Konecky Lab – n-Alkanes Analysis
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Required PPE:

- **Lab Coat**
- **Goggles**
- **Nitrile Gloves**

Overview: This is a protocol for the analysis of n-alkanes from a total lipid extract (TLE) via our 'ASE Total Lipid Extraction' protocol. While the 'raw' TLE could be run for n-alkanes, there are far too many compounds present and will very likely lead to both inlet and column contamination from less volatile components. Thus, the TLE is typically split into 'acid' and 'neutral' fractions (See 'Fatty Acid Extraction & Derivatization' protocol), then the 'neutral' fraction is split into 'apolar' and 'polar' fractions (See 'TLE Neutral Fraction' protocol). The 'apolar' fraction is then used by this method, whereby n-alkane analysis is accomplished by gas chromatography coupled to either a flame ionization detector or a quadrupole mass spectrometer.

Materials needed (HPLC+ grade solvents; all aluminum and glassware combusted at 500°C):

- 2 mL GC Vials (for blanks and large samples)
- Fused 400 µL Insert GC Vials (1 per sample and 9 for standards; Thermofisher Cat. No. 6PSV9-03FIVP)
- GC Caps with PTFE-lined septa
- Hexanes (HPLC grade or better)

Part 1: Preparation of Samples & Blanks

1. The dried, neutral apolar fraction should be dissolved in 150 µL of hexane and transferred to a GC insert vial. This volume should be manually rinsed or the vial should be 'rolled' on its side to rinse the walls. Repeat this process twice. This nominally exceeds the volume of the insert, but sufficient hexane will evaporate during rinsing and transfer to prevent overfilling.
2. Repeat step 1 for each sample to be analyzed, up to 20 in one batch. Then, all sample should be gently dried and re-dissolved in a known volume of hexane. Typically, 100 to 200 µL is a good start and can be adjusted based on expected yield.
3. Label two 2 mL vials as "blank" and fill with 1.5 mL of hexane.

Part 2: Preparation of Standards

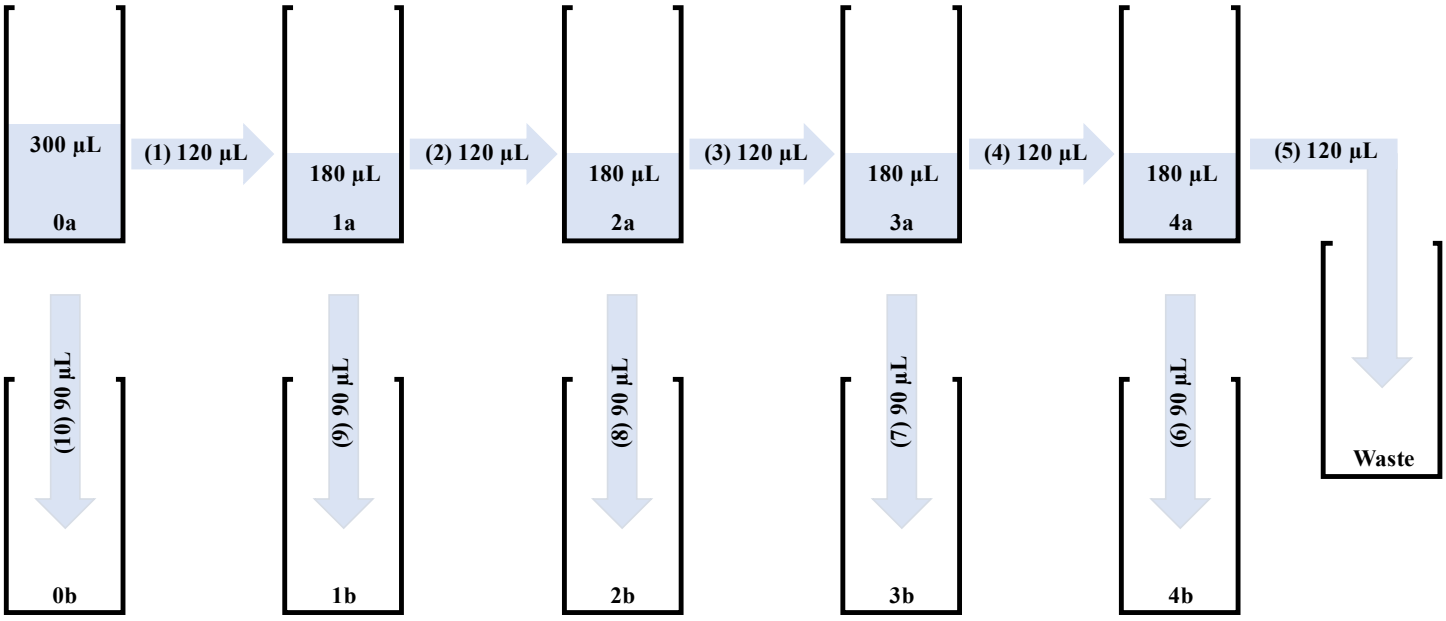
1. The mixed standard is the Sigma C7-C40 Saturated Alkanes Standard (Cat. No. 49452-U). The original 1000 µg / mL in 1 mL vial was diluted to 10 mL and then dispensed 75 µL at a time into 300 µL GC inserts and gently dried, resulting in each insert containing 7.5 µg (or 7,500 ng) of each alkane. To prepare two calibration curves (a and b), perform the following (carefully!). Note that, with care, a single pipette tip can be used for the entire operation.
    1. Take **one** dried aliquot and re-dissolve in 300 µL of hexane, resulting in a concentration of 25 ng / µL. Label the vial '0a' to indicate it is the start of the calibration curve.
        - **Important!** The dry standard does not dissolve immediately. After dispensing the initial 300 µL of solvent, allow to stand for at least 1 minute, most easily achieved by performing this step before preparing the vials in the following step.
    2. Gather four additional vials with 300 µL inserts and dispense 180 µL of hexane into each. Label them 1a through 4a, indicating their position on the calibration curve. Loosely place a GC cap on each vial to prevent evaporation.
    3. Gather five additional vials with 300 µL inserts and label them 0b through 4b, indicating their position on the *second* calibration curve.
    4. The following 10 transfer steps are shown in the diagram below for clarity.
        (1) Pipette 120 µL from vial 0a into 1a, actuating the pipette several times in vial 1a to ensure complete mixing. Vial 1a is now at 10 ng / µL.
        (2) Pipette 120 µL of vial 1a into vial 2a, actuating the pipette several times in vial 2a to ensure complete mixing. Vial 2a is now at 4 ng / µL.

