Glycosphingolipid enrichment by mild alkaline hydrolysis

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Reagents:
-KOH pellet
-Methanol
-HPLC grade Water
-Acetic acid

Equipment/Apparatuses:
- Sonicator
- Vortex mixer
- Incubator or heating block
- Ice bucket
- Glass pipette

Procedures:

The following protocol describes a method for elimination of glycerolipids by mild alkaline hydrolysis.
After allowing the mild alkaline hydrolysis reaction to proceed for a minimum 6hr or up to 18hr, the desalting process must be performed in a same day.

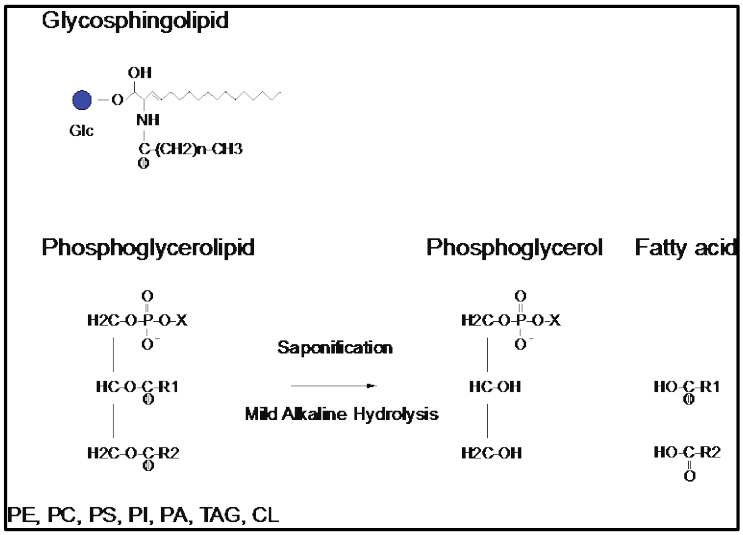

Figure 1. Saponification of glycero-type lipids by mild alkaline hydrolysis

1. Prepare a solution of 0.5M potassium hydroxide (KOH) using KOH pellets (Fisher Scientific). Dissolve 560mg of KOH in 20 ml of aqueous 95% MeOH to make 0.5 M KOH aq-MeOH solution. Briefly, dissolve the KOH pellet in 1ml of water and then add 19 ml of MeOH.

2. Add 500 µl of 0.5M KOH aq-MeOH solution to the dried lipid material and sonicate to dissolve the lipids. Apply 0.5ul of the solution onto a pH test strip to make sure the pH is in strong basic range.

3. Incubate at 37 °C for minimum 6hr or up to 18 hr to destroy glycerolipids. The reaction can be performed in a well-equilibrated incubator/oven or in a heating block. The glass tube must be equipped with a screw top cap with PTFE liner.

4. Stop the reaction by removing the sample tube from the incubator or heating block and placing it on ice.

5. While maintaining the sample tube on ice to prevent excessive heat formation, slowly add 0.5ml of 5% acetic acid (AcOH) dropwise to neutralize. Gently mix the sample tube between additions of 5% AcOH.

6. Apply 0.5ul of the solution onto a pH test strip to confirm the solution is in mild acidic range. If the pH is still in basic range, add additional drops of 5% AcOH.

Note:
The solution after go through the above-steps contains high concentration of salts (Potassium acetate) and it should be desalted prior to the analysis – See the protocol "Desalting by tC18 Sep-Pak chromatography".