoring?

It reduces expectation-related assessment bias.

What is a confounder?

A factor that can explain the endpoint apart from the proposed effect.

Does a positive screen prove clinical efficacy?

Further relevant evidence is required.

CNS Stimulant Depressant Anxiety and Seizure Screens

Practical 11 - Pharmacology simulation and data practical

Aim

To analyse a supplied pharmacological screening dataset with appropriate controls.

Principle

Screening endpoints are operational measurements. Blinding, randomisation, baseline comparability, welfare and confounding effects determine what the results support.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Identify the named screen, species in the source study, randomisation, blinding, endpoint definitions and humane limits.
4. Review raw time or individual-unit records and baseline comparability before deriving summary scores.
5. Calculate only the screen-appropriate effects, retain missing observations and evaluate confounders such as sedation, motor changes or collection loss.
6. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- For an appropriate continuous endpoint: relative reduction (%) = (control - test)/control x 100
- Do not apply ratio arithmetic automatically to ordinal scales.

Worked Example

Illustrative test movement count 60 versus control 100 is a 40% count reduction. It is not by itself evidence of anxiolytic efficacy.

CNS stimulant, depressant, anxiety and seizure records

• Prepare separate worksheets for locomotor activity, behavioural anxiety endpoints and seizure-related observations; include motor and sedation confounding controls.
• A behavioural change must be assessed against general motor function, blinding, welfare and the actual model.

Result and Interpretation

• Present independent-unit results, uncertainty and a conclusion limited to the model and endpoint.

Precautions

• Apply replacement, reduction and refinement to study planning.
• Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
• Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

Why blind scoring?

It reduces expectation-related assessment bias.

What is a confounder?

A factor that can explain the endpoint apart from the proposed effect.

Does a positive screen prove clinical efficacy?

Further relevant evidence is required.

Analgesic Inflammatory Local-Anaesthetic and Pupil-Response Screens

Practical 12 - Pharmacology simulation and data practical

Aim

To analyse a supplied pharmacological screening dataset with appropriate controls.

Principle

Screening endpoints are operational measurements. Blinding, randomisation, baseline comparability, welfare and confounding effects determine what the results support.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Identify the named screen, species in the source study, randomisation, blinding, endpoint definitions and humane limits.
4. Review raw time or individual-unit records and baseline comparability before deriving summary scores.
5. Calculate only the screen-appropriate effects, retain missing observations and evaluate confounders such as sedation, motor changes or collection loss.
6. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- For an appropriate continuous endpoint: relative reduction (%) = (control - test)/control x 100
- Do not apply ratio arithmetic automatically to ordinal scales.

Worked Example

Illustrative swelling is 0.80 units in control and 0.50 in test: reduction = 37.5%. Compare matched baselines and measurement uncertainty.

Analgesic, inflammatory, local-anaesthetic and pupil-response records

• Separate nociceptive latency, inflammatory swelling, local sensory-response and pupil-diameter datasets, preserving each method and welfare endpoint.
• Avoid equating motor suppression with analgesia or pupil change with a unique receptor mechanism.

Result and Interpretation

• Present independent-unit results, uncertainty and a conclusion limited to the model and endpoint.

Precautions

• Apply replacement, reduction and refinement to study planning.
• Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
• Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

Why blind scoring?

It reduces expectation-related assessment bias.

What is a confounder?

A factor that can explain the endpoint apart from the proposed effect.

Does a positive screen prove clinical efficacy?

Further relevant evidence is required.

Diuretic Activity Evaluation

Practical 13 - Pharmacology simulation and data practical

Aim

To analyse a supplied pharmacological screening dataset with appropriate controls.

Principle

Screening endpoints are operational measurements. Blinding, randomisation, baseline comparability, welfare and confounding effects determine what the results support.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Identify the named screen, species in the source study, randomisation, blinding, endpoint definitions and humane limits.
4. Review raw time or individual-unit records and baseline comparability before deriving summary scores.
5. Calculate only the screen-appropriate effects, retain missing observations and evaluate confounders such as sedation, motor changes or collection loss.
6. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- For an appropriate continuous endpoint: relative reduction (%) = (control - test)/control x 100
- Do not apply ratio arithmetic automatically to ordinal scales.

Worked Example

Illustrative 4 mL urine at sodium 50 mmol/L contains 0.20 mmol sodium; 2 mL at 80 mmol/L contains 0.16 mmol. Concentration alone reverses the apparent comparison.

