Science Education Collection

Soxhlet Extraction of Lipid Biomarkers from Sediment

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Overview

Source: Laboratory of Jeff Salacup - University of Massachusetts Amherst

Every lab needs standards that track the performance, accuracy, and precision of its instruments over time to ensure a measurement made today is the same as a measurement made a year from now (**Figure 1**). Because standards must test the performance of instruments over a long period of time, large volumes of the standards are often required. Many chemical standards can be purchased from retail scientific companies, like Sigma-Aldrich and Fisher. However, some compounds that occur in nature and that are relevant to paleoclimatic studies have not yet been isolated and purified for purchase. Therefore, these compounds need to be extracted from natural samples, and because of the large volumes of standards required, large volumes of sediment need to be extracted. The Accelerated Solvent Extraction (Dionex) and sonication extractions are not appropriate for the extraction of such large sediment volumes. In these circumstances, a Soxhlet extraction is used.

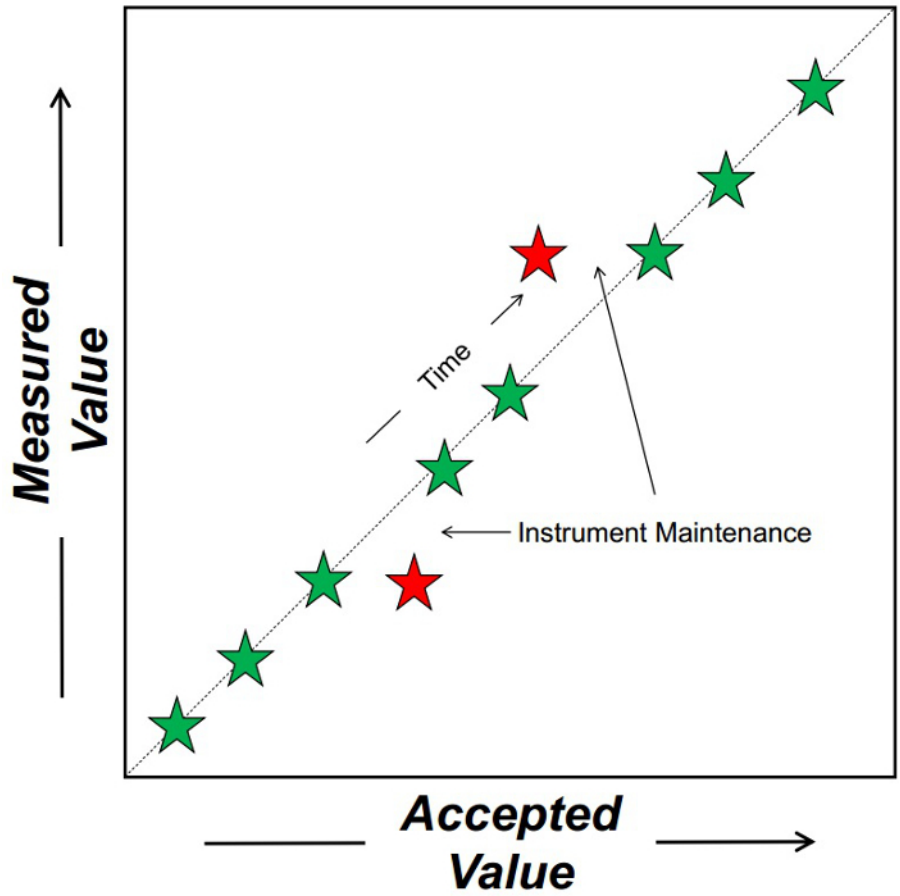

Figure 1. Schematic depicting how chemical standard tracks the performance of an instrument through time. The dashed line represents a 1:1 relationship between the accepted and measured (on the instrument) value of a variable. Each star is a weekly measurement of the chemical standard. Green stars represent standards that are accurate. Red stars reflect those that are not accurate indicating that the instrument requires corrective maintenance.

Principles

Soxhlet extraction is likely the oldest form of organic matter extraction. Archeological evidence from Mesopotamia places the use of a Soxhlet-like device that utilized hot water at ~3,500 BC[1,2]. Modern Soxhlets use sophisticated blown glass condensers and organic solvents in this "continuous" extraction method (**Figure 2**). Solvent is refluxed from a round-bottomed flask upward into a condenser with a recirculating cold-water coil. When the gaseous solvent contacts the coil, it condenses into a chamber with a glass fiber thimble holding the sample. This chamber is set with a recirculator, and when a certain volume is reached (generally a volume large enough to submerge the whole sample), the chamber

is flushed back into the round-bottomed flask via a built-in siphon, where the lipid extract accumulates while the solvent becomes part of the next cycle. Hence, the term "continuous" extraction. Soxhlet extraction is often used for the extraction of larger (>10 g) samples.

