**Author:** Jeff Salacup, Ph.D, UMass – Amherst **Title:** Extraction of Lipid Biomarkers from Geological Archive Sediments – 3. ASE

**Overview:**

The material comprising the living “organic” share of any ecosystem (leaves, fungi, bark, tissue; **Figure 1**) differs fundamentally from the material of the non-living “inorganic” share (rocks and their constituent minerals, oxygen, water, metals). Organic material contains carbon linked to a series of other carbon and hydrogen molecules (**Figure 2**), which distinguishes it from inorganic material. Carbon’s wide valency range (-4 to +4) allows it to form up to four separate covalent bonds with neighboring atoms, usually C, H, O, N, S, and P. It can also share up to three covalent bonds with a single other atom, such as the triple bond in the often poisonous cyanide, or nitrile, group. Over the past 4.6 billion years, this flexibility has led to an amazing array of chemical structures which vary in size, complexity, polarity, shape, and function. The scientific field of organic geochemistry is concerned with the identification and characterization of the whole range of detectable organic compounds, called biomarkers, produced by life on this planet, as well as others, through geologic time.   
 **Principles:**

Accelerated solvent extraction is a trademarked (Thermo Scientific Dionex) method of extraction that utilizes high temperatures (~100 °C) and pressures (~1200 psi) to increase the kinetics of the extraction process. The extractor, called an Accelerated Solvent Extractor, or ASE (**Figure 3**), can hold up to 24 individual samples. Once the ASE is loaded and set to run, it is completely automated. The ASE allows electronic control of the entire extraction process: extraction temperature, pressure, solvent volume, solvent mixture, duration, rinse, and repetition are all adjustable from sample to sample. Most organic geochemistry laboratories now use the ASE as the standard method of solvent extraction.

Biomarkers contained in the sample dissolve into the organic phase based on the rules of solubility, which with organic compounds, are controlled primarily by the polarity of both the biomarker and the solvent. This is summarized by the so-called “like dissolves like” rule, whereby relatively apolar biomarkers (those containing exclusively C and H; isoprene) dissolve in apolar solvents (such as hexane, polarity = 0.1) and more polar biomarkers (those containing O, N, S, P; GDGTs) dissolve in more polar solvents (such as methanol or dichloromethane, polarity = 5.1 and 3.1). A mixture of dichloromethane and methanol (9:1) is commonly used.

