Recrystallization of SA CHCA DHB

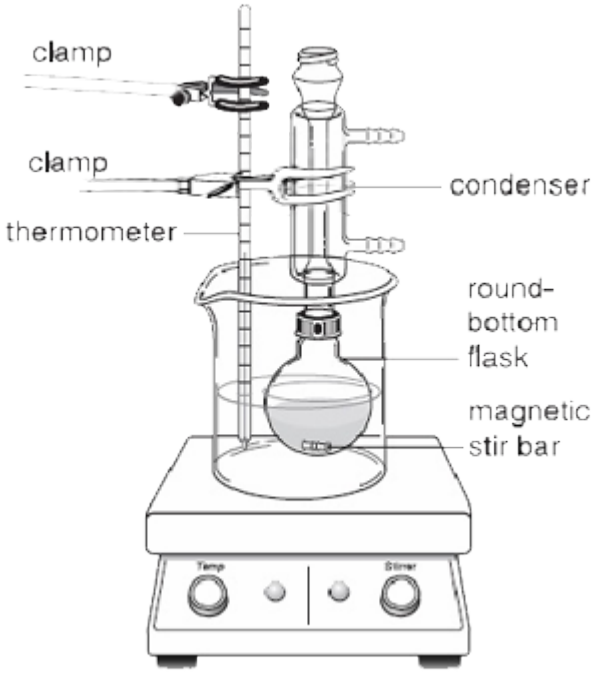
Health and Safety:

Wear nitrile gloves, laboratory coat and safety glasses. The process should be done with fume hood ventilation.

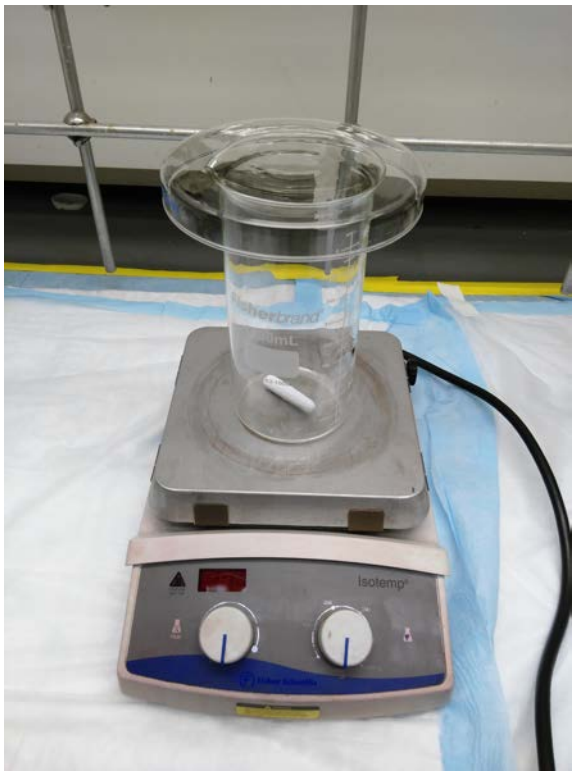
Equipment and Materials:

Concept

1) Ideally, for a small amount of matrix (5-50 gram):



2) For a large quantity of matrix (50+ gram) or if it is too difficult to set up as in 1), it is preferred to use a beaker and a petri dish (serving as a condenser). This setup will not be as efficient as in (1) as far as condensation goes, but it is easier to handle for large amount of matrix and for personnel who are not familiar with organic lab setups.



Equipment (for a large amount of matrix)

1. 1000 mL beaker,
2. hot plate with magnetic stirring,
3. 3-inch magnetic bar (Fisher, 14-513-68),
4. Buchner funnel (diameter size from 60mm to 100mm, e.g., Fisher FB-966-F),
5. 1000 mL filtering flask,

6. filter paper, coarse
7. 1000 Erlenmeyer flask,

8. vials (20 mL Glass Scintillation Vials are recommended) for aliquot
9. Aluminum foil for cover the vials.

