SOP: SP032

Preparative Thin-Layer Chromatography

Materials and Reagents:

1. Thin layer chromatography plates (note 1)
2. Organic solvents (note 2)
3. TLC tank (note 3)
4. Scissors or paper cutter
5. Foil
6. Ruler
7. Pencil
8. Glass Pasteur pipet
9. Pasteur pipet bulb
10. Plastic wrap
11. Lead weight
12. Chemical fume hood
13. Metal spatula
14. TLC sprayer, 250 ml (Kontes 422530-0250)
15. TLC developer (note 4)
16. Heat gun, 200-300^{o}F
17. Scanner
18. 16 x 100 mm glass tubes with Teflon-line caps
19. Vortex
20. Benchtop centrifuge
21. N_2 bath

Protocol:

1. _____ Using scissors or paper cutter, cut TLC plate to appropriate size and place on foil (note 5).

2. _____ Using a ruler and pencil, draw a line 1 cm above and parallel to TLC plate bottom (note 6).

3. _____ Using a glass Pasteur pipet and pipet bulb, add compound(s) to TLC plate (note 7).

4. _____ Let all compounds applied to TLC plate dry completely.

5. _____ Thoroughly wash TLC tank (note 8).

6. _____ Make fresh TLC solvent and add to tank (note 9).

7. _____ Cover tank opening with plastic wrap, followed by tank lid, and place lead weigh on top. Allow the tank to equilibrate for 5-10 minutes (note 10).

8. _____ Place plate(s) in equilibrated TLC tank.

9. _____ Let plate(s) sit in tank until solvent front reaches top of plate.

10. _____ Remove plate and let dry completely on foil in chemical fume hood.

11. _____ Place plate in TLC spraying area (note 11).

12. _____ Thoroughly spray TLC plate with desired developer in TLC sprayer attached to compressed air line in chemical fume hood (note 12).

13. _____ If necessary, heat TLC plate with heat gun to complete plate development.

14. _____ Immediately scan developed TLC plate to preserve results (note 13).

15. _____ Using a metal spatula, scrape desired silica from TLC plate and transfer to a clean 16 x 100 mm glass tube.

16. _____ Add 8 to 10 ml of $CHCl_3$-CH_3OH (2:1) to each tube and vortex vigorously.

17. _____ Centrifuge at 3000 RPM, room temperature for 15 minutes.

18. _____ Using a glass Pasteur pipet, transfer organic supernatant to a clean 16 x 100 mm glass tube and dry under a stream of N_2.

19. _____ Repeat steps 16 to 18 twice more.

20. _____ Re-suspend extracted compound in a minimal amount of $CHCl_3$-CH_3OH (2:1) and analyze by TLC (note 14).

Notes:

1. Depending on the type of preparative TLC needed, there are two types of TLC plates to use. For small preparative TLC, use plastic-backed EM Science F254 plates (EM Science 5735/7). For large preparative TLC, use glass-backed EM Science F254 plates (EM Science 13792/7).
2. Fresh HPLC-grade chemicals, preferably Burdick & Jackson, make the best TLC solvents systems. Commons systems are $CHCl_3$-CH_3OH-H_2O (60:30:6) for PIM preparation/analysis and $CHCl_3$:CH_3OH:NH_4OH (80:20:2) for TDM preparation/analysis. Fresh solvents are also important for solubilization of lipids for TLC analysis.
3. For small TLC (5 x 10 cm or 10 x 10 cm) use a small Kontes tank (Kontes 416180-1020). For large TLC (20 x 10 cm or 20 x 20 cm) use a large Kontes tank (Kontes 416180-0000).
4. There are numerous detection sprays, based on what molecule is to be detected. See SOP for charring spray, α-napthol and Dittmer-Lester for details. Additionally, F_{254} plates may be viewed under low and high wave ultraviolet light, and all plates may be placed in a tank with iodine crystals, which will temporarily stain some compounds.
5. For small preparative TLC analysis, cut plastic-backed plates to 10 x 10 cm or use in original 20 x 20 cm size. Glass-backed TLC plates are to be use in their original 20 x 20 cm size.
6. It is best to apply compound as one thin band, and no closer than one centimeter to plate edges (to prevent "smiling" of compound). Glass-backed TLC plates have a cellulose loading zone for application of compounds, which acts much like the stacking gel on an acylamide gel.
7. Ideally, compound should be suspended in organic solvent at a high concentration. Approximately 10 mg may be applied to a 10 x 10 cm plastic TLC plate, 20-30 mg to a 20 x 20 cm plastic TLC plate, and 25-200 mg to a glass-backed TLC plate.
8. Wash tank using mild detergent soap. Rinse each side three times with hot water, three times with deionized water, once with HPLC-grade CH_3OH and dry under compressed air. Then, rinse each surface with HPLC-grade acetone and dry under compressed air.
9. Make approximately 10 ml for small TLC tanks and 100 ml for large TLC tanks.
10. Tank should be sealed as best as possible to minimize leakage of solvent fumes. Preferably, place tank in area with low air flow.
11. Place a partially open box in a chemical fume hood and cover with foil. This will provide a place to set the TLC plate while spraying, and protect the inside of the hood from oxidizing chemicals.
12. If separated compounds cannot be visualized by iodine or ultraviolet light, a small amount of developer may be sprayed on plate sides. Use a metal spatula to remove silica from the TLC plate to provide a barrier between the developed section and the preparative section, as any chemical modification to the desired compound will render it useless. Additionally, a glass plate may be applied to the silica-free channel, perpendicular to the plate, to prevent spray from drifting to the preparative section of the plate.
13. All TLC plate developers will fade with time; Dittmer-Lester may disappear within minutes, so it is important to document TLC results as soon as possible.
14. See SOP SP033.

References:

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Slayden, RA and Barry 3rd, CE (2001). Analysis of the Lipids of Mycobacterium tuberculosis. Mycobacterium tuberculosis Protocols (Parish T and Stoker, NG ed), Humana Press Inc, Towata NJ, pp 229-246.

Belisle, JT, Vissa VD, Sievert T, Takayama K, Brennan PJ, and Besra GS. Role of the Major Antigen of Mycobacterium tuberculosis in Cell Wall Biogenesis. Science (276): pp 1420-1422.

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