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Isolation and Characterisation of a Natural Constituent

Practical 20 - Practical study

Aim

To isolate or evaluate a natural constituent and build a justified identity record.

Principle

Isolation, purity, chemical identity and biological activity are separate claims. Chromatographic fractionation and complementary spectra support a structural assignment; an active crude fraction can contain multiple contributors.

Materials and Requirements

- Authenticated source material and approved extraction/fractionation record
- Reference constituent and suitable chromatography
- Orthogonal spectral and purity measurements
- 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. Trace the botanical source, extraction and fractionation through a labelled material balance.
3. Compare fractions by an approved chromatographic method and select the candidate based on the stated objective.
4. Purify through the authorised procedure and measure recovered mass and analytical purity.
5. Combine formula, UV/IR, proton and carbon NMR and mass evidence with a suitable reference; record unresolved isomeric or stereochemical ambiguity.
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 / constituent	Mass / recovery	Chromatographic evidence	Spectral assignment
Crude extract	Record	Fingerprint	Assess
Selected fraction	Record	Purity profile	Assign
Purified candidate	Record	Orthogonal check	Conclude

Calculations

- Isolation yield (%) = isolated constituent mass / stated dry-source mass x 100

Worked Example

Illustrative 50 mg candidate isolated from 10.0 g dry material gives 0.50% yield.

If analytical purity is 90%, this is not automatically 50 mg of pure constituent; specify the reporting basis.

Result and Interpretation

• Report the isolated fraction, yield, purity basis and strength of identity evidence; assess activity separately.

Precautions

• Use authenticated and approved materials.
• Keep fractions and solvents traceable.
• Do not administer isolated teaching constituents.

Viva Questions and Answers

Does a pure-looking crystal prove identity?

No; chemical characterisation is needed.

Why use carbon NMR?

It adds carbon-environment constraints to the structure.

Does an active fraction identify the active molecule?

Contributors need separate confirmation.

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