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Analysis by Gas Chromatography

Practical 4 - Bench practical

Aim

To quantify a suitable volatile analyte using gas chromatography and evaluate separation and detector response.

Principle

A vaporised preparation is carried through a temperature-controlled column by a gas stream. Compounds separate according to volatility and interactions with the stationary phase.

Quantification may use an external standard or an internal-standard area ratio. The analyte must be sufficiently volatile and stable for the selected method; derivatisation, where needed, is a separate validated operation.

Materials and Requirements

- Gas chromatograph with the selected detector and column
- Qualified carrier-gas supply and gas-specific leak-check equipment
- Reference analyte and internal standard where specified
- Suitable diluent, crimped vials and calibrated glassware

Procedure

1. Record detector, column, gas identities, flow or pressure mode, inlet mode, temperature program and acquisition settings from the selected method.
2. Confirm instrument readiness and gas-supply checks with the trained operator. Inspect blanks before introducing test material.
3. Prepare standards and test solutions in closed containers, controlling evaporation and using the same internal-standard concentration where applicable.
4. Establish calibration using the intended response or area ratio and verify a separate check preparation.
5. Inject the analytical sequence with blanks and suitable reference checks. Assess retention repeatability, separation and carryover.
6. Review chromatograms for interference and integration consistency. Retain the temperature program and raw data with the report.
7. Calculate the measured concentration, apply dilutions and assess suitability using the method-specific limits.

Observations and Record

Enter actual measurements or the supplied teaching data. Record the method, material identity, conditions and units.

Preparation	Analyte area	Internal-standard area	Ratio / concentration
Blank	Record	Record	Check interference
Calibrators	Record	Record	Fit relationship
Independent check	Record	Record	Recovery
Test solutions	Record	Record	Final result

Calculations

- Response ratio R = analyte area / internal-standard area

- If R = mC + b, then C = (R - b) / m
- Original concentration = measured concentration x documented dilution factor

Worked Example

Illustrative calibration: R = 2.50C with C in mg/mL. A test ratio of 1.20 gives C = 1.20/2.50 = 0.480 mg/mL. With a 20-fold dilution, the original concentration is 9.60 mg/mL.
A consistent internal-standard amount and acceptable recovery are assumed; the numerical example does not specify a universal GC program.

Result and Interpretation

- Report concentration with the calibration model, dilution and sequence checks.
- Consider evaporative losses and inlet discrimination when recoveries differ between preparations.
- Interpret identity with the specificity available from the detector and method.

Precautions

- Observe instrument-specific precautions for hot inlets, detectors and pressurised gases.
- Hydrogen-containing systems require the prescribed leak checks and shutdown procedure.
- Keep volatile preparations tightly closed.
- Use the appropriate gas regulator and approved exhaust arrangements.

Viva Questions and Answers

Why use an internal standard?

A suitable standard can compensate for some preparation and injection variability.

Can every drug be analysed directly by GC?

No; volatility and thermal stability may limit direct analysis.

Why include blanks after concentrated injections?

They help assess carryover and contamination.

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