Diuretic activity records

- Review timed urine volume, body-mass and intake records together with electrolyte concentration and total electrolyte excretion.
- Differentiate water volume from sodium or potassium excretion, hydration effects and collection completeness.

Result and Interpretation

- Present independent-unit results, uncertainty and a conclusion limited to the model and endpoint.

Precautions

- Apply replacement, reduction and refinement to study planning.
- Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
- Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

Why blind scoring?

It reduces expectation-related assessment bias.

What is a confounder?

A factor that can explain the endpoint apart from the proposed effect.

Does a positive screen prove clinical efficacy?

Further relevant evidence is required.

Antiulcer Activity in Pylorus-Ligation Records

Practical 14 - Pharmacology simulation and data practical

Aim

To analyse a supplied pharmacological screening dataset with appropriate controls.

Principle

Screening endpoints are operational measurements. Blinding, randomisation, baseline comparability, welfare and confounding effects determine what the results support.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Identify the named screen, species in the source study, randomisation, blinding, endpoint definitions and humane limits.
4. Review raw time or individual-unit records and baseline comparability before deriving summary scores.
5. Calculate only the screen-appropriate effects, retain missing observations and evaluate confounders such as sedation, motor changes or collection loss.
6. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- For an appropriate continuous endpoint: relative reduction (%) = (control - test)/control x 100
- Do not apply ratio arithmetic automatically to ordinal scales.

Worked Example

Illustrative mean lesion score 8 in control and 4 in test gives a 50% score reduction on that defined scale; it does not establish human antiulcer efficacy.

Pylorus-ligation study records

• Use supplied historical study records only. Review blinded lesion scoring, gastric-volume and acidity measurements, welfare endpoints and the authorised design; do not perform or describe the surgery.
• Ulcer score, acid output and a protective-effect hypothesis are separate outcomes with model-specific limitations.

Result and Interpretation

• Present independent-unit results, uncertainty and a conclusion limited to the model and endpoint.

Precautions

• Apply replacement, reduction and refinement to study planning.
• Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
• Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

Why blind scoring?

It reduces expectation-related assessment bias.

What is a confounder?

A factor that can explain the endpoint apart from the proposed effect.

Does a positive screen prove clinical efficacy?

Further relevant evidence is required.

Oral Glucose Tolerance Data Analysis

Practical 15 - Pharmacology simulation and data practical

Aim

To analyse supplied oral-glucose-tolerance concentration-time data.

Principle

A glucose-tolerance curve describes the measured time-dependent response under a defined challenge. Baseline, timing, fasting and the original protocol influence interpretation; animal-model data are not human diagnostic thresholds.

Materials and Requirements

- Supplied anonymised teaching traces or datasets
- Study design, controls, units and instrument calibration records
- Ethics and welfare records where the original study involved animals
- 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 study question, experimental unit, controls and original approval or data provenance. This notes exercise uses records or simulation; it is not authorisation to perform animal procedures.
3. Review supplied challenge, baseline and sampling-time records without performing live administration or blood collection.
4. Plot individual glucose-time profiles using consistent concentration and time units.
5. Calculate total AUC by the selected trapezoidal method and distinguish it from incremental AUC, stating treatment of values below baseline.
6. Compare independent groups with baseline and missing-data assessment.
7. Summarise independent experimental units, variability, exclusions and limitations; retain all original traces and data.
8. 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.

Group / preparation	Condition / time	Raw endpoint	Derived result / interpretation
Vehicle / baseline	Record	Enter	Evaluate
Reference condition	Record	Enter	Evaluate
Test condition	Record	Enter	Calculate

Calculations

- Trapezoid area = (C1 + C2)/2 x (t2 - t1)

Worked Example

Illustrative times 0, 1, 2 h and glucose 5, 9, 6 mmol/L give total AUC = 7 + 7.5 = 14.5 mmol.h/L. Baseline-subtracted signed area is 14.5 - 5 x 2 = 4.5 mmol.h/L.

Result and Interpretation

- Report curves, chosen AUC convention and study-specific interpretation without making a patient diagnosis.

Precautions

- Apply replacement, reduction and refinement to study planning.
- Any live work requires the current institutional and Indian CCSEA/IAEC framework, trained staff and an approved protocol.
- Do not convert a teaching calculation into an unapproved animal or patient procedure.

Viva Questions and Answers

What distinguishes total and incremental AUC?

Incremental AUC uses a defined baseline subtraction convention.

Why retain actual sampling times?

Timing affects area estimates.

Can animal thresholds diagnose humans?

Human diagnosis requires the applicable clinical criteria and setting.

