INDIAN INSTITUTE OF TECHNOLOGY KANPUR
DEPARTMENT OF CHEMICAL ENGINEERING
PG Research lab

STANDARD OPERATING PROCEDURE

High Performance Liquid Chromatography

1. Switch ON the all the power switches.
2. Switch ON the components of the HPLC module by the push power buttons situated at the left bottom side (It gives green/yellow light indication by switching 'ON' the modules).
3. Start the PC and wait until all the indicators turn OFF in HPLC Module.

Status Light Color	**Status**
No light (and Module Power switch light is ON)	**Ready**
Yellow	**Not Ready condition**
Green	**Run mode/ Analysis running**
Red	**Error**

4. Prepare mobile phase as described in the analytical method and fill in the reservoir. Always use freshly prepared solvent (HPLC grade); especially use demineralized water which was filtered through about 0.2 µm filters.
5. Go to DESKTOP →Double click on CONTROL PANEL → Launch HPLC →New window will open.
6. Click on STATUS icon → Click **ON** at RIGHT SIDE in the instrument STATUS Tab & Make sure that all the modules are in **IDLE** state(In case any module is not showing idle then Go to particular tab of that module & click **OFF** then after 5 sec click **ON**).

7. Purging of lines: Open the purge valve of pump (by turning it anticlockwise (do not open fully otherwise black knob will come out).
8. Right click on Quat Pump Tab → go to Method → **Set FLOW RATE 5ml/min.**
9. Now select required solvent & make it 100% then APPLY. Wait until flow reaches to 5ml/min & then purge for 2 min.
10. Right click on Quat pump tab → go to Method → **Set FLOW RATE 0ml/min** → Wait until flow reaches to 0ml/min.
11. Do the same for all required solvent lines for purging. After purging each line close the purge Valve by rotating Clockwise direction (do not over tight Purge Valve).
12. Ensure that the required column is placed on the heat exchanger assembly in column compartment and connect the tubing in such direction that the flow is from pump to detector & before running sample the front cover of the column compartment should be close.
13. Right click on pump →Go to method → Set FLOW RATE 0.1-1ml/min.
14. Equilibrate the system for at least 30 minutes with required mobile phase (according to your sample run method) & Check baseline in software. If found stable, you can run the sample.

Prepare required standard and test solutions as described in the analytical method and fill the sample vials and keep in the sample tray.

(SAMPLE MUST BE FILTERED FROM 0.22micron SYRINGE FILTER).

Running of Samples

1. Click on STATUS icon→ Create new method→ Go to instrument setup→ Quat pump Set required flow rate→ Set Required solvents.

2. Go to sampler→ set injection volume as per requirement →set needle wash either flush port & set time 3 sec (make sure that outlet of needle wash is dipped in organic solvent reservoir like ACN).
3. Go to column compartment →enter the method parameters (like Temperature, etc.) as per the requirement.
4. Go to the detector → enter the method parameters (like wavelength) as per the requirement.
5. Save as the method with appropriate file name.
6. Go to SINGLE RUN and FILL the correct location of vial & sample name SELECT the ACQUISITION method. THEN RUN.
   (In case you want to abort the run then go to RUN QUEUE and click on STOP sign & DO NOT start next sample run until system becomes IDLE)
7. Go to STATUS tab and monitor the run.

SHUT DOWN PROCEDURE

1. Go to PUMP tab → Method → Set flow rate 0.5ml/min of ACN or required organic solvent.
2. Stabilize the column for 40-45 min.
3. Again go to PUMP tab → Method → Set flow rate 0ml/min of ACN & wait until it reaches to 0ml/min.
4. Click OFF at the right side of screen.
5. Switch OFF all HPLC Modules either in ascending or descending order.
6. Turn OFF Mains Switches at the back side of machine.
7. Turn OFF PC.