Drug Assay by Fluorimetry

Experiment 8 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine the assigned fluorescent drug by fluorescence calibration.

Principle

A suitable analyte fluoresces under specified excitation and emission conditions. A calibrated response is useful only in its validated concentration range.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed fluorescence calibration method and record blank-corrected fluorescence signal. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
blank-corrected fluorescence signal	_____
Calculated content and unit	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- For F = mC + b, C = (F − b)/m; include test dilution.
- Example: F = 55, b = 5 and m = 10 signal units per microgram/mL give 5 micrograms/mL before dilution correction. Record excitation/emission wavelengths and matrix.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

A suitable analyte fluoresces under specified excitation and emission conditions. A calibrated response is useful only in its validated concentration range.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

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