DNA Isolation from Plant and Supplied Tissue Preparations

Practical 16 - Approved nucleic-acid isolation practical

Aim

To isolate DNA from an approved plant model and assess recovery and integrity.

Principle

Cell disruption releases nucleic acids; controlled lysis and purification separate DNA from proteins, polysaccharides and other contaminants. Precipitated threads alone do not prove DNA identity or purity.

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. Use cauliflower or another authorised plant model; animal-tissue comparisons use supplied records unless separately approved.
3. Record input mass and use the adopted plant-DNA kit or validated lysis and clarification method.
4. Purify through the specified binding/wash/elution or approved precipitation workflow, retaining extraction blanks.
5. Measure DNA concentration and recovered volume and assess integrity by a suitable gel and purity by complementary checks.
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

- DNA yield per gram = measured DNA mass / input tissue mass
- DNA mass = concentration x elution volume

Worked Example

Illustrative DNA concentration 80 ng/microlitre in 100 microlitres gives 8,000 ng = 8 micrograms. From 0.20 g plant material, yield is 40 micrograms/g.

Report the actual assay specificity and extraction-blank result.

Four source-comparison records

• Bacteria: use only an approved certified nonpathogenic teaching source or supplied extraction record. Document cell-wall disruption and chromosomal/plasmid DNA interpretation.
• Cauliflower and onion: record botanical source, cell-wall disruption and polysaccharide/secondary-metabolite removal in separate extraction worksheets.
• Goat liver: use supplied authorised tissue-extraction records for this comparison; assess tissue provenance, nucleases, protein removal and the approved tissue-DNA method.
• Compare concentration, input-normalised yield, contamination and gel integrity for each source; visible precipitate alone is not specific DNA identification.

Result and Interpretation

• Present extraction flow, recovery, purity indicators and gel 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 avoid harsh handling after lysis?

It can shear high-molecular-weight DNA.

Why include an extraction blank?

It checks contamination introduced during purification.

Why do plant matrices need care?

Polysaccharides and secondary metabolites can interfere.

RNA Isolation from Yeast

Practical 17 - Approved nucleic-acid isolation practical

Aim

To isolate RNA from approved teaching yeast and assess concentration and integrity.

Principle

RNA purification requires effective nuclease control and separation from DNA, proteins and other matrix components. The extraction method and downstream use determine whether a DNA-removal step is required.

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. Use approved teaching yeast or supplied extraction records and document starting material.
2. Prepare a nuclease-controlled workspace and apply the adopted yeast-disruption and RNA-purification kit method.
3. Include extraction blanks and apply the justified DNA-removal and cleanup steps.
4. Measure concentration using an RNA-appropriate method and assess integrity by the selected electrophoretic or instrument method.
5. Record yield, purity, integrity and suitability for the planned downstream application.

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

- RNA concentration = A260 x 40 micrograms/mL x dilution, for 1 cm-normalised absorbance
- RNA yield = concentration x recovered volume

Worked Example

Illustrative A260 = 0.25 at dilution 5 gives 50 micrograms/mL RNA. A 0.20 mL recovery contains 10 micrograms.

Absorbance alone cannot distinguish residual DNA from the intended RNA.

Result and Interpretation

- Present extraction flow, recovery, purity indicators and gel evidence.
- Report biological and technical replication separately and explain control performance.

Precautions

- Use the approved RNase-control practices and compatible nuclease-free materials.
- Do not treat an acceptable absorbance ratio as proof of intact RNA.
- Retain the RNA-specific quality and DNA-contamination controls.

Viva Questions and Answers

Why control RNases?

RNA can degrade rapidly through nuclease activity.

Why assess DNA contamination?

It can bias concentration and downstream amplification.

What confirms RNA integrity?

A suitable electrophoretic or equivalent integrity method.

Protein Estimation by Bradford and Lowry Methods

Practical 18 - Analytical practical

Aim

To estimate protein concentration using Bradford or Lowry calibration and suitable matrix controls.

Principle

Bradford measurements depend on dye binding, while Lowry measurements involve copper chemistry and a subsequent colour reaction. Response varies with protein composition and can be affected by detergents, reducing agents and other matrix components.

