Konecky Lab – Urea Adduction
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Contact: Jack Hutchings (jackh@wustl.edu)

Required PPE:

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

Overview: Bulk hydrocarbon extracts (fatty acids, alkanes, or others) contain a mixture of compounds. A distinction can be made between 'straight-chain' (i.e., a single unbroken link of C atoms), 'branched' (e.g., containing methyl groups within the chain), and 'cyclic' (e.g., containing pentane or hexane moieties). For alkanes and fatty acids, we are interested in 'straight-chain' compounds, and so removing branched and cyclic compounds may resolve coelution problems. Urea adduction accomplishes this by including (mostly) only straight chain compounds within its crystal structure during precipitation, while excluding branched and cyclic compounds. Thus, we perform urea adduction, rinse the crystals off (the non-adducted branched/cyclic compounds), and then dissolve the crystals to release the adducted (straight chain) compounds. This is a modified method that replaces the original liquid-liquid extraction step with a supported-liquid extraction. This method requires about the same amount of time but reduces the skill and variability associated with liquid-liquid extraction.

Materials needed (HPLC+ grade solvents; all aluminum and glassware combusted at 550°C):

- n-Hexane (Hexanes are also acceptable)
- Acetone
- Methanol
- Urea-saturated methanol (solubility is 166 mg/mL, so target 175 mg/mL)
- 5% (w/v) NaCl in DI water (e.g., 10 g sodium chloride in 200 mL deionized water)
- SLE Media (Agilent Chem Elut S, diatomaceous earth probably also acceptable)
- 4 mL vials & caps
- Short-form Pasteur pipettes
- Glass Wool

Equipment needed:

- N_2 Dry-down Station (Flexivap, using gentle N_2 and 25-30°C)
- Column Chromatography Racks
- Small, Aluminum Funnels (Commonly sold for perfume or essential oil purposes, ours have a tip end diameter of ~3 mm and fit within the opening of 4 mL vials)

Day 1: Urea Adduction #1

1. Transfer your samples into 4 mL vials. If your sample is already in a 4 mL vial, then urea adduction can proceed in that vial.
    - If your sample is already in a 4 mL vial, then dry the sample under N_2 and re-dissolve in 0.4 mL hexane and 0.2 mL acetone.
    - If your sample is in a different type of vial, then dry the sample under N_2, and perform repeated transfers using 0.2 mL hexane, 0.2 mL hexane, and 0.2 mL acetone. These transfers triple rinses your original vial and satisfies the solvents needed for the adduction process.
2. For each sample, add 0.2 mL urea-saturated methanol.
3. Cap and allow to react at room temperature overnight.

Day 2+: Optional Additional Urea Adductions

This day is optional to perform a second (or third or fourth…) urea adduction. Each time, the adducted fraction will become increasingly pure. Typical samples will require only a single adduction and so this section can be skipped entirely, but challenging samples may require multiple adductions for sufficient purity.

1. Uncap vials and dry under N_2 without heating. After visibly dry, you may apply ~30 °C heat and then allow the samples at least 10 minutes beyond apparent 'dryness' to ensure complete removal of residual solvents.
    - Your target compounds are trapped within the crystal structure so there is no risk of loss due to excessive drying.
2. For each sample, label a 4 mL vial to collect the urea non-adduct (UNA) fraction.

- If doing sequential adductions, then simply dry the existing UNA vial and continue collecting the UNA fraction.

3. Pipette 1 mL hexane onto the dry crystals, roll the vials to ensure contact with all crystals, and then slowly pour into the matching UNA vial using the small metal funnels to prevent any spilling.
    - Pouring is done in place of pipette rinsing because the crystals are fragile and tend to break and become stuck in the pipette.
4. Repeat step 3 twice for a total of 3 hexane rinses of the UNA fraction.
5. Re-dry your crystals on the Flexi-vap to remove any residual hexane from UNA rinsing.
6. Redissolve crystals in 0.2 mL of methanol. Roll the vial to ensure dissolution.
7. For each sample, add 0.2 mL acetone.
8. For each sample, add 0.4 mL hexane.
9. Cap and allow to react at room temperature overnight.

Day 2 (or 3+ if you performed additional adductions): Adduct Fraction SLE

Part 1: UNA Collection

1. Uncap vials and dry under N_2 without heating. After visibly dry, you may apply ~30 °C heat and then allow the samples at least 10 minutes beyond apparent 'dryness' to ensure complete removal of residual solvents.
    - Your target compounds are trapped within the crystal structure so there is no risk of loss due to excessive drying.
2. For each sample, label a 4 mL vial to collect the urea non-adduct (UNA) fraction.
    - If you performed sequential adductions, then simply dry the existing UNA vial and continue collecting the UNA fraction.
3. Pipette 1 mL hexane onto the dry crystals, roll the vials to ensure contact with all crystals, and then slowly pour into the matching UNA vial using the small metal funnels to prevent any spilling.
    - Pouring is done in place of pipette rinsing because the crystals are fragile and tend to break and become stuck in the pipette.
4. Repeat step 3 twice for a total of 3 hexane rinses of the UNA fraction.
5. Re-dry your crystals under N_2 to remove any residual hexane from UNA rinsing.

Part 2: Supported-Liquid Extraction (SLE) Columns

6. Prepare a set of pipette columns plugged with glass wool and filled with 1.25 mL SLE media. Place the columns over 4 mL vials labeled with the sample identifier, fraction (apolars, FAMEs, etc.) and either UA (urea-adduct) or UAS (urea-adduct saturated –if you have already performed silver nitrate).
7. Dissolve the crystals in 200 µL 5% NaCl DI water and roll vial for complete dissolution and then transfer into the appropriate SLE column using a Pasteur pipette.
    - Slowly draw the water into the pipette to keep the transferred water within the lower portion of the pipette.
    - Take care when dispensing the water onto the SLE column so that the majority of the dispensed water is pipetted onto the SLE media and not the walls of the pipette column.
8. Repeat the previous step twice for a total of 3 transfers that total 600 µL of water.
9. Wait 15 minutes to allow the sample water to fully saturate throughout the SLE media.
10. Add 1 mL of hexane to the original sample vial, rinse, and transfer into the SLE column. Allow to drain via gravity – do **not** use positive pressure.
    - You may use a small burst of positive pressure if you get a small 'bead' of air at the base of the pipette that results in very low flow.
11. Repeat the previous step three times for a total of 4 mL of hexane.
    - The fourth rinse can go directly on the SLE column – your original vial will have no remaining sample material.
12. Once gravity flow of the final transfer has ceased, use positive pressure to blow out any remaining solvent into the sample vial.
13. Reduce the volume under N_2 and transfer into appropriately sized and labeled GC vials. Be sure to do at least 2 additional rinses after the first transfer.
14. Dry the GC vials and re-dissolve in an appropriate amount of hexane and analyze via GC to quantify yields.