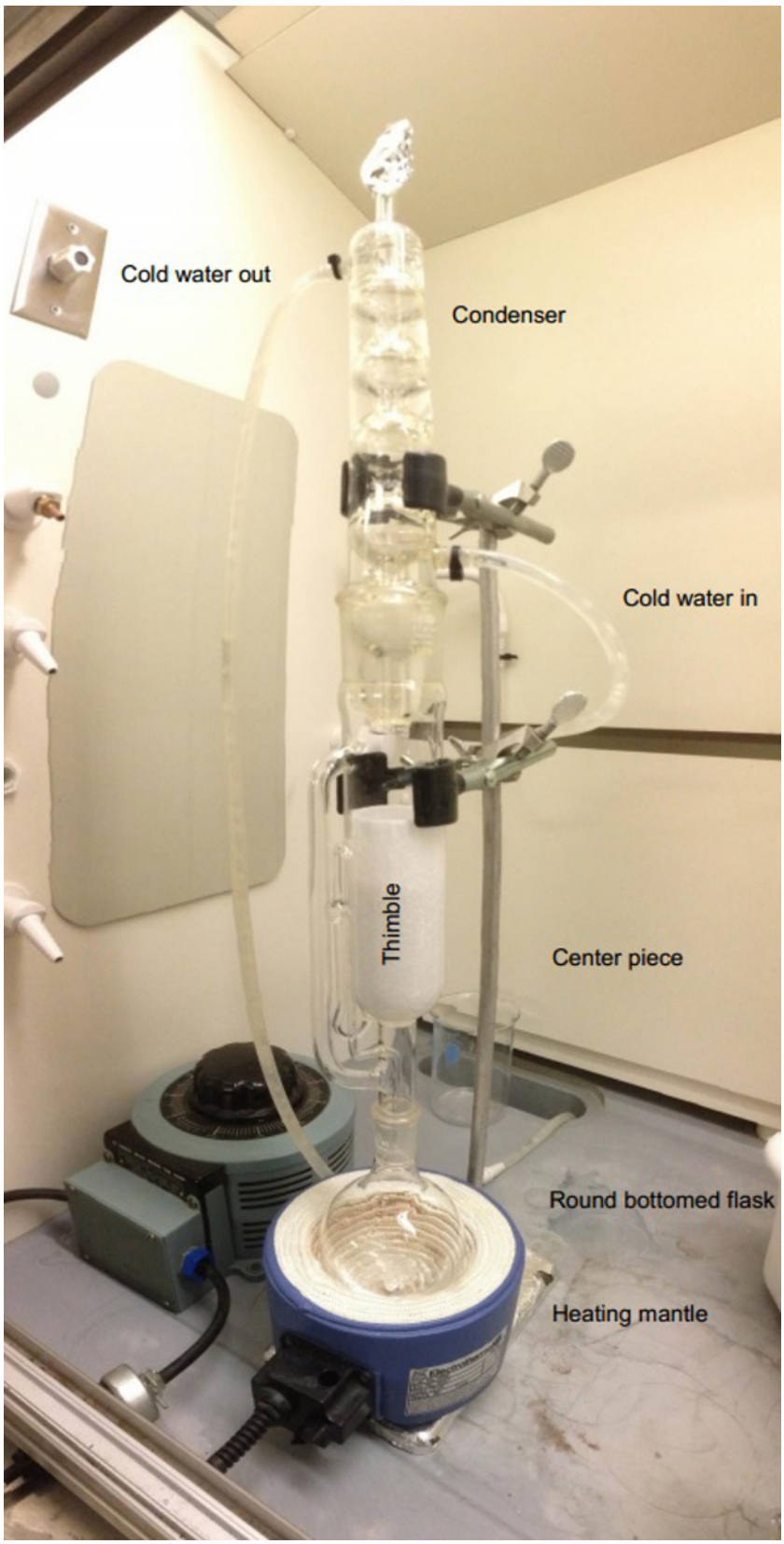

Figure 2. A soxhlet apparatus.

Procedure

1. Setup and Preparation of Materials

1. Collect a sample of frozen, freeze-dried, crushed, and homogenized marine sediment. A sample like this contains many of the compounds needed for standards.
    1. Standards are often made from sediments that are left over after a coring expedition or analysis. For example, in this experiment, sediment that was obtained from the 'Mud Patch' located just south of Cape Cod is extracted. This sediment was taken as part of a coring expedition but will not be used to answer scientific questions. We can therefore use it to make a standard.
    2. Place a ~100 g chunk of the sediment into the freezer overnight so that it freezes through.
    3. Once the sediment is completely frozen, turn on the freeze dryer (available from many scientific equipment retailers like Fisher) and wait until the condenser reaches it setpoint (often ~-30 °C).
    4. Load the sediment sample into the freeze dryer and close the purge to begin pulling a vacuum on the sample.
    5. Depending on the amount of water in the sediment, and the size of the sample, it may take several days for the sample to dry.
    6. Once the sample is dry, turn off the freeze dryer, vent it, and remove the sample.
    7. Place the sample in a solvent rinsed mortar and grind to a powder using a pestle. Do this to the entire sample and store in a glass jar in the freezer until ready to extract.
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 the vials, borosilicate glass pipettes, and weighing tins at 550 °C for 6 h prior to ensure removal of possible organic contaminants.
3. Obtain dichloromethane and methanol (both are common in most organic geochemistry laboratories), then 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. Acquire a soxhlet apparatus to use in this experiment (these can be purchased from Fisher Scientific or other science retailer), then wash and combust it at 550 °C for 6 h prior to use.
5. Obtain glass fiber thimbles (can be purchased from Whatman) and combust them at 550 °C for 6 h prior to use.