**Procedure:**

1. Collect the necessary materials:
   1. Extract 22 samples. Samples (leaves, dirt, fungi, bark, tissue), usually frozen, freeze-dried, crushed, and homogenized prior to extraction, are extracted in groups to maximize efficiency.
   2. Depending on the size of the sample, use vials with volumes ranging from 4-60 mL. For this experiment, use borosilicate glass vials (40 mL) and solvent safe caps. Combust vials, borosilicate glass pipettes, and weighing tins at 550 °C for 6 hr prior to ensure removal of possible organic contaminants.
   3. Dichloromethane and methanol are common in most organic geochemistry laboratories. Use them individually to rinse lab tools and glassware before use. A mixture of dichloromethane (DCM) to methanol (MeOH; 9:1) is used in many labs to efficiently extract biomarkers with a wide range of polarities. Solvents should be free of organic contaminants.
   4. Use an Accelerated Solvent Extractor (Thermo Scientific Dionex) for this experiment.
   5. Gather the remaining materials: ASE sample cells and collection vials (cell and collection vial volume varies according to sample size), combusted (14 mm diameter) glass fiber filters (such as Whatman GF/F), an ASE filter plunger, lab spatula(s), a lab scale, and combusted diatomaceous earth or Ottowa sand (Fisher Scientific).
2. Preparation of sample cells.
   1. Assemble a sample cell for each sample to be extracted, plus one blank.
      1. For each cell, screw an end cap onto one end of the cell body.
      2. Place a combusted glass fiber filter on top of each cell using solvent-rinsed tweezers. Then, gently and slowly press the filter down into the cell using the filter plunger.
      3. Label the cell bodies by number (1 – 22) for each sample and write ‘blank’ on the blank.
      4. Fill the blank with diatomaceous earth (or sand) and cap with a second end cap. Tighten by hand.
3. Preparation of Sample.
   1. Place a combusted weighing tin on the lab scale and then tare.
   2. Rinse the lab spatula with solvent, then use it to transfer an appropriate mass of sample into the weighing tin, and record the mass.  
      1. The mass of the sample varies depending on its organic matter content. Relatively organic matter lean material (marine mud) may require several grams, while organic matter rich material (leaf tissue) may require much less.
   3. Transfer all of the material in the weighing tin into a prepared ASE cell.
   4. Place another glass fiber filter onto the top of the cell, then slowly and gently press down until it reaches the top of the sample using the filter plunger.
   5. Add diatomaceous earth (or sand) to the cell until it is almost full. Be careful to remove any debris from the cell body threads.
   6. Cap the top of the cell with another end cap.
   7. Repeat steps 3.1 – 3.6 for each sample.
4. Preparation of collection vials.
   1. Label each vial with the number of a corresponding cell (1 – 22 or blank) and cap with ASE collection vial cap.
5. Extraction.
   1. Place each sample cell into a numbered slot on the upper ASE tray.
   2. Place the corresponding collection vial in the same number slot on the lower ASE tray.
   3. Create the extraction method using the keypad on the ASE. Extract at 100 °C and 1200 psi. Extract each sample three times with a static hold of 10 min and flush the cell body with 50% of its total volume between static holds.
   4. Make sure the solvent bottle contains enough solvent to extract all of the samples.
   5. Rinse the ASE three times before starting the run by pushing the “rinse” button on the ASE control pad.
   6. Press start.

**Representative Results:**

At the end of the extraction, there will be a total lipid extract (TLE) for each sample. Each vial now contains the extractable organic matter from a sediment, soil, or plant tissue. These TLEs can be analyzed, and their chemical constituents identified and quantified.

**Applications:**Different classes of biomarkers impart information on specific aspects of the Earth system. For example, in its infancy, organic geochemistry was primarily concerned with the formation, migration, and alteration of petroleum, and many of the chemical tools organic geochemists use today are based on those initial investigations. It was through the investigation of a class of compounds called isoprenoids, having a repeating five carbon pattern (**Figure 2**), that scientists discovered petroleum comprised the chemically-altered remains of ancient primary producers, such as plankton in the ocean (converting to oil)(**Figure 4**) or peat bogs on land (coal)(**Figure 5**). Chemists at large oil companies used the ratios of a variety of compounds, each with its own known rate of alteration, to estimate how old petroleum was, where it came from, and if it was worth exploiting. Today, new biomarkers are being discovered, identified, and characterized in modern and ancient samples analyzed in organic geochemistry labs around the world. Many of today’s applications seek to extract environmental information from biomarkers obtained in modern samples (leaves, soil, microbes, water samples, etc.) in order to extend the biomarker’s utility to ancient sediments in an effort to reconstruct the climates, environments, and ecosystems of the past. For example, the distribution of a group of biomarkers called glycerol-dialkyl glycerol-tetraethers (GDGTs for short), produced by a suite of archaea and bacteria, were found in modern sediments to change in a predictable manner in response to air or water temperature. Therefore the distribution of these biomarkers in ancient sediments, or through a series of sediments of known age, can be used to reconstruct air and water temperature back several million years.

**Legend:**

Figure 1: Organic material, such as trees, leaves, and moss, are chemically and visually distinct from inorganic material, such as pavement.

Figure 2: Isoprene comprises five carbon atoms and two double bonds. When added together in biosynthesis reaction, they can form complex molecules diagnostic for the presence of life. For example, 2, 6, 10, 15, 19-pentamethyleicosane, commonly found in cyanobacterial mats.

Figure 3. An Accelerated Solvent Extractor (ASE).

Figure 4: Illumination of plankton at Maldives.   
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Figure 5: Peat bog at 4500 m elevation in the Ecuadorian Andes.  
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