TLC TEST PROTOCOL
(Thin Layer Chromatography)
What’s TLC and what’s it used for?

Thin Layer Chromatography is a technique used to separate the pure components present in a mixture. This separation is possible due to the difference on the adhesion force of the molecules that are present in the mixture to a mobile phase (normally a solvent) and to a stationary phase (called thin layer, silica gel). This difference translates into more or less movement of each individual component, which allows its separation and identification.

Materials needed

Acetone
Agitator or vortex
25% ammonia solution (NH3)
Sodium bicarbonate
Pirex jars or similars: 1 l, 0’5 l, 0’25 l.
TLC plate (silica gel in an aluminium support)
2 big and 1 small glass containers used for TLC
Funnel
1,5ml eppendorfs
Tweezers, spatulas, scissors, pencils and rulers.
Protection eye glasses
Support for eppendorfs
Latex gloves
Ultraviolet lamp, of 24 nm, with protection screen
Marquis reactive
Methanol (MeOH).
Capillary micropipets
Blotting paper
Substance patterns, dissolved in methanol
p-DMAB reactive
1ml pipet
Pasteur pipettes
50ml test tubes
5 ml vials
Small glass dishes (watch glass)
Step 1: Preparing the glass containers

1.1 - Cut blotting paper:

- 15 x 15 cm (big container)
- 5 x 15 cm (small container)

1.2 - Preparing the solvent:

- *Amphetamines and derivatives system (MeOH: NH3)* for the big container. Measure 29.25 ml in the test tube. Add 0.75 ml of NH3. Mix it with the test tube well closed.
- *Cocaine system (ACETONE 100%)* for the other big container. Measure 30 ml.
- *Ketamine system (MeOH)* for the small container. Measure 20 ml.

1.3 - Add the solvent into the container, and make it soak the blotting paper. The paper should be lying against the glass wall. Cover the container.

1.4 - Leave it for 30 minutes, so the solvent vapours can saturate the glass container.
Step 2: Preparing the samples

2.1 - Place 5mg of the sample in an eppendorf.
(Use an small plastic 5mg spatula completely full)

**NOTE:** 5 mg is the double or triple of what is needed for Marquis colorimetric reaction.

* Cocaine is an exception in which is better to fill the eppendorf with half of the quantity that’s used for Marquis. With LSD blotters or other substances use ¼ of the blotter. In case of LSD microdots, use ¼ of the pulverized microdot.

2.2 - Add 0.5 ml of methanol in the eppendorf with the sample.

2.3 - Shake the sample energetically (manually or with an electric shaker).
Method III

Step 3: Preparing the TLC plate

3.1 - Cut the thin layer plate:

\[(\text{nº of punctures} = \text{nº of samples} + \text{nº of patterns}) + 1\text{cm} \times 10\text{cm} \text{height}.\]

3.2 - Draw with a pencil a parallel line at 1.5 cms above the lower border. Divide it with separate marks of 1 cm of distance between them and separate 1 cm of both sides of the plate.

3.3 - Draw another line of 0.5 cms in the upper border scratching the silica with the spatula tip until the aluminium is seen.

3.4 - Write with a pencil the initials of the sample patterns below of each mark.

Maximum lengths of the TLC plate:

- Big container: 16 cm length x 10 cm height
- Small container: 6 cm length x 10 cm height

TLC plate dimensions and distance between marks.

Sample of cocaine TLC plate with the initials of the substance and its usual adulterants: Fe (phenacetin), Pa (paracetamol), Co (cocaína), Pr (procaína), Tr (tetracaine), Be (benzocaine), Li (lidocaine), Caf (caffeine).
Method IV

Step 4: Spotting the samples

4.1 - Drop 3 µl of the sample on the TLC plate using a capillary micropipet.

4.2 - Drop the needed µl of the pattern or patterns with different capillary micropipets.

4.3 - Let the solvent evaporate.

4.4 - Look at the stains that the samples and patterns have left in the TLC plate using the ultra violet developing camera. If the procedure has been done correctly and the filling has been enough, dark stains must be seen where puncture has been done. If they can not be seen, do the puncture again until the stains are visible.
**Method V**

**Step 5: Elution**

5.1 - **Introduce the TLC plate, using the tweezers, into the glass container,** and let the top of it lean against the dryer paper while the bottom is fixed in the small glass lump of the container.

5.2 - **Seal the glass container with its lid.**

5.3 - **Leave the TLC plate inside the container with the elution** until the solvent has gone up until reaching the upper line (previously drawn with pencil onto the TLC plate: See Step 3).

5.4 - **Take the TLC plate out of the container** and **let it dry** in a ventilated place.
**Step 6: Analyzing the stains/spots**

6.1 - Introduce the **dry TLC plate** into the **ultra violet developing camera**.

6.2 - Compare the **sample** stains with the **pattern** ones.

6.3 - Circle with a pencil the **top of the stains**.
Method VII

Step 7: Colour testing revelation

7.1 - Add onto the stains a drop of Marquis Test. After a few seconds the sample should get colored depending on the different substances. This process is useful for MDMA and other amphetamines.

7.2 - Add onto the developed stain a drop of p-DMBA Test. After a few seconds the sample must get colored depending on the different substances. This test is useful for indolamines (LSD, magic mushrooms, foxy, 4-ac-dipt, etc) and for some phenetilamines (2CT2, 2CB, 2CI, DOB, DOM, etc).