

## **Practical Work n°4: Qualitative analysis of analgesic drugs by Thin Layer Chromatography (TLC)**

### 1. OBJECTIVES

- Identify the active ingredients contained in pain medications.
- Compare the unknown substances with reference substances.
- Determine the values of the retention factor ( $R_f$ ).
- Understanding the principle of chromatographic separation.

### 2. THEORETICAL PRINCIPLE

Thin Layer Chromatography (T L C) is a qualitative analysis method used to separate the components of a mixture.

TLC allows for rapid, simple and economical identification of the active ingredients of analgesic drugs by comparing  $R_f$  values with those of reference substances.

It is based on:

- A stationary phase: plate covered with silica gel ( $SiO_2$ ) .
- A mobile phase: Eluent (mixture of solvents).

**The constituents migrate differently depending on:**

- Their polarity
- Their affinity for the stationary phase
- Their solubility in the mobile phase

The retention factor ( $R_f$ ) is defined by:

$$R_f = \text{Distance traveled by the substance} / \text{Distance traveled by the solvent front}$$

### 3. MATERIALS AND REAGENTS

#### Materials

- Silica TLC plates
- Chromatographic tank with lid
- Capillaries or micropipettes
- Pencil, Ruler, Pliers
- UV lamp (254 nm), Beaker
- Hot plate

#### Reagents

- Drug sample analgesic (tablet)
- Reference substances:
  - Paracetamol, Doliprane and – Aspirin
- Extraction solvent: ethanol or methanol
- Eluent (example): **mixture 1** : ethyl acetate/ acetic acid/water (**8ml :1ml :1ml**)
- mixture2** : chloroform and methanol in the proportions **60% - 40%** .

**mixture 3:** Ethyl acetate / Methanol / Acetic acid (**8ml : 1ml : 1ml**) or Ethyl acetate / Acetic acid (**9ml : 1ml**)

#### 4. OPERATING CONDITIONS

- Ambient temperature
- Tank saturated with Eluent vapors
- Migration height: approximately 8 cm
- Migration time: 10–20 minutes

#### 5. SAMPLE PREPARATION

a) Preparation of the solution of the unknown sample drug

1. Finely crush one tablet.
2. Dissolve a small amount in 10 mL of ethanol.
3. Filter to remove insoluble excipients.
4. Keep the filtered solution.

b) Preparation of reference solutions

Dissolve a small amount of each pure substance in ethanol.

#### 6. EXPERIMENTAL PROTOCOL

**Step 1:** Preparing the plate

- Draw a deposit line with a pencil 1 cm from the bottom.
- Mark the drop points (references + sample).

**Step 2:** Sample Submission

- Using a capillary tube, apply:
  - Paracetamol (reference)
  - Aspirin (reference)
  - Doliprane (reference)
  - Unknown sample
- Let it dry.

**Step 3:** Development

- Pour the Eluent into the tank (0.5 cm high).
- Insert the plate without immersing the deposits.
- Close the tank.
- Allow migration up to 8 cm.

**Step 4:** Revelation

- Remove the plate.

- Immediately mark the solvent front.
- Observe under UV lamp or by diiodine vapor.
- Circle the visible spots.

## 7. RESULTS

Fill in the table

Substance	Spot distance (cm)	Front distance (cm)	$R_f$
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Paracetamol			
Aspirin			
doliprane			
Sample			

## 8. ANALYSIS OF RESULTS

- Compare the  $R_f$  values.
- If  $R_f$  sample =  $R_f$  reference  $\rightarrow$  probable presence of the substance.
- If several spots  $\rightarrow$  medication composed of several active ingredients.

## 9. INTERPRETATION

- A single task  $\rightarrow$  simple medication.
- Multiple tasks  $\rightarrow$  combination of analgesics.
- Difference in  $R_f$   $\rightarrow$  different substance.

## 10. QUESTIONS

1. Why do we use a pencil and not a pen?
2. What is the role of silica?
3. Why is it necessary to saturate the tank?
4. How does polarity influence migration?
5. Why do we observe under UV light?
6. Conclusion