

Amino Acids by Paper Chromatography

Experiment 1 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To separate and compare the assigned compounds by amino acids by paper chromatography.

Principle

Differential distribution in the paper/mobile-phase system produces different migration of the assigned amino acids.

Requirements

- Assigned chromatography paper and development chamber.
- Assigned standards, coded mixture, prescribed mobile phase, application tools and visualisation method.

Procedure

1. Plan the separation

Record the analytes, stationary phase, mobile-phase composition and prescribed visualisation method.

2. Apply the material

Mark an origin in pencil and apply small compact standard and mixture spots; allow each application to dry as assigned.

3. Develop

Place the paper in the prepared prescribed solvent system with the origin above the liquid, and allow the assigned development.

4. Visualise and measure

Mark the front, dry and apply the prescribed amino-acid visualisation method under supervision; record spot positions and colours.

5. Compare

Compare mixture spots with standards on the same run. Record number of spots, migration and limitations; matching migration alone is not absolute proof of identity.

Pharmaceutical Analysis

Observations and Record

Lane / spot	Distance from origin	Rf / appearance
Standard 1	_____	_____
Standard 2	_____	_____
Assigned mixture	_____	_____
Solvent front	_____	Not applicable

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- $R_f = \text{distance travelled by the spot centre} / \text{distance travelled by the solvent front}$, both measured from the same origin.
- Example: $4.0 \text{ cm} / 8.0 \text{ cm} = 0.50$. Compare only under the same separation conditions.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Keep the origin above the solvent level and mark the front promptly.
- Use small compact applications and the authorised ventilation/visualisation precautions.

Viva Questions and Answers

1. Why compare standards on the same run?

Migration depends on the separation conditions.

2. What can a second spot indicate?

A mixture, impurity or other separated component; interpretation needs the method context.

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