



Facility: Ball Laboratory
Seaver North Chemistry 209
Pomona College College Department of Chemistry
Claremont, CA 91711

Principal Investigator: Professor Nicholas D. Ball
Seaver North Chemistry Laboratory 208
(413)-542-5650

Author(s): Ariana Tribby

Scope: This SOP details the procedure and safety considerations for the CombiFlash Chromatography Purification System.

Kind of SOP: Technique.

Last Revision: 11/12/2015

Introduction and Purpose:

The CombiFlash Purification System effectively improves the resolution for the compound by eliminating reaction byproducts in order to obtain an ultra-pure final product. It is generally performed after celite-filtering technique has been completed in order to obtain purer yields.

Necessary Materials:

- Appropriately-sized column and cartridge.
- 8mL glass test tubes
- Silica gel powder
- Round sponge filters
- Hexane
- Ethyl Acetate
- DCM or 90/10 hexane/ethyl acetate
- Or needed eluent (solvents)

Step-by-step setup: (Note: see picture on page 4 for setup)

1. Cartridge Preparation

- Fill approximately half of the cartridge with silica gel powder in the hood, taking care to avoid inhaling the powder.
- Insert a white sponge filter to the top of the silica gel using the solid, white cylindrical block plunger, making sure that there are no air spaces in between the filter and the gel powder.
- Using DCM or 90/10 hexane/ethyl acetate, or whichever volatile solvent your compound has solubility (e.g., MeOH, DCM, Hexane, ethyl acetate). You want to make sure you use the least amount of solvent as possible for an effective column. **Avoid solvents like DMSO, water, and acetone they undergo an exothermic reaction with the silica gel.** Transfer your product using a glass bulb pipet to the cartridge by spreading the product evenly over the packed sponge filter. Rinse the round bottom flask that contained your product with DCM or 90/10 to extract as much product as possible. Finally, rinse sides of the cartridge to ensure all of your product reaches the filter.
- Using acetone, chimwipe the CombiFlash injection valve assembly with acetone. Once the valve assembly dries, push it through the cartridge so that there is no space between the tip of the injector and the filter. Lock the injection valve assembly by turning the top clockwise until there is a click. (It is locked when a "cross" is made between the metal injection valve assembly and the plastic lips of the cartridge on the underside of the valve assembly.)
- Place the locked cartridge-valve assembly upside-down by gently pushing the solvent line through the metal ring located on the adjustable flash column mount and balancing the cartridge against the metal ring.
- On the left-hand corner of the computer screen, either select File, "New" (if planning to construct your own solvent gradient), or select "Open" and "extract" (if planning to extract a previous gradient). Save the new file under desired name.
- Select Tools, Manual Control, and then "air purge cartridge" while the cartridge is still upside-down for 5-10 minutes. (The program will not time it.) In the meantime, prepare the fraction collection tubes by inserting new 8mL glass collection tubes into the rack.
- When purge is finished, click "stop" and turn the locked cartridge-valve assembly upright, and screw the tip of the cartridge into its appropriate metal fitting.

2. Column Preparation

- Selecting the appropriate sized column is critical. On the main screen, select the "help" bar and search "column size." Locate "RediSep Rf Column Selection Guide" to determine your column size based on your predicted yield size in mg (sample load range).
- Remove the two red caps from the appropriately selected column and insert the by raising the adjustable flash column mount and inserting the column in its appropriate fitting. Make sure both the cartridge and column are screwed in tightly.

3. Computer Program Set-Up

- After exiting air purge, return to the main screen.

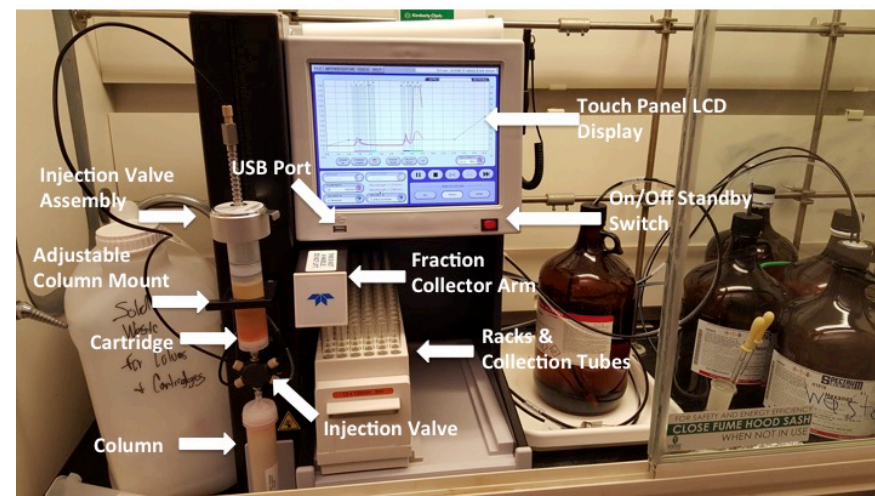
- On the main screen, click on "Tools", then "manual control" to change the percentage of solvent B or to change solvents altogether.
- **Method Editor:** To create or edit the gradient method, click on "Method editor", then "gradient optimizer." Use the Rf values from the product's crude TLC to complete the required sections. Alternatively, you may manually construct your own gradient by selecting the curve on the gradient graph and dragging the curve to generate the desired slope.
 - Under "Peak Detection", select "details" for each wavelength and change "Signal Gain" to 4x (to get a sharper and better separation). In the same window, ensure "slope-based" and "threshold" are selected.
 - Still under Method Editor, ensure that the flow rate, run length, solvents, and column size are correct. Under "Peak Collection" ensure that "non-peak" is set at max, and "peak" is set at the desired collection volume.

4. Initiating a Run

- Select the large "play" button.
- Enter the appropriate information for Loading Technique. (For our purposes, the loading technique is solid, 25g pre-filled). Also, ensure that the machine has detected the correct collection tube rack to deposit the product in.
- You can access your run live, or at any time by internet searching the IP address: 134.173.85.230.
- During a run, the gradient can be held constant by selecting "pause" button. The run can be completely stopped by selecting the "stop" button. If CombiFlash system does not begin to collect fractions when it needs to, click "collect all" so that it will immediately collect.

5. Completing a Run

- After the run is complete, (and before running a subsequent experiment), select "fast forward" to air purge the cartridge before unlocking and separating the injection valve assembly from the cartridge.
- Dispose appropriately. Chimwipe the plunger.



Safety Considerations and Precautions:

1. Avoid inhaling silica gel powder when preparing the cartridge by using a hood and/or wearing a protective mask.
2. Take special care to ensure the injection valve assembly is locked when starting a run. (If not, the machine will not automatically stop and solvent/product will overflow and spill.) In addition, if the solvent line is not screwed on properly to the injection valve assembly, there is a risk of flooding the machine and hood.
3. It is good precaution to remain close-by during a run, especially during the beginning to ensure the machine collects the fractions properly.
4. See the Safety Data Sheet Documentation Binder to reference Safety Data Sheet and physical and health hazards associated with the chemicals used with the CombiFlash system.