        (3) Pipette 120 µL of vial 2a into 3a, actuating the pipette several times in vial 3a to ensure complete mixing. Vial 3a is now at 1.6 ng / µL.
        (4) Pipette 120 µL of vial 3a into 4a, actuating the pipette several times in vial 4a to ensure complete mixing. Vial 4a is now at 0.64 ng / µL.
        (5) Pipette 120 µL from vial 4a into waste.

(6) Pipette 90 µL from vial 4a into 4b. Cap both vials.
(7) Pipette 90 µL from vial 3a into 3b. Cap both vials.
(8) Pipette 90 µL from vial 2a into 2b. Cap both vials.
(9) Pipette 90 µL from vial 1a into 1b. Cap both vials.
(10) Pipette 90 µL from vial 0a into 0b. Cap both vials.

- Some helpful tips:
    - Perform this quickly *and* carefully, but prioritize care over speed when developing your technique.
    - Try to insert the tip to the same depth for each vial. This attempts to normalize the amount of carryover between vials and should improve accuracy.
    - Place a cap on each vial with solvent in it to minimize any evaporation during the procedure
    - During steps 1-4, dispense the solvent back into the current vial completely and then draw the intended amount for the next dilution.
    - During initial filling, use the reverse pipetting technique where you draw to the second stop of the pipette and then dispense to the first stop. This improves dispensing accuracy.

5. **Congratulations!** You now have two 5-point 60% dilution calibration curves starting at 25 ng / µL. Vials should be capped tightly so that the septum makes a slight, semi-circular indentation on the insert, which ensures a good, air-tight seal between the insert and the vial.



Part 3: Sequence Assembly

1. The sequence should use the following pattern:
    a. The start of the sequence should use 2 blank injections following by the first calibration curve. Short sequences (less then 10-12 samples) may use only one blank.
    b. The body of the sequence should include 1 blank injection for every 6-8 samples, spaced as evenly as possible.
    c. The end of the sequence should use a single blank injection followed by the second calibration curve.
    d. The first blank vial should be used for the first half of the sequence and the second blank vial should be used for the second half of the sequence.