## **Biotage**

## **Sample Preparation**

- 1. Concentrate your sample into a solid or an oil. (You can also dry load your sample onto silica and pack it into one of the SNAP columns.)
- 2. Run a TLC on your compound that needs purification and determine its Rf value along with the Rf values of all impurities.

## Running a Sample

- 1. On main menu, press **New**, lamp will be turned on. (Note that the lamp has a 7.5 min warm-up time, can set up method during this time.)
- 2. Select **User** (McNeil Lab) and **Type** (either optimize previous run or start a new method), then enter the total mass of sample being purified.
- 3. For Previous run, select run then click **Optimize**. Adjust threshold, select product peak and then **Create** method. Column flushing will stop after collecting the selected peak.
- 4. For new method, select TLC. Enter strong solvent percentage used for TLC along with the Rf values you calculated or move the Rf bar according to TLC plate. Then Create method. A linear gradient method will be created when one TLC is entered. A step gradient method can be created by adding another TLC result.



5. At the **Parameters** tab, select the solvents (can have A/B, B/C, C/D as eluent), cartridge type, flow rate and rack type to be used. Enter a sample name for the run.

- a. To determine which cartridge type will be appropriate: For SNAP KP-Sil, it is supposed to be good for mixture that is 1 wt% of the column (3 wt% for Ultra). If we use a column that is not listed, we can always set it up using the appropriate volume and max pressure.
- b. Or while in the **Optimize** tab, click on cartridge a list will appear with column sizes and anticipated amount of desired product. Select column that predicts a larger amount of product that what you plan to have.



6. At the **Collection** tab, select the UV wavelengths and start threshold (can select collect all for UV inactive compounds).



- 7. At the **Gradient** tab, review the elution gradient and if needed, drag the blue line to modify the gradient. (Select the **equilibrate** button if elution of the column is desired.)
- 8. Load the cartridge and racks onto the system. Ensure that the tubing is securely attached.
- 9. Select **Setup** (on bottom right) and **Solvents** tab and make sure the solvents are filled. Check also to see how full the waste bottle is at this time.
- 10. Press Run.
- 11. In the **Run Parameters** dialog box, select the appropriate rack positions.
- 12. To start the run or equilibration, press Run.

- a. equilibration will prep your column with the eluent you have chosen.
- b. after this is complete check the column to make sure it has been completely prepped. If so, continue to step 13. if parts of it remain dry see step 12c for further instruction.
- c. Run more eluent through your column.
  - i. Select the Setup tab
  - ii. Select **Prime** (at the top) modify flow rate, cartridge type, and eluent system desired, and volume of eluent you deem necessary to sufficiently prime your column
  - iii. Press Start
- d. Check your column again. If sufficiently primed move to step 11. if not repeat steps 12c until column is ready.
- 13. After equilibration is done. Select the **Load sample** button and inject your sample onto the column. Afterwards, select **Gradient** to run the sample.
- 14. When column is running, click **Edit** to adjust elution gradient if needed. Air flush will start when the gradient is done. If the column is done early, click **Stop** to end the elution.

## **Processing the Data**

- 1. When the run is complete all the data will be stored under the **Results** tab.
- 2. In the **Results** tab select the different fractions collected, the gradient used, and the time of the entire run.