TLC:Separation of Amino Acids Cheat Sheet

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Aim

To separate and identify amino acids in a mixture using thin layer chromatography (TLC).

Introduction

Chromatography

Chromatography is a technique used for separating compounds in a mixture based on differences in their distribution between a stationary phase (like silica) and a mobile phase (solvent mixture).

Thin Layer Chromatography (TLC)

TLC separates compounds based on

- ✔ solubility,
- ✔ interaction with the stationary phase, and
- ✔ molecular size.

It's useful for qualitative and quantitative analysis.

Chromatographic Separation of Amino Acids

Amino acids, having different R groups, interact variably with silica, affecting their movement on a TLC plate. Ninhydrin is used to visualize amino acids, forming purple spots on reaction.

Materials Required

Plant Material

- ☐ Green Mung Beans (Vigna radiata) extract.

Reagents

{fa-square-o}} Standard solutions of individual amino acids (Known Concentrations).

- ☐ Solvent mixture (butanol:acetic acid:water in 12:3:5 ratio).
- ☐ Ninhydrin reagent (2% in acetone).
- ☐ Silica gel for TLC

Equipments

- ☐ Glass Plates for TLC / Readymade TLC plate.
- ☐ TLC chamber.
- ☐ Capillary tubes.
- ☐ Reagent spray bottle.
- ☐ Spreader for silica gel.
- ☐ Conical flasks,
- ☐ beakers.
- ☐ Conical flasks
- ☐ Measuring Cylinder
- ☐ Weighing Balance
- ☐ Oven
- ☐ Ruler

Procedure

1. TLC Plate Preparation:

- ✔ Clean the glass plates thoroughly.
- ✔ Prepare a slurry of silica gel with water (1:2) in a beaker.
- ✔ Pour the silica gel slurry onto the glass plate and spread evenly using a spreader.
- ✔ Allow the silica layer to dry and then activate it by heating in an oven at 110°C for 1 hour.

2. Solvent Preparation: Pour solvent mixture into TLC chamber and let it saturate for 30 minutes.

3. Sample Application: Use capillary tubes to spot amino acid solutions onto the baseline (approximately 2 cm from the bottom of the plate). Allow to dry.

4. Development: Place the plate in the TLC chamber ensuring the baseline is above the solvent. Let the solvent ascend to about 1 cm from the top.

5. Drying: Remove the plate and mark the solvent front with a pencil. Dry the plate under a hood.

6. Detection: Spray the dry plate with ninhydrin and dry in an oven at 105°C for 5 minutes.

7. Analysis: Measure the distances moved by the solute and solvent, and calculate Rf values.

Rf Value Calculation:

Rf = Distance moved by solute / Distance moved by solvent X 100

Observation Table

**S.N.	**Std AA/ Sample	Distance Travelled By Compound (cm)	Distance Travelled by Solvent (cm)	Rf value
1	Std AA 1	----	----	----
2	Std AA 2	----	----	----
3	Sample Band 1	----	----	----
4	Sample Band 2	----	----	----
5	Sample Band 3	----	----	----

Std AA -Standard Amino acid

Sample Band 1- Separated Amino acid in Plant sample numbered accordingly as Band 1, Band 2 etc

Results

Calculate and record the Rf values for each amino acid to identify them in the mixture.

Perform the experiment and fill in the Rf values and the identified amino acids.

Figure TLC Chromatogram of Amino Acids

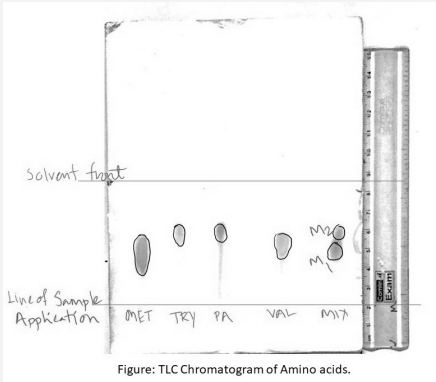

Figure: TLC Chromatogram of Amino acids.

MET- Methionine; TRY-Tyrosine; PA- Phenyl alanine, VAL-Valine
MIX- Mixure (Sample)
M1-Mixture Band 1
M2- Mixure Band 2

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