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Extraction of Analgesics from Biological-Matrix Models

Practical 9 - 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.

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