
Acid hydrolysis of organic materials

Introduction

Many polymeric materials can be decomposed into smaller subunits that are suitable for GC/MS analysis by acidic or basic hydrolysis. Ester and amide bonds are particularly susceptible, and some ether-linked lipids can be saponified in acidic solution. Basic hydrolysis (saponification) is more commonly used for ester-linked polar lipids. The products of ester hydrolysis will be alkanols plus either free alkanolic acids (at low pH) or alkanoates (at moderate to high pH) that need to be derivatized before analysis. This procedure is most commonly applied to total lipid extracts, but can also be applied to solid materials such as sediments, biomass, or extracted residues.

Materials

- 0.5N HCl in H₂O (**note A**)
- 5% NaCl in H₂O
- MTBE (GC grade)
- 40 mL VOA vials
- Teflon capliners
- pH paper
- heating block (70°C)
- TurboVap

Special Hazards and Warnings

- ☒ You are working with a moderately strong acid. Be sure to wear gloves and safety glasses. If you don't want small holes to appear in your clothes, a lab coat is recommended.
- ☒ Capped vials can sometimes burst while being heated. Keep the hood sash lowered while samples are heating, and wear safety glasses.

Procedure

1. Transfer sample to a VOA vial using an appropriate solvent (DCM, ether, etc.) and dry in the Turbovap. Add 5mL of 0.5N HCl solution, cap the vial tightly using a Teflon capliner, and heat in the heating block at 70°C for up to 24 hours (**note B**).
2. Allow to cool, then add 5mL of NaCl solution. (**note C**) Shake well.
3. Add 10mL of MTBE and shake vigorously for 2 minutes. Allow the two phases to separate (**note D**), then pipette off the organic layer (top) and collect it in a second VOA vial. Repeat 2 more times with fresh 10mL aliquots of MTBE, collecting a total of ~25mL of extract. Dry the extract to ~1 mL under N₂ in the TurboVap.
4. Depending on the next step, you may want to filter the extract through anhydrous sodium sulfate to eliminate any water that might have been collected with the sample.

5. **Important!** You are extracting a strongly acidic solution with a moderately polar solvent (MTBE). It is likely that your extract will be slightly acidic. If the next step is methylation, this is not a concern. However, if you plan on making TMS derivatives and then injecting directly into the GC/MS, this will cause problems with excessive GC column bleed. Neutralize your sample first by shaking with a small amount of 5% NaHCO_3 solution, then dry over anhydrous Na_2SO_4 . Alternatively, you could extract the acidic solution with hexane, which will not dissolve any appreciable amount of acid. When in doubt, check the solution pH before injecting into the GC.

Notes

A. Hydrolysis can be conducted in methanol, water, or any other protic solvent. Methanol is somewhat better at solubilizing polymeric organic materials, but there is a chance that some carboxyl groups will be converted to methyl esters. Adding a small amount of water to the reaction will help to limit their formation, as will shaking with acidic water (step 2). If you want to form methyl esters, see the method for alkaline transesterification.

B. Methods from various labs differ widely in the temperature and time used for hydrolysis. In general, longer is better for very thick, gooey sediment extracts. For fresh biomass, a couple hours at 60°C is probably sufficient.

C. The NaCl solution is added to partition the HCl into the aqueous phase, away from the organic phase. In a pinch, you can get away with using pure water for this step. However, a NaCl solution is more efficient at this, so we recommend its use when possible.

D. Often the hydrolyzed products of fresh biomass produce a thick emulsion that resists separation. Some tricks that may help, in order of increasing effectiveness: *i)* gently stir the emulsion with the tip of a long Pasteur pipette or glass stir rod; *ii)* add more NaCl to the aqueous layer, in an effort to 'salt out' the more polar materials; *iii)* freeze the sample, then allow it to slowly thaw while gently stirring; *iv)* transfer the whole mess to a centrifuge tube and spin at 2000 rpm for 10 minutes. This last option always works, but is slow going.

Troubleshooting

There is not much that can go wrong with this reaction, aside from the formation of emulsions that are difficult to extract. If the hydrolysis is incomplete, you can increase any of the following: temperature, time, HCl concentration. At some point you will begin oxidizing organic materials, so try not to overdo it. The most common problem with this procedure is insufficient removal of HCl from your sample (step 5), which leads to other problems in subsequent steps.