Sulphonamides by Thin-layer Chromatography

Experiment 2 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To separate and compare the assigned compounds by sulphonamides by thin-layer chromatography.

Principle

Adsorption and distribution between the stationary layer and mobile phase separate the assigned sulphonamides.

Requirements

- Assigned TLC plate and 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

Use a pencil origin and apply compact standard and test spots without damaging the layer.

3. Develop

Develop in the assigned mobile phase with the application points above the solvent; protect the plate from uneven wetting.

4. Visualise and measure

Mark the front, dry and use the assigned UV or chemical detection method; record each spot and migration.

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.

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.

- Rf = distance travelled by the spot centre / distance travelled by the solvent front, both measured from the same origin.
- Example: 4.0 cm / 8.0 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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