Thin Layer Chromatography of Brain Gangliosides

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Materials & Reagents:

- Distilled methanol
- Distilled chloroform
- 0.25% aqueous KCl
- TLC plates (EMD Milllipore 105635)
- Glass cover plates (can be prepared from used TLC plates)
- 10 µl spotting syringe (Hamilton 80366)
- Glass TLC developing chamber (e.g. Camag 022.5155)
- Resorcinol Reagent: For 100 ml add 64.7 ml water, 5 ml 6% aqueous resorcinol, 0.31 ml 1% $CuSO_4 \cdot 5H_2O$, and 30 ml concentrated HCl. Store refrigerated and replace monthly.
- Reagent Sprayer (e.g. Kontes K422530-0125)
- Compressed inert gas (e.g. dry nitrogen)
- 125°C oven

Method:

1. Place a TLC plate in the 125°C oven for 10 min.
2. Prepare running solvent, e.g. chloroform-methanol-0.25% aqueous KCl (60:35:8).
3. Place running buffer ~0.5-mm deep in the bottom of the developing chamber. Place the chamber on the benchtop, away from sources of heat and protected from drafts.
4. Remove TLC plate and mark loading 5-mm lanes 1 cm above the bottom and no closer than 0.75 cm from each vertical edge using a soft pencil. Avoid “wounding” the surface.
5. Apply 1 µl (or as desired) of standards and samples on the loading lanes using a Hamilton syringe. Apply in as thin a line as readily feasible, and avoid “wounding” the surface.
6. Allow all lanes to dry, using a blower (no heat) as needed.
7. Place the TLC plate in the developing chamber and allow to develop until the solvent front reached ~1 cm from the top.
8. Remove the TLC plate and allow to dry using mild heat (e.g. top of oven).
9. Inside of a fume hood spray plate evenly with Resorcinol Reagent in two directions. Wear latex gloves to avoid exposure to the acid spray, and keep the hood sash low. Use a strong spray, but do not leave the surface visibly wet.
10. Cover with clean glass cover plate (free of lint, no Kimwipes!) and clip in place.
11. Heat at 125°C for 20 min.
12. Remove and allow to cool. The plate may be stored by removing the clips and taping the edges.
13. Sialoglycans (gangliosides) appear blue-purple against a white background.

NOTES:

- Preheating the TLC plate prior to sample spotting ensures that atmospheric water adsorbed to the silica surface, which alters migration, is removed.
- Running solvent can be stored in a well-sealed glass bottle with a Teflon-lined cap indefinitely. Small volumes in large bottles, however, are susceptible to differential solvent evaporation during use and should be avoided.
- Once placed in the developing chamber, the running solvent should be used the same day, and then discarded. Evaporation, storage without a thorough seal, or repeated use for TLC development changes the proportion of solvents and diminishes resolution.
- The detection limit for ganglioside sialic acid using resorcinol reagent is ~25 pmol. Quantitative analysis can be performed in the range of ~25-250 pmol sialic acid, keeping in mind that staining is proportional to both the ganglioside concentration and the number of sialic acids per ganglioside.
- Non-ganglioside lipids appear yellow or brown against a white background.
- Bands can be quantified by scanning and using image analysis (e.g. ImageJ) to provide pixel area x intensity.