Standard operation procedure for HPLC (Small molecule column)

1) **Turn ON the machine** (switch on left side), choose **Manual** and **start the computer**.
2) **Refill the solvent bottle** (you are only allowed to start your experiment with a full solvent bottle)
3) **Empty the waste bottle** (check the provided waste canister)
4) **Carry out the clean up cycle** (follow instruction provided on the screen. Remember:
    1) Column valve must be in REJECT position
    2) Injection Valve is in LOAD position
    3) Add a flask below the cleaning ports (the last two tubes
5) Next, turn the **injection valve to LOAD position** and turn the valve for the Small Molecule **column to CONNECT**
6) **Place your glassware under the collection ports** (1-9, or as much as you will need, use your own glassware)
6) **Turn on the Laptop, start the program** 'JAJ Scan' to establish communication the with instrument.
7) **Connect the computer to instrument** ( ), open the settings () and set the detector to four wavelengths (

)between 200 and 800 nm) at which your moelcule absorbs (take a UV Vis spectrum with a different spectrometer before you, you won't be able to change this during the run)

Standard operation procedure for HPLC

1) **Dissolve your chemical compound in 5-7 mL** of chloroform (or other solvent) – For Chloroform, use the provided HPLC grade with Ethanol as stabilzier, chloroform with amylene is not allowed!)
2) **Filter your solution** at room temperature, using a filter with **pore size of 0.45 µm**
3) **Start the pump**: Set the flow rate of the pump and start the pump
    1) **10 mL/min for small molecule column**, write down the pressure in the provided HPLC notebook.

Load your sample :

1) **Wash the needle the eluent solvent :** Attach the needle to a syringe (10 mL) with a Luer Lock and take up 2-4 mL of chloroform and discharge the solvent (carfully wipe off the outside of the needle to remove any residues).
2) **Take up the filtered solution using the rounded needle.** Remove the air bubble using the provided stand with the filter paper, following the tasked carried out during training.
3) Remove the read cap from the injection valve at the HPLC. **In the LOAD position of the injection valve**, fully inject the needle into the valve (do NOT bend the needle at any time). Close the Sash (if possible) and slowly add the solution into the loop (watch the dicharge port, you will need to see solvent coming).

Standard operation procedure for HPLC

Add your compound to the HPLC column (Start separation)

1) While the **syringe is immersed into the valve**, turn the valve quickly to INJECT position, the sample is now added to the column, wait at least for 1 min* until your product is fully load onto the column.
2) **During this time, wash the injection port:** In the INJECT position of the injection valve, attach the provided white nozzle to a syringe (no Luer Lock) and wash the injection port (the Sash must be closed during this task)
3) **After cleaning, add the red cap** to back on the injection valve
4) **After the waiting time (>1 min),** turn the injection valve to the LOAD position.

*The waiting time for the injection can be a couple of minutes, if but don't use it during the reycling mode

Wait at the machine until the moelcule arrives at the detector:

4) **Monitor the detector signal:** Send the solvent to the waste container until the first product arrives at theUV Vis detector.
5) **RECYCLING MODE:** When the first compound is detected at the UV – Vis detector, press the RECYCLING botton to reinject the commpound into the column.
6) In this position, the compound will be recycled and will arrive at the detector every 20-25 mins (you are allowed to leave the room but you will need to check in every hour to monitor the separation – overnight operation is not allowed!)

Standard operation procedure for HPLC

Sample Collection

Once you are satisfied with the separation, you can collect your sample (whenever it arrives again at the detector):

1) Press the RECYCLING button to stop recycling mode, then hit by the **COLLECT** button to collect the chemical product in the flask (the machine will collect in Position 1, can be manually edited the colelction port in the dector setting)
2) Press **NEXT** if you want to move to the next position, press RECYCLE if you want to start the recyling again
3) Collect all compounds on the column (or send the peaks to waste in case you do not want to collect the molecule)

Notes for better sepearation:

After the first recycyling round, it is adviced to remove compounds from the column in case there is a clear separation.

If you see the compound arrarivg at the detecotr, hit the RECYCLING botton, followed by COLLECT. The solution will be colelcted in postion 1.

After the compound is collected (UV Vis trace is low), press the RECYCLING botton for purifying the other compounds on the column

Standard operation procedure for HPLC

Cleaning and shutting down

1) When you have completed the sepeartion, stop the pump and move the column valve to REJECT position
2) Run the Clean up cycle, following the provided instructions on the screen
3) **CLEANING**: Carefully remove the Teflon tubing and wash ALL teflon tubings you have used with chloroform and acetone
4) Empty the waste bottle of the HPLC
5) Turn off the computer and turn off the HPLC (switch on the left)
6) Close the Sash

Don’t forget to log your experiment in the notebook