Separation of Plant Pigments by Column Chromatography

Practical 10 | Instrumental Methods of Analysis and Novel Drug Delivery Systems

Aim

To study Separation of Plant Pigments by Column Chromatography, and interpret the recorded observations.

Principle

- Pigment bands separate because of differences in interaction with the packed stationary phase and mobile phase. A visible band colour is useful for tracking but is not complete identity evidence.
- Compounds separate because they interact differently with the stationary and mobile phases.
- Identity assessment uses an appropriate comparison standard and method conditions; one migration or retention value alone is not conclusive.
- Observe the packed bed and continuous solvent path so channel formation can be distinguished from normal band movement.
- Keep fraction labels linked to collection sequence; compare fractions using the assigned independent identity test.

Materials and Apparatus

- Prepared pigment mixture, packed teaching column, assigned mobile phase and collection vessels.
- Assigned test extract or mixture, comparison standards and the specified stationary/mobile phase system.
- Appropriate chromatographic apparatus, application equipment, developing chamber or instrument and the assigned detector.

Method

1. Record the stationary phase, mobile phase and the purpose of the separation.
2. Prepare test and comparison solutions at suitable concentrations for the assigned method.
3. Introduce measured test and comparison portions without overloading the separation system.
4. Develop or run the separation under the assigned conditions, recording the relevant operating details.
5. Locate separated bands, spots or peaks using the specified observation method.
6. Compare the test pattern with the standard and calculate the appropriate separation metric.

Observation Record

Complete this record using actual laboratory findings or the provided teaching data.

Observation / parameter	Record / finding
Fraction label	_____
Band colour	_____
Elution order	_____
Collected amount	_____
Comparison evidence	_____

Calculations and Interpretation

- Record fraction order, volume and the assigned identity observations.
- For any recovery calculation, use a defined starting amount and verified recovered amount.

Result

Record the actual finding, calculated value with units where relevant, and a conclusion supported by the observations.

Finding: _____

Conclusion: _____

Precautions

- Keep application concentrated and avoid overloading.
- Record the solvent front immediately for planar chromatography.
- Use retention or migration data only with the stated system and relevant identity evidence.

Viva Questions

1. Why does overloading impair separation?

It broadens bands or spots and can change apparent migration or retention.

2. Why run a comparison standard?

It supplies a relevant reference pattern under the same analytical conditions.

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