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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.

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