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

- Selected Bradford or Lowry reagent method
- Bovine serum albumin or the stated protein reference
- Blank, independent check and suitable readout system
- 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 one method for the stated matrix and document its compatible concentration range.
4. Prepare a reference calibration and a separate check in the relevant buffer conditions.
5. Treat standards, blanks and test dilutions using the selected reagent order and timing.
6. Measure at the adopted wavelength, apply the appropriate blank and verify that dilution gives a consistent result.
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
Buffer / reagent blank	Record	Record	Calculate

Preparation / attribute	Method condition	Raw measurement	Calculated result
Reference levels	Record	Record	Calculate
Test dilutions	Record	Record	Calculate
Protein-equivalent concentration	Record	Record	Calculate

Calculations

• Protein concentration = concentration obtained from the selected calibration x dilution factor

Worked Example

Illustrative calibration A = 0.400C + 0.020, where C is mg/mL of the reference protein.

A test absorbance of 0.220 gives 0.500 mg/mL in the measured dilution. A ten-fold dilution gives 5.00 mg/mL in the original preparation.

Report the reference and method because responses are not identical for every protein.

Result and Interpretation

• State the method, buffer, reference equivalent and interference assessment.

• Report replicate variability and the evidence that the method can measure the intended attribute in this matrix.

Precautions

• Use compatible buffers and validated assay timing.

• Avoid extrapolation beyond the working range.

• Prepare dilution checks for concentrated or interfering matrices.

• Use the adopted laboratory procedure, suitable controls and the designated waste arrangements.

Viva Questions and Answers

Do Bradford and Lowry have the same chemistry?

No; their response mechanisms and interference profiles differ.

Why identify the reference protein?

Protein composition influences the assay response.

What can a dilution check show?

Unexpected concentration dependence may reveal interference.

UV Estimation of DNA and RNA

Practical 19 - 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.

Gene Amplification by PCR

Practical 20 - Approved PCR practical

Aim

To plan and interpret PCR amplification of a benign teaching target.

Principle

PCR repeats denaturation, primer annealing and extension to amplify a defined region. Primer specificity, template quality and reaction controls determine whether an observed product supports the intended target.

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. Use a known benign plant, yeast or certified teaching-DNA target and the selected polymerase protocol.
3. Record primer identities, expected product size, template input, reagent master mix and the protocol-specific thermal programme.
4. Include no-template, positive-template and extraction controls where applicable; separate preparation and post-amplification handling.
5. Run the authorised programme and assess product size and nonspecific bands by a suitable method, retaining all controls.
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

- Idealised copies after n perfect doublings = initial copies x 2^n; real PCR efficiency and plateau limit this model.

Worked Example

Illustrative 100 starting copies after 10 perfect doublings would give 102,400 copies.

A band endpoint does not establish that this ideal amplification occurred or provide reliable original-copy quantitation.

Result and Interpretation

- Deliver a reaction map, thermal programme rationale, control results and target-specific interpretation.
- 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 include a no-template control?

It checks reagent or handling contamination.

Why must annealing be target specific?

Primer binding depends on sequence and reaction chemistry.

Does endpoint band brightness give original copy number?

Not without a validated quantitative method.

Protein Quantification by Western Blotting

Practical 21 - Biological teaching-data practical

Aim

To quantify a target protein from a controlled Western-blot dataset.

Principle

Western blotting combines separation, transfer and antibody-based detection. Specificity and a nonsaturated linear response are needed; normalisation corrects selected loading effects but cannot repair poor specificity.

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. Use approved protein records with target and loading or total-protein controls.
3. Assess full-lane separation, transfer controls, antibody specificity and the absence of saturated exposure.
4. Apply consistent regions and background subtraction and normalise with a justified stable loading measure.
5. Compare independent biological replicates and retain uncropped images and processing settings.
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

- Normalised target = background-corrected target signal / justified loading signal
- Relative fold = normalised test / normalised reference

Worked Example

Illustrative target/loading signals are 200/100 in control and 300/100 in test: normalised values 2 and 3 give a 1.5-fold difference.

Do not interpret a saturated band or unstable loading control quantitatively.

Result and Interpretation

- Report target specificity, linear range, normalisation and biological variability.
- 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 keep uncropped images?

They preserve lane context and additional signals.

What invalidates densitometry?

Saturation or an unvalidated response range can distort ratios.

Why distinguish biological replicates?

They represent independent biological observations rather than repeated readings.

MPO AChE Amylase and Glucosidase Enzyme Assays

Practical 22 - Biological teaching-data practical

Aim

To analyse MPO, acetylcholinesterase, amylase and glucosidase assay records.

Principle

Enzyme inhibition is measured against a matched enzyme reaction, but coloured compounds, direct reagent reactions and nonlinearity can mimic inhibition. Each named enzyme needs its own substrate, measurement and control method.

