Purpose: To separate neutral lipids from *C. elegans* lipid extracts.

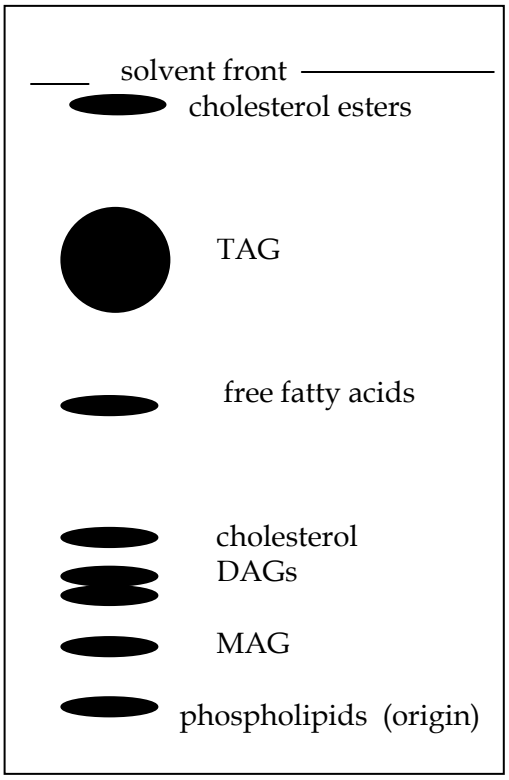
Procedure:

Plate preparation: use Silica Gel HL plates (Analtech 40611). Mark lanes needed for samples and standards, make the loading mark several inches from the bottom of the plate to ensure that samples are not immersed in the solvent. Using a razor blade, scrape vertical straight lines between lanes to avoid contamination of lanes. Activate the plates by placing in a 100°C oven for one hour.

Solvent preparation: Mix 80 ml hexane, 20 ml diethylether, and 2 ml acetic acid in the TLC tank. Put the lid on the tank and let the solvent equilibrate for at least 30 min.

Running the TLC plate: Load 30 µl of the lipid extract per lane, using a Hamilton syringe. Apply 10 µl at a time to allow the solvent to evaporate, so that the sample is loaded in a discreet spot. Run at least three replicates of each sample. After samples have dried, place the plate in the TLC tank and cover it tightly. Run the plate until the solvent reaches 1-2 cm of the top of the plate. Remove the plate and let the solvent evaporate in the hood.

Visualize the lipids on the TLC plate: Spray the plate lightly with primulin (0.005%) dissolved in acetone/water (80/20). Visualize bands under UV light and photograph. Circle the fractions of interest with a pencil. Neutral lipids will separate according to this figure: Typically most *C. elegans* fatty acids are found in the triglyceride (TAG) and phospholipid fractions.



Analysis of lipid fractions: Using a razor blade, carefully scrape the circled area of the TLC fraction of interest. Scrape the silica into a labeled 13 x 100 mm glass tube. Add 30 µl of 15:0 free fatty acid (0.1 mg/ml dissolved in hexane). Using a glass pipette, add 1 ml of of 2.5% sulfuric acid (v/v) in methanol, put screw caps on tightly and incubate the samples for 45 min. in a 70° water bath. Remove samples from the water bath and let them cool at room temperature for five minutes. To each tube, add 1.5 ml of water and 200-300µl of hexane. Recap the tube and shake vigorously, then centrifuge for one min. at low speed. Carefully remove 50-100µl of the top hexane layer and transfer to a gas chromatography vial insert.

Inject samples (2 µl) on a gas chromatograph equipped with a polar column. We use an Agilent 6890 series gas chromatograph equipped with a 20m x 0.25mm SP-2380 column (Supelco, Bellefonte, PA) and quantified using a flame ionization detector. The gas chromatograph program used to *C. elegans* FAME analysis starts with an initial temperature of 120° for 1 min followed by an increase of 10°/min to 190° followed by an increase of 2°/min to 200°.

Peak identity can be determined by comparison to authentic standards. Alternatively, a mass spectrometer detector can be used instead of the flame ionization detector. The mass spectrum of each fatty acid peak can be compared to that of authentic standards. The lipid extract concentration can be calculated by comparing the total peak areas to the 15:0 internal standard.

Reference:

Adapted from: Christie, W. W. (2003) LIPID ANALYSIS – 3rd edition. Oily Press.