

**Name of Procedure:**

Thin-Layer Chromatography  
4:1 Developing Solvent

**Suggested Uses:**

The 4:1 Thin-Layer Chromatography Developing Solvent is used to separate and identify organic compounds. Suggested uses of this TLC solvent are cannabis alkaloids.

**Apparatus Needed to Perform Procedure Including Preparation of Reagent:**

Thin-Layer Chromatography Plates  
    Silica gel GF - fluorescent indicator  
    Thickness: 250 microns  
Developing tank  
Micro pipets  
UV light (long and short wave)  
Fume hood  
Gloves  
Eye protection  
Laboratory coat  
Graduated cylinders  
Reagent bottle  
Funnel  
Porcelain spot plate  
Spatula  
Ethyl ether  
Hexane

**Formula for Preparing Reagent:**

1. Measure out 40 milliliters of hexane and 10 milliliters of ethyl ether.
2. Pour measured solvents into reagent bottle and mix well.
3. Properly label reagent bottle.

**Expiration Date of Chemical Reagent:**

The solvent can be used until depletion provided it is stored in an airtight reagent bottle in a cool place.

**Application of Procedure on Evidence:**

1. A sample of the unknown (approximately 1-2 milligrams) is placed in the well of a porcelain spot plate and several drops of a suitable solvent are added to dissolve sample.
2. A known standard (approximately 1-2 milligrams) is also dissolved in the well of a spot plate with several drops of a suitable solvent. (Known standard is selected after visual observation and/or a series of preliminary tests).
3. With a capillary pipet, several microliters of the unknown and standards solutions are placed side by side approximately 5 millimeters from the bottom of the TLC plate.
4. Add 4:1 solvent to the developing tank to a depth of approximately 2 millimeters and allow several minutes for atmosphere to equilibrate.
5. Allow all the spotting solvent to evaporate from the TLC plate.
6. Place the TLC plate in the developing tank and close the lid.
7. Allow the TLC plate to develop to the top of the plate.
8. Remove the TLC plate from the developing tank and allow the solvent to dry.
9. Visualize with UV light or an appropriate visualizing reagent.
10. Record results.

**Safety Concerns:**

Always wear eye protection, gloves and a laboratory coat when preparing this TLC solvent.

**Literature References:**

Randerath, Kurt, **Thin-Layer Chromatography**, New York, Academic Press, 1968

Moffat, A.C., **Clarke's Isolation and Identification of Drugs**, 2nd Ed., The Pharmaceutical Press, 1986.

Parker, James M. and Fiske, Harold L., "Thin Layer Chromatography of Marihuana," **Journal of Forensic Sciences**, Vol. 24, No. 4, 1979.