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Column Chromatographic Separation

Practical 4 - Practical study

Aim

To separate a model mixture by column chromatography and assess fraction identity and recovery.

Principle

Differential interactions with the stationary and mobile phases cause compounds to migrate at different rates. Loading, solvent strength and flow influence band width and separation.

Materials and Requirements

- Approved model mixture and chromatographic materials
- TLC method for fraction monitoring
- Fraction containers, balance and identity checks
- 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. Screen the mixture by TLC and select a justified stationary phase and eluent programme.
3. Prepare and equilibrate the column through the authorised procedure, avoiding cracks or dry channels.
4. Load the mixture in a narrow compatible band and collect labelled fractions at recorded volumes.
5. Compare fraction TLC patterns with references, pool only justified fractions and measure recovery and purity.
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.

Fraction / volume	Eluent composition	TLC / identity	Recovered mass
Early fractions	Record	Compare	Measure
Target fractions	Record	Compare	Measure
Late fractions	Record	Compare	Measure

Calculations

- Component recovery (%) = recovered component mass / known input component mass x 100

Worked Example

Illustrative input contains 100 mg of target component; isolated target of suitable purity is 80 mg: recovery = 80%.

Total recovered mass must be reconciled separately from component purity.

Result and Interpretation

• Present the fraction map and justify pooling using analytical evidence.

Precautions

• Avoid column drying and channel formation.
• Keep solvent and sample loading compatible.
• Do not equate one TLC zone with absolute purity.

Viva Questions and Answers

Why use a narrow loading band?

It limits initial band broadening.

Why analyse fractions?

It identifies where components elute and supports pooling decisions.

Why can stronger eluent reduce resolution?

Components may move too quickly and overlap.

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