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. Prepare a four-assay worksheet specifying enzyme, substrate, detection principle, units and validated initial-rate interval.
3. Review enzyme-free, substrate or reagent blanks and vehicle and reference-inhibitor controls for each assay.
4. Calculate blank-corrected rates and compare test with vehicle under matched substrate conditions.
5. Fit a concentration-response model only over valid data and distinguish IC50 from a mechanism-specific inhibition constant.
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

- Inhibition (%) = 100 x [1 - corrected test rate / corrected vehicle rate]

Worked Example

Illustrative corrected vehicle rate 0.100 absorbance units/min and test rate 0.040 give 60% apparent inhibition. If the compound independently changes the detection reagent, this number is not valid enzyme inhibition.

Four assay-specific principles

• MPO: use a defined peroxide-dependent chromogenic method. One authorised example measures o-dianisidine oxidation near 460 nm; other kits have different probes and readouts, so keep the chosen method intact.
• AChE: an Ellman-type assay uses acetylthiocholine and DTNB, with the thiocholine-related colour read near 412 nm. Include compound/reagent interference controls.
• Alpha-amylase: a starch-hydrolysis assay can quantify reducing-sugar products with a calibrated DNS read near 540 nm. Native reducing compounds and colour require controls.
• Alpha-glucosidase: a p-nitrophenyl-alpha-D-glucopyranoside method measures the released p-nitrophenol response near 405 nm under the adopted pH conditions.
• For each assay retain enzyme/source, substrate, buffer/pH, reaction time, temperature and detector settings from the selected validated protocol; do not transfer an extinction factor or unit definition between assays.

Result and Interpretation

• Deliver separate MPO, AChE, amylase and glucosidase records with assay-specific interference checks.
• 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

Is IC50 always Ki?

No; IC50 depends on assay conditions and inhibition mechanism.

Why measure initial rate?

Endpoint saturation can obscure rate changes.

Why separate detection controls?

An optical or chemical artifact can mimic inhibition.

MTT Trypan-Blue and SRB Viability Assays

Practical 23 - Biological teaching-data practical

Aim

To compare cell-viability measurements and retain valid subculture records.

Principle

Trypan-blue exclusion assesses membrane integrity, MTT reflects a metabolic reduction response and SRB measures a protein-associated signal after the selected procedure. These endpoints are related but not interchangeable.

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. Use an approved cell line with identity and contamination-control records or a supplied dataset; review passage and subculture history.
3. Record treatment, vehicle, cell-free interference controls and assay-specific positive controls.
4. For dye exclusion count viable and nonviable cells using the defined chamber and dilution; for MTT/SRB verify blank correction and linear response.
5. Calculate each endpoint separately and compare independent biological replicates, recognising compound colour or chemical-reduction interference.
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

- Dye-exclusion viability (%) = viable cells / total cells x 100
- Relative corrected assay response (%) = (test - relevant blank)/(vehicle - relevant blank) x 100

Worked Example

Illustrative 180 viable and 20 nonviable cells give 90% viability. MTT corrected signals 0.40 test and 0.80 vehicle give 50% relative metabolic response.

Those values need not agree because they measure different attributes; an IC50 requires a justified concentration-response fit.

Result and Interpretation

• Report the endpoint, controls, replicates and interpretation without equating metabolic response directly to cell number.
• 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

What does trypan blue assess?

Loss of membrane exclusion under the defined timing.

Why use cell-free compound controls?

The compound may alter colour or assay chemistry.

Is an MTT reduction direct proof of apoptosis?

A mechanism-specific assessment is needed.

DNA Fragmentation by Agarose Electrophoresis

Practical 24 - Electrophoresis practical

Aim

To assess supplied DNA-fragmentation gel patterns with suitable controls.

Principle

DNA cleavage can alter size distribution and produce ladders or smears. A pattern is not specific for one cell-death mechanism and is affected by extraction, nuclease activity and gel conditions.

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. Select an appropriate fragment range, gel, buffer and approved stain; record electrophoresis settings.
3. Use a size ladder, extraction or loading controls and the supplied DNA preparations.
4. Run only through the authorised electrical and imaging procedure; retain the original unsaturated image.
5. Fit log10 fragment size against migration within the supported ladder range and estimate unknown linear-fragment sizes.
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

- Example calibration: log10(size in bp) = intercept + slope x migration distance

Worked Example

Illustrative log10(bp) = 4.0 - 0.030d with d in mm. At d = 40 mm, size is 10^2.8 = 631 bp approximately.
Do not apply this linear-fragment calibration directly to uncut supercoiled plasmids.

