Konecky Lab – Combined Urea Adduction + Argentation Column Chromatography

Updated 2024-01-11
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Required PPE:

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

Overview: This method is a concatenation of our urea adduction and silver nitrate column protocols. See those protocols for extended background and rationale. In this protocol, it is assumed that both unsaturated **and** either UCM or branched/cyclized compounds are present and interfere with analysis of either n-alkanes or n-acids. In this case, the two clean-up methods of urea adduction and silver nitrate column chromatography can be performed sequentially, saving some time, solvent, glassware, and sample dry-downs/transfers. It is expected that this concatenation will save ~30 minutes/batch of user time while also increasing yields by a minor (0-3%) amount due to the elimination of transfer steps.

Materials needed (HPLC+ grade solvents; all aluminum and glassware combusted at 450°C):

- Silver nitrate silica gel (10% w/w) [Sigma Cat. No. 248765]
    - With customized, stop-cocked lid for flushing with Ar after usage
- n-Hexane
- DCM
- Acetone
- Urea-saturated methanol (solubility is 166 mg/mL, so target 175 mg/mL)
- 4 mL vials & caps
- 20 mL scintillation vials
- Glass Pipettes
- Glass Wool

Equipment needed:

- N_2 Dry-down Station (Flexivap, using gentle N_2 and 30°C)
- Ar tank with ¼" tubing
- Sonication Bath
- Vortex Mixer

Protocol:

Day 1

1. Transfer your samples into labeled 4 mL vials and dissolve in appropriate solvents using one of the following methods:
    a. If your sample is already in a 4 mL vial, then dry the sample using the Flexivap and re-dissolve in 0.4 mL n-hexane and 0.2 mL acetone.
    b. If your sample is in a different type of vial, then dry the sample using the Flexivap, and perform repeated transfers using 0.2 mL n-hexane, 0.2 mL n-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

1. Uncap vials and dry using the Flexivap. Allow the samples at least 15 minutes beyond apparent 'dryness' in order to ensure complete removal of residual solvents. Because your target compounds are trapped within the crystal structure, there is no risk of loss due to excessive drying.
2. For each sample, label a 20 mL scintillation vial to collect the urea non-adduct (UNA) fraction.
3. Pipette 1 mL n-hexane onto the dry crystals, roll the vials to ensure contact with all crystals, and then pour into the matching UNA scintillation vial. This 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 6 twice for a total of 3 n-hexane rinses of the UNA fraction.
5. Dissolve the crystals in 1 mL DI water.

6. Prepare up to 3 silver nitrate columns to receive liquid-liquid transfers from the samples. Construct pipette columns using short-form pipettes stuffed with glass wool. Add ~1.0 g of 10% silver nitrate silica gel to each column.
    a. Silver nitrate is light and oxygen sensitive. Prepare your columns immediately prior to using and only prepare up to 3 columns at once.
7. Prepare two collection vials for each sample: saturated [**Sat**] and unsaturated [**US**].
8. Pre-rinse each column with 3 mL hexane and discard eluent to waste.
9. Place the columns over the **Sat** vials.
10. Working with 1-3 samples at a time, perform 4 liquid-liquid extractions from each sample into the corresponding silver nitrate column collect the urea adduct fraction. To perform the liquid-liquid extractions:
    a. Add 1 mL n-hexane to the aqueous solution. Cap.
    b. Vortex for 20 sec, sonicate vials for 20 sec, and then vortex again for 20 sec.
    c. Pipette the upper organic layer of each vial into the corresponding silver nitrate column.
    d. Between liquid-liquid transfers, be sure that the previous transfer in the silver nitrate column has drained to near the surface of the column media. If it is draining slowly, use the N2 column pusher to accelerate elution.
11. After performing the 4 liquid-liquid extractions, move columns over the **US** vials and elute each column with ~3 mL DCM.
12. When finished performing columns, tightly close and parafilm the silver nitrate container. Bring the container to the Ar tank and attach the tubing to one of the lid's valves. With both valves open, initiate a **gentle** flow for Ar and let flow for 2-3 minutes. When finished, close the outlet (unattached) valve first, then close the inlet valve and remove the tubing. The silver nitrate is now ready for storage.
13. Dry **UNA**, **Sat**, and **US** vials using the Flexivap and transfer to fused insert GC vials using three, sequential transfers of 0.15 mL hexane, being sure to either roll or rinse in walls of the 4 mL vial before transferring.
    a. If using larger GC vials (MRQ30 with capacity of 1.2 mL or standard GC with capacity of 2 mL), then you may increase the volume of each transfer. However, 3 x 0.15 mL hexane is sufficient for complete transfer.
14. The saturated, straight chain n-alkanes/acids will be in the **Sat** vial. Unsaturated, straight chain compounds will be in the **US** vial. Branched/cyclized compounds of varying degrees of unsaturation will be contained within the **UNA** vial.
15. Dry the GC vials and re-dissolve in 0.4 mL hexane.
16. Analyze via GC-FID to calculate yield.