

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 R_f values.

R_f Value Calculation:

$R_f = \text{Distance moved by solute} / \text{Distance moved by solvent} \times 100$

Observation Table

**S.N.	**Std AA/ Sample	Distance Travelled By Compound (cm)	Distance Travelled by Solvent (cm)	R _f 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 R_f values for each amino acid to identify them in the mixture.

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

Figure TLC Chromatogram of Amino Acids

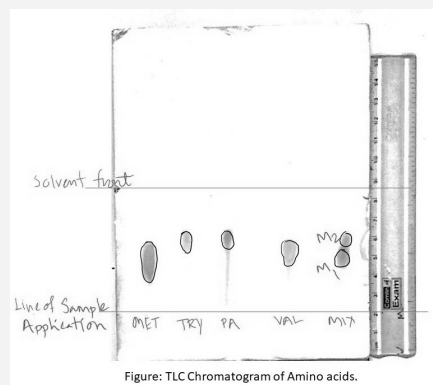


Figure: TLC Chromatogram of Amino acids.

MET- Methionine; TRY-Tyrosine; PA- Phenyl alanine, VAL-Valine

MIX- Mixure (Sample)

M1-Mixture Band 1

M2- Mixure Band 2



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