Fragmentation interpretation

- Compare untreated, reference and test lanes at a justified DNA-input basis. Retain extraction blanks and the full image.

• Report the pattern descriptively and seek orthogonal apoptosis or damage evidence rather than diagnosing mechanism from a smear.

Result and Interpretation

• Provide a labelled lane map, control assessment and approximate fragment sizes with calibration limits.
• 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 a ladder?

It provides size references in the same run.

Why avoid saturated images?

They obscure quantitative intensity and band detail.

Why can plasmid forms migrate differently?

Conformation changes mobility independently of length.

Comet-Assay Data Interpretation

Practical 25 - Biological teaching-data practical

Aim

To quantify supplied comet-assay images with a documented scoring rule.

Principle

Single-cell gel electrophoresis assesses migration of DNA under the selected assay conditions. The alkaline and neutral methods have different sensitivities; image metrics are assay-dependent and do not alone identify the damage mechanism.

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. Review supplied approved-system images and the exact assay variant, controls and image-quality criteria.
3. Select fields by a predeclared rule, blind scoring where possible and exclude artifacts only with documented reasons.
4. Measure the prescribed metrics consistently and summarise cells within independent biological replicates.
5. Compare groups with an analysis respecting the replicate structure and discuss cytotoxicity and handling artifacts.
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

- Tail DNA (%) = tail fluorescence / total comet fluorescence x 100

Worked Example

Illustrative total signal 1,000 units and tail signal 250 give 25% tail DNA.

Scoring 100 cells from one preparation does not create 100 independent biological replicates.

Result and Interpretation

- Provide representative full-field images, scoring rules, control results and replicate-level summaries.
- 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

What does tail DNA express?

The proportion of measured fluorescence in the defined tail.

Why avoid cell-level pseudoreplication?

Cells from one preparation are not independent experimental preparations.

Does the metric identify a mutation?

It measures assay-defined DNA migration, not a specific mutation.

Fluorescence-Based Apoptosis Assessment

Practical 26 - Biological teaching-data practical

Aim

To interpret fluorescence-based apoptosis markers with orthogonal controls.

Principle

Annexin-associated binding and membrane-impermeant dyes report different cellular attributes under the selected method. Quadrant patterns are time- and method-dependent and do not by themselves prove a complete cell-death mechanism.

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. Use supplied approved-system fluorescence images or cytometry records with unstained, single-stain and appropriate reference controls.
3. Review compensation, gating, debris and doublet handling for cytometry or prespecified image-scoring criteria.
4. Quantify the defined cell populations with the correct denominator and retain the full gating or scoring record.
5. Compare time points and independent preparations with viability and another suitable mechanistic endpoint.
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

- Population (%) = events meeting the defined gate / valid analysed events x 100

Worked Example

Illustrative 200 events in a marker-positive gate among 2,000 valid events give 10%.

Do not include debris inconsistently or label every marker-positive event as definitively apoptotic without context.

Result and Interpretation

- Report markers, controls, time, gating and the strength and limits of the mechanistic inference.
- 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 single-stain controls?

They support assessment of spectral overlap and compensation.

Why specify time point?

Marker patterns can change through the death process.

Why use another endpoint?

Independent evidence strengthens the interpretation.

Pharmacokinetic Analysis of Different Administration Routes

Practical 27 - Software and data-analysis practical

Aim

To compare pharmacokinetic profiles after different administration routes using a supplied dataset.

Principle

Noncompartmental analysis estimates exposure from the concentration-time profile without first assuming a fixed compartmental structure. Terminal extrapolation requires a defensible terminal phase. IVIVC additionally needs an appropriate relationship between in-vitro release and in-vivo input or response; two similar-looking curves do not establish it.

Keep the supplied data, transformations, software version, analysis settings and complete output together. Record units explicitly and distinguish assumptions from observations.

Materials and Requirements

- Supplied concentration-time data, dose, route and subject identifiers
- Dissolution profiles where an IVIVC investigation is intended
- Spreadsheet or institution-approved analysis software
- Raw-data dictionary and analysis worksheet

Procedure

1. Define the analysis question and the variables, units, identifiers and missing-data conventions in the dataset.
2. Import a working copy and check values against the original file. Inspect ranges, duplicates, timing and unit consistency.
3. Map concentration, time, dose and subject columns and select the intended route and exposure calculation.
4. Inspect the profile and choose the integration method, terminal observations and treatment of below-quantitation values through the stated analysis plan.
5. Calculate Cmax, Tmax, AUC, terminal rate where justified, half-life and extrapolated fraction. Review terminal-fit diagnostics.
6. In WinNonlin or the selected implementation, preserve the model and settings report. For IVIVC, identify the development and validation datasets and the appropriate input-estimation method.
7. Retain diagnostics, intermediate calculations and the selected analysis settings. Explain exclusions and sensitivity checks.
8. Write a conclusion that addresses the stated question and the limitations of the design rather than relying on one fitted statistic.