2. 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 combusted glass fiber thimble.

3. Extraction

1. Transfer ~400 mL of the DCM:MeOH (9:1) mixture into the round-bottomed flask (flask should be more than half full) and put in heating mantle. Add several (5-10) solvent-rinsed boiling chips.
2. Place the sample thimble, open-end up, into the centerpiece of the Soxhlet apparatus.
3. Place the centerpiece on top of the round-bottomed flask and secure with a glassware clamp.
4. Install the condenser on top of the centerpiece of the Soxhlet and secure with a glassware clamp.
5. Attach one of the cold water lines from the condenser to the cold water line in the hood using a hose clamp. Route the other into the drain.
6. Turn on the water to ensure proper circulation and drainage.
7. Turn on the heating mantle and adjust the temperature until the solvent in the round-bottomed flask is lightly boiling.
8. Monitor the extraction a few times over the next hour.
    1. Check to make sure the temperature is properly set at a low boil, the solvent is condensing in the condenser and dripping into the center piece, the center piece is filling and emptying properly, and the water is properly draining into the hood drain.
9. Monitor the extraction over the next 36 h.
    1. Ensure the temperature is properly set at a low boil, the solvent is condensing in the condenser and dripping into the center piece, the center piece is filling and emptying properly, the water is properly draining into the hood drain, and the solvent level in the round-bottomed flask is still about half full.
10. After 36 h, stop the extraction by turning off the heating mantle.
11. Label the flask "TLE".

Results

At the end of extraction, a total lipid extract (TLE) for the sample is produced. The round-bottomed flask contains the extractable organic matter from the sediment sample. This TLE can now be analyzed, and its chemical constituents identified and quantified.

Applications and Summary

The extract from the marine mud contains compounds called alkenones, which are used in paleoceanography. Alkenones are long-chained alkyl-ketones produced by certain classes of haptophyte algae that live in the sunlit surface ocean[3] (**Figure 3**). The two most common alkenones are 37 carbon atoms long and have two or three double bonds in them. The haptophytes adjust the ratio of these two alkenones in their cells according to the temperature of the water they live in. The ratio of the two alkenones defines the $U^{k'}_{37}$ ratio:

Equation 1) $U^{k'}_{37} = (C_{37:2}) / (C_{37:2} + C_{37:3})$ [4,5]

Culture[6,7] and core-top sediment[8] calibration studies led to the development of the $U^{k'}_{37}$ Index as a quantitative SST proxy. In this work we use:

Equation 2) $U^{k'}_{37} = 0.034(SST) + 0.039$; ±1.4 °C from 0 to 28 °C [culture-based[7]]

Alkenones are preserved in sediments dating as far back as the Early Eocene (~56 million years ago)[9]. Knowing the distribution of alkenones in a sediment core through time relates information on the evolution of sea surface temperature at that location. However, it's necessary to first make sure the instrument accurately and precisely measures the ratio of the two alkenones, and that is why standards are needed.

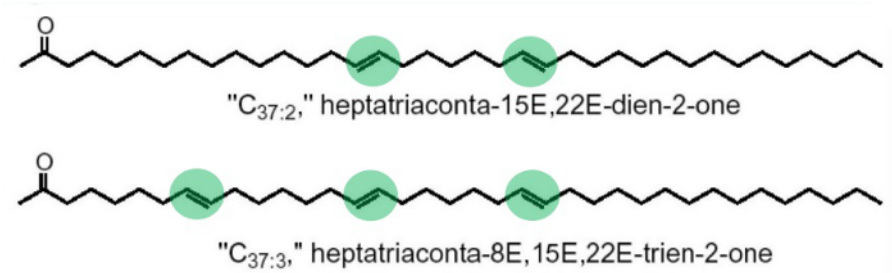

Figure 3. Alkenones with 2 (C37:2) and 3 (C37:3) double bonds (left) are produced by certain haptophyte algae that live in the sunlit surface ocean (right). (Photo courtesy of Tim I. Eglinton, Woods Hole Oceanographic Institution)

References

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