Chemicals

MALDI Matrices (from any source with 95%+ purity)

Alpha-cyano-4-hydroxycinnamic acid (CHCA); 2,5-Dihydroxybenzoic acid (DHB); 3,5-Dimethoxy-4-hydroxycinnamic acid (SA)

Acetonitrile, HPLC grade; Acetic acid

Procedure

Recrystallization CHCA

1. For a 25-gram batch of CHCA, use 700 mL of 90% ACN (0.1% acetic acid)
2. CHCA with the solvents described in step 1 is placed in a 1000mL of beaker with a magnetic stirring bar and with heating until 85°C. The beaker should be covered with a glass petri dish. When all the CHCA dissolves to get a clear solution, another 50 mL of 90% ACN is added.
3. Set the stirring motion at a fast speed. Then turn off the heating. Wrap up the flask with aluminum foil and leave the flask on the same hot plate with no heating. The fast stirring and slow cooling down process combine to give a slow crystallization of SA. During this process, the crystals tend to have a very small size and contain minimum impurity. This process will take couple hours for the crystals to start to form, and 6 hours or overnight to complete.
4. Filter the crystals of SA, rinse with ice cold 90% ACN and dry in a hood with dimmed light if light is needed at all.
5. Repeat the recrystallization again with the collected crystals from step 4, adjust the volume of solvent needed per the weight of the crystals. Store the CHCA at -20 to -80 °C.

Recrystallization of SA

1. For 25 g SA, use 400 mL 70% ACN (0.1% acetic acid).
2. SA with the solvent is placed in a 1000mL beaker with a magnetic stirring bar and with heating at 85°C. The beaker should be covered with a glass petri dish. When all the SA dissolves to get a clear solution, another 50 mL of 70% ACN is added.
3. Set the stirring motion at a fast speed. Then turn off the heating. Wrap up the flask with aluminum foil and leave the flask on the same hot plate with no heating. The fast stirring and slowly cooling down process combine to give a slow crystallization of SA. During this process, the crystals tend to have a very small size and contain minimum impurity.

This process will take couple hours for the crystals start to form, and 6 hours or overnight to complete.

4. Filter the crystals of SA, rinse with ice cold 70% ACN and dry in a hood with dimmed lighting.

5. Repeat the recrystallization again with the collected crystals from step 4, adjust the volume of solvent needed per the weight of the crystals. Store the aliquot of SA at -20 to -80 °C.

Recrystallization of DHB

1. Place 100 g of DHB into a 500 mL clean Pyrex beaker with a stirring bar and heating at 90°C. Slowly add MilliQ water to the solid with stirring until all the crystals are dissolved. The goal is to saturate the solution at an elevated temperature. The beaker should be covered with a glass petri dish.

2. Heat the solution to 95°C and until a clear solution is obtained. This should take about 1 to 2 hours.

3. Add 50 mL of water to prevent the crystal formation during the filtration and add 500 µL of acetic acid to keep the solution acidic and reduce the sodium or potassium adduction to the matrix. The solution should be clear. Then turn off the heat and set the stirring at a fast speed.

4. Crystallization should take overnight.

5. Wash the crystals with a minimal volume of ice-cold MilliQ water. Collect the crystals with glass Petri dish and dry the crystals in the hood or desiccator. Cover the Petri dish with Kimwipes so that crystals are not contaminated by the dust during the drying process.

6. Repeat one more time of recrystallization with collected crystals from 5.2.6, adjust the volume of solvent needed per the weight of the crystals.

7. Store the aliquot of DHB at -20°C or -80°C.

Expected outcome

Sinapinic acid and DHB crystals are snow white color when are at highest purity, while CHCA crystals should be bright yellow.

Reference: please see any basic organic laboratory training book for the recrystallization procedure and mechanism.

Note:

1. It is critical to do the recrystallization at least twice to obtain MALDI quality matrix.
2. The recrystallization is not necessary for commercial products made for MALDI MS that have very high purity and are in small vials (~20 mg/per vial)
3. Because there is no filtration step of the hot matrix solution to remove the non-dissolved particles during the recrystallization, any particles that are not dissolved in the crude matrix will stay in the final product. Hence, it is necessary to use a syringe filter for spraying matrix solution that will be used on a sprayer, e.g., TM sprayer. The recommended syringe filter is PTFE filter from Fisher Scientific (09-719H) which is: Nonsterile; Pore size: 0.45 µm; Diameter: 25mm
4. The filtration step of the hot matrix solution is skipped because it is difficult to do for a large amount matrix solution: the hot solution tends to boil under vacuum and evaporate resulting crystals crushing out and clogging up the funnel.
5. Recycled matrices from sublimation can be purified using this protocol as well.