Observations and Record

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

Parameter	Input or method	Calculated value	Diagnostic / units
Cmax and Tmax	Observed profile	Record	State units
AUC	Selected integration	Record	Check sampling

Parameter	Input or method	Calculated value	Diagnostic / units
Terminal slope	Justified points	Record	Review fit
IVIVC	Development and validation design	Record	State evidence

Calculations

- Linear trapezoid area = (C1 + C2)/2 x (t2 - t1)
- AUC_infinity = AUC_last + C_last/lambda_z, where terminal extrapolation is justified
- Half-life = ln(2)/lambda_z
- After extravascular dosing, dose/AUC estimates CL/F rather than true clearance unless F is known.

Worked Example

Illustrative oral profile: times 0, 1, 2, 4, 6 and 8 h; concentrations 0, 8, 4, 2, 1 and 0.5 mg/L.

Linear trapezoidal AUC to 8 h is 20.5 mg.h/L. The illustrated terminal decline from 2 to 8 h halves every 2 h, giving lambda_z = 0.3466 h^-1.

Tail area = 0.5/0.3466 = 1.443 mg.h/L; AUC_infinity is about 21.943 mg.h/L and half-life is 2.00 h.

A formal analysis uses adequate observations, prespecified methods and appropriate terminal-fit assessment.

Route comparison

- Compare dose-normalised exposure, Cmax, Tmax and the assumptions needed for absolute or relative bioavailability.
- Identify absorption-related differences without equating a later Tmax with proof of one particular absorption mechanism.

Result and Interpretation

- Report the exposure parameters and their data basis. Evaluate extrapolated exposure and the adequacy of terminal sampling.
- State separately whether an IVIVC was developed, tested or only proposed.

Precautions

- Do not mix concentration or time units.
- Distinguish observed from model-derived values.
- A regression between dissolution and plasma concentration is not automatically a validated Level A IVIVC.
- Preserve the original dataset and a reproducible record of the analysis.

Viva Questions and Answers

Why select the terminal phase carefully?

The selected slope affects half-life and extrapolated exposure.

What is CL/F?

Apparent clearance after an extravascular dose when absolute bioavailability is not established.

Why use separate IVIVC validation evidence?

The fitted relationship must predict data beyond the observations used to develop it.

Enzyme Inhibition and Induction Data

Practical 28 - Biological teaching-data practical

Aim

To distinguish enzyme inhibition from induction in supplied activity data.

Principle

Inhibition can reduce measured catalytic activity without reducing enzyme abundance. Induction concerns an increase in expression or functional capacity over an appropriate time course. Activity alone may not distinguish them.

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. Use supplied approved-system records with immediate activity and expression or protein measurements.
3. Identify time course, concentration, viability, vehicle and suitable reference controls.
4. Calculate residual activity and fold expression on their respective normalised bases.
5. Assess whether the evidence supports inhibition, induction, toxicity or an unresolved combination.
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

- Residual activity (%) = corrected test activity / corrected vehicle activity x 100
- Expression fold = normalised test expression / normalised control expression

Worked Example

Illustrative immediate activity falls from 100 to 40 units (40% residual), while a separate later expression measure rises from 1.0 to 2.0 (twofold).

These findings can coexist and do not establish an in-vivo interaction magnitude.

Result and Interpretation

- State the endpoint and time basis and avoid extrapolating a clinical interaction from one assay.
- 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

Can inhibition and induction coexist?

They can affect different endpoints or time scales.

Why measure viability?

Toxicity can reduce apparent enzyme activity.

Does increased transcript guarantee increased activity?

Protein expression and function need separate assessment.

Biological-Matrix Drug Extraction with UV Analysis

Practical 29 - Analytical practical

Aim

To extract an analyte from an approved biological-matrix model and evaluate recovery and analytical performance.

Principle

Extraction separates an analyte from matrix components before UV or chromatographic measurement. Protein precipitation, liquid-liquid extraction and solid-phase extraction rely on different separation properties. Recovery and matrix effect are distinct attributes.

