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% $CuSO4 \cdot 5H2O$, 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.

TLC Read-out: Resorcinol Spray Reagent

64.7 ml of water

5 ml of 6% resorcinol in water (should be clear to beige…….. not brown. If you make the 6% new, the resorcinol is on the dry chemical shelf and it should be a white powder)

0.31 ml of 1% $CuSO_4 \cdot 5H_2O$

30 ml of concentrated HCl

NOTE: Make fresh every 2 weeks

Let the plate cool before spraying. Make sure to put the lane origins (where you drew the lines in pencil) at the top of the box, so the front of the plate is resting on the bottom of the box. This insures a good spray where your sample will be.

When you turn on the gas the PSI should be about 25 with a good “whooshing” sound to get an even spray. You can test it before putting the plate in the box – there should be a visible mist when you spray.

Spray plate in two directions (NOT until wet!) This makes it uniformly covered.

Cover with glass plate and clip together – the cover plate should be the same size as the TLC plate.

Heat at 125°C for 20 min

Spray pattern: Make “turns” off the plate so as not to have a buildup!