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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.

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