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

- Synthetic matrix, qualified surrogate or institution-supplied approved material
- Selected extraction procedure and analyte reference
- Blank, pre-extraction spike, post-extraction spike and neat reference
- 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. Define the analyte and matrix model. Record ethics and biosafety authorisation when real biological material is supplied by the institution.
4. Prepare matrix blanks and independent spikes at relevant levels, including pre- and post-extraction controls.
5. Perform the selected qualified extraction and record actual recovery volumes and any dilution or concentration step.
6. Measure the extracts with the specified UV or HPLC method. Compare response in the appropriate controls rather than calculating recovery from an uncorrected matrix response.
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
Matrix blank	Record	Record	Calculate
Pre-extraction spike	Record	Record	Calculate
Post-extraction spike	Record	Record	Calculate
Neat reference	Record	Record	Calculate

Calculations

• Extraction recovery (%) = response of pre-extraction spike / response of post-extraction spike x 100, for matched analyte amounts and valid response conditions
• Matrix factor = post-extraction spike response / neat reference response

Worked Example

Illustrative responses at matched amounts: pre-extraction spike 8000, post-extraction spike 10000 and neat reference 12500.
Recovery = 80.0%. Matrix factor = 10000/12500 = 0.80, suggesting suppression in this response model.
These metrics answer different questions and should not be merged into one correction without a validated approach.

Result and Interpretation

• State matrix, concentration levels, extraction method and the distinction between recovery and matrix response.
• Report replicate variability and the evidence that the method can measure the intended attribute in this matrix.

Precautions

• Use approved supplied material; no student collection is required for the model exercise.
• Prevent cross-contamination and misidentification.
• Assess analyte stability during the complete preparation.
• Use the adopted laboratory procedure, suitable controls and the designated waste arrangements.

Viva Questions and Answers

Is recovery the same as matrix effect?

No; recovery concerns extraction, while matrix effect concerns the measurement response.

Why use a matrix blank?

It reveals endogenous interference and contamination.

Why test several levels?

Recovery or interference can depend on concentration.

Biological-Matrix Drug Extraction with HPLC Analysis

Practical 30 - Analytical practical

Aim

To extract an analyte from an approved biological-matrix model and evaluate recovery and analytical performance.

Principle

Extraction separates an analyte from matrix components before UV or chromatographic measurement. Protein precipitation, liquid-liquid extraction and solid-phase extraction rely on different separation properties. Recovery and matrix effect are distinct attributes.

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

- Synthetic matrix, qualified surrogate or institution-supplied approved material
- Selected extraction procedure and analyte reference
- Blank, pre-extraction spike, post-extraction spike and neat reference
- 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. Define the analyte and matrix model. Record ethics and biosafety authorisation when real biological material is supplied by the institution.
4. Prepare matrix blanks and independent spikes at relevant levels, including pre- and post-extraction controls.
5. Perform the selected qualified extraction and record actual recovery volumes and any dilution or concentration step.
6. Measure the extracts with the specified UV or HPLC method. Compare response in the appropriate controls rather than calculating recovery from an uncorrected matrix response.
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
Matrix blank	Record	Record	Calculate
Pre-extraction spike	Record	Record	Calculate
Post-extraction spike	Record	Record	Calculate
Neat reference	Record	Record	Calculate

Calculations

• Extraction recovery (%) = response of pre-extraction spike / response of post-extraction spike x 100, for matched analyte amounts and valid response conditions
• Matrix factor = post-extraction spike response / neat reference response

Worked Example

Illustrative responses at matched amounts: pre-extraction spike 8000, post-extraction spike 10000 and neat reference 12500.
Recovery = 80.0%. Matrix factor = 10000/12500 = 0.80, suggesting suppression in this response model.
These metrics answer different questions and should not be merged into one correction without a validated approach.

Result and Interpretation

• State matrix, concentration levels, extraction method and the distinction between recovery and matrix response.
• Report replicate variability and the evidence that the method can measure the intended attribute in this matrix.

Precautions

• Use approved supplied material; no student collection is required for the model exercise.
• Prevent cross-contamination and misidentification.
• Assess analyte stability during the complete preparation.
• Use the adopted laboratory procedure, suitable controls and the designated waste arrangements.

Viva Questions and Answers

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No; recovery concerns extraction, while matrix effect concerns the measurement response.

Why use a matrix blank?

It reveals endogenous interference and contamination.

Why test several levels?

Recovery or interference can depend on concentration.

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