

### 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<sub>f</sub> values.

R<sub>f</sub> 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 <sub>f</sub> 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<sub>f</sub> values for each amino acid to identify them in the mixture.

Perform the experiment and fill in the R<sub>f</sub> values and the identified amino acids.

### Figure TLC Chromatogram of Amino Acids

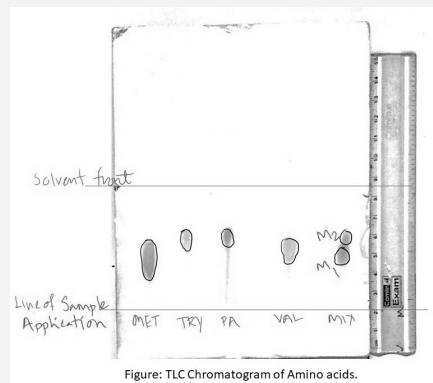


Figure: TLC Chromatogram of Amino acids.

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

MIX- Mixure (Sample)

M1-Mixture Band 1

M2- Mixure Band 2



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Page 1 of 2.

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