
Fast Extraction and Methylation of Fatty Acids

Introduction

Preparation of fatty acids from biological materials for GC/MS analysis is typically time consuming, requiring separate extraction, hydrolysis, and derivatization steps. For samples of bacterial biomass, the workup can be combined into a single rapid, quantitative step resulting in fatty acid methyl esters (FAMES) ready for GC/MS or GC/IRMS analysis. The following procedure is our adaptation of the method published by Rodriguez-Ruiz et al (Biotechnology Techniques, Vol 12, No 9, September 1998, pp. 689–691). Note that the procedure is not recommended for plant materials, where hydrolysis of the more recalcitrant structural carbohydrates is required to get good lipid yields. For those samples, we recommend overnight saponification followed by extraction.

Materials

- Acetyl chloride
- Anhydrous methanol
- Hexane (GC grade)
- Dionized water
- Ice
- Heating block (100°C)
- 40mL VOA vials
- GC vials
- Silica-gel short column

Special Hazards and Warnings

- ☒ Acetyl chloride is very reactive with substances containing acidic protons, including water, methanol, etc. The reaction is highly exothermic, and can result in rapid boiling and/or explosions of an acidic solution. Always wear gloves and safety glasses, and add acetyl chloride to methanol slowly while keeping the mixture cold on ice. Be especially careful to keep acetyl chloride away from water.
- ☒ Capped vials can sometimes burst while being heated, especially at the high temperature (100°C) used here. Keep the hood sash lowered while samples are heating, and wear safety glasses.

Procedure

1. Samples must be thoroughly dried for this procedure. We typically freeze-dry biomass overnight in the culture tubes used for extraction (**note A**). Add internal standard as needed.
2. Prepare a mixture of 20:1 v/v anhydrous methanol/acetyl chloride as follows: transfer methanol into a clean 40mL vial, cap, and set to cool in an ice bath for ~5 minutes. Be sure not to get any water around the vial top or cap, where it might drip into the

- methanol when it is opened. Add acetyl chloride dropwise from a pipette, swirling the mixture as you add to prevent boiling. Return to ice frequently to keep cold. Failure to follow this procedure will result in spattering and/or boiling of the mixture, which should be avoided. The mixture needs to remain anhydrous, so limit exposure to air as much as possible.
3. To each sample, add 2 mL of the methylation mixture (above) and 1 mL of hexane. Cap the tube tightly and heat at 100C for 10 minutes (**note B**). A single phase should form. This volume works well for biomass samples containing <100mg dry weight; scale volume up as needed.
 4. Let the sample cool to room temperature. Add 2 mL deionized water and 2 mL hexane. Two phases should form. Remove the hexane (top) phase and collect in a vial. To maximize extraction yield, you can repeat the extraction with further 2 mL additions of hexane, if desired (**note C**).
 5. The hexane extract can now be concentrated into a GC vial and injected directly on the GC/MS. To provide a cleaner FAME sample, we recommend first cleaning up the extract on a short silica-gel column using hexane to elute the FAMES. This is a useful safeguard because it also prevents inadvertent injection of acidic water into the GC/MS if the extraction was not done carefully..

Notes

A. Biomass can be transferred into culture tubes with distilled water. Depending on how much water is in the culture tubes, they will sometimes break as the water expands when freezing. For small samples (a few mL), laying the tubes at an angle in the freezer is usually sufficient. For larger volumes of water, we recommend freezing by swirling the culture tube in a dewar of liquid N₂, slowly lowering the tube so that the water freezes from the bottom up. Once fully frozen, you can transfer to the freezer for storage.

B. We are heating the reaction mixture to well above its boiling point. If the cap is not perfectly sealed, the solvent will all evaporate during the 10 minute heating time. We typically use Teflon capliners, but still occasionally have tubes leak. Check the tubes after several minutes, and recap any that appear to be leaking. Add more acetylation reagent as necessary.

C. If you shake the mixture vigorously after adding hexane, many samples will produce a dense emulsion that is hard to separate. The easiest approach is to avoid vigorous shaking in the first place. If you do get an emulsion, spinning down the sample in a centrifuge, or else just being really patient, are your two best options.

Troubleshooting

The most most likely place to go wrong is getting water in your reaction mixture, which will result in bare fatty acids (ie, hydrolysis products) rather than transesterified FAMES. Make sure all glassware is dry, you are using anhydrous methanol, and that your samples are very dry.