Anti Oxidants for long term storage

* BHT @ 10μg/mL
  + For long period of storage, it can be useful to flush vials or tubes with nitrogen before closing to prevent fatty acid oxidation. For the same purpose, a low amount of antioxidant such as BHT (butylated hydroxytoluene or 2,6-di-tert-butyl-4-methoxyphenol) or ethyl gallate (i.e. about 50 µg BHT/ml) may be added in the solvent if the natural antioxidant amount is estimated too low (purified extracts). The antioxidant is used as a concentrated solution in ethanol (10 mg/ml).
    - <http://www.cyberlipid.org/extract/extr0001.htm>
* **Glassware**: Since all solvents may extract also some contaminants from containers or apparatus, only Teflon lined stoppers and clean glassware must be used. During extraction take care of any contact between solvent and washers or greased bearings in your mechanical device.  
    
  **Antioxidant**: When fatty acid analyses are planned, it is advisable to add an antioxidant such as butylated hydroxytoluene (BHT) (about 100 mg per liter).  
    
  **Lyophilized tissues**: They are difficult to extract, thus it is recommended to rehydrate them with distilled water before extraction (about 5 ml per g dry material).  
    
  **With some tissues**: it is recommended to add first the required methanol amount during the homogenization to prevent clogging, this is currently the case with liver, muscle, blood. chloroform is then added before the last mixing and the true extraction step.  
    
  **Evaporation of solvents**: When a large amount of solvent must be evaporated, it should done in a rotary film evaporator, the flask containing the extract being maintained at no more than 50°C with a water bath. The reduced pressure must be generated by a pump running without oil (motorized pump with metallic or Teflon membranes) or conveniently with a water aspirator achieving a vacuum of 10-20 mm Hg. Last traces of water may be removed by adding 1 or 2 ml ethanol and evaporating again.
* The first description of that concentration device was made by Craig LC (*Craig LC et al.,*[*Anal Chem 1950, 22, 1462*](http://www.cyberlipid.org/extract/craig1.pdf)), the famous inventor of countercurrent distribution. The diagram given in the Craig's paper (see below) shows clearly the principle of the device found on the market as yet.

Solvent and concentrations to use for neutral lipids

* Hexanes
  + Among hydrocarbons, **hexane** is the most popular but is a good solvent only for lipids of low polarity. Its main use is to extract neutral lipids from mixtures of water with alcohols. A mixture of isomers, called "hexanes", can be used for the same purpose. Hexane can be replaced by petroleum ether which is a mixtures of various hydrocarbons with 5 to 8 carbon atoms.
    - <http://www.cyberlipid.org/extract/extr0001.htm#5>
* 0.02μgmL to 100.0μg/mL for calibration curve standards
  + For each level of calibration, all FAME were present at equal concentrations ranging from 0.02 to 100.0 µg/mL across the series. As an internal standard, 21:0 FAME was added to each mixture at a concentration of 50.0 µg/mL. All standards were analyzed in quadruplicate by GC–FID and each of the GC–MS methods.
    - QUANTIFICATION OF FAME: GC–FID VS. GC–MS Lipids, Vol. 40, no. 4 (2005)
* Stock solutions should be made to 10mg/10ml and diluted down working concentrations

Solvent for lipid standards

* Lipids are aliquoted into 100ug/ml portions into hexane following the same procedure as Sigma Aldrich and how they store their FAME derivatives for shipping
  + <http://www.sigmaaldrich.com/catalog/product/supelco/49453u?lang=en&region=US>

Insect homogenate

* Stabilizers
* Sample number in pool
* Life stage to sample the insects