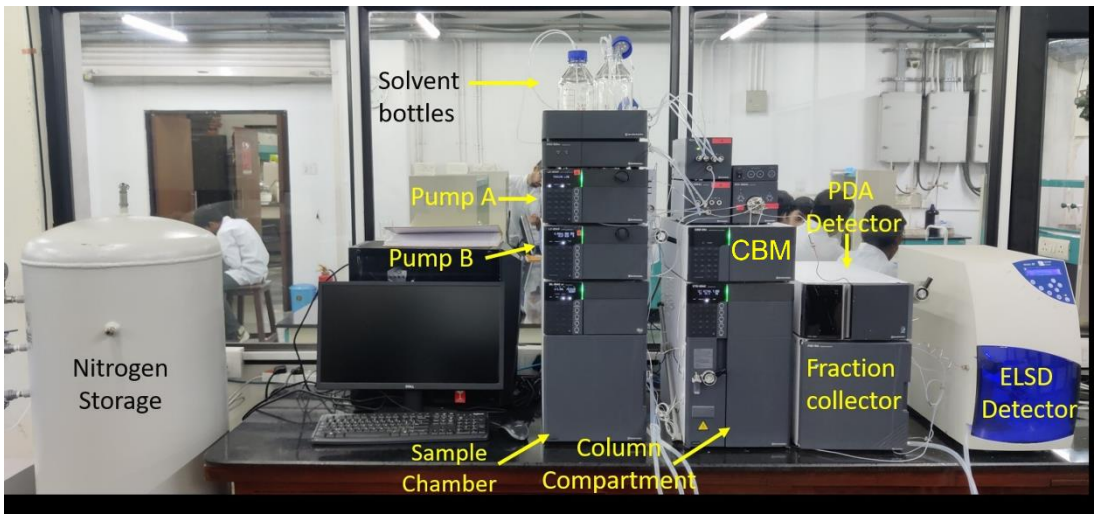
Standard Operating Procedure for GPC

A GPC (Gel Permeation Chromatography) instrument consists of the following key components:

1. **Solvent bottles:** Store the solvents used in the analysis
2. **Dual Pumps (A and B)**: Deliver the mobile phase
3. **Sample Chamber**: Holds the sample to be analyzed
4. **Controller and Balance Module (CBM)**: Manages the instrument's operating parameters, monitors the system's balance, and controls the flow rates, pressures, and temperatures to ensure precise and accurate analysis.
5. **Column**: Separates the sample components based on size
6. **PDA (Photodiode Array Detector)**: Detects and measures the absorbance of the separated components.
7. **Fraction Collector**: Divide the eluent into discrete fractions based on time or volume
8. **ELSD (Evaporative Light Scattering Detector)**: ELSD detects and measures components that don't absorb UV light, using scattered light to identify and quantify them



Protocols to run a sample in a GPC (Gel Permeation Chromatography) instrument:

1. Verify that the solvent level is above the filter level; if not, add the required solvent (ensure mobile phase sonication for 10 minutes prior to use).
2. Initialize the instrument by switching on the module switches and subsequently CBM.
3. Open the pump valve and purge the lines for 5-10 minutes to eliminate air bubbles. Similarly, purge the injection line to ensure bubble-free operation.
4. After purging is finished, close the pump valve, power on the PC, and launch LabSolution software; navigate to Instrument and HPLC, and set the flow rate to 0.2 mL/min and increase it gradually to 1 mL/min over 10 minutes.
5. Allow the solvent to run for a few minutes while monitoring the baseline on both detectors.
6. If the ELSD detector is not in use, maintain it in the off mode.
7. For ELSD detector operation, switch on the nitrogen generator, set the pressure to 3.5 bar and temperature to 50 °C, and enable the LED and nitrogen valve.
8. Once a stable baseline is achieved and the system is functioning nominally, connect the column and closely monitor the baseline and backpressure (70 – 80 kgf for 600 mm MIXED-D column) to ensure optimal system performance and maintain equilibrium.
9. Verify the parameters in the existing method file 'GPC-Org-THF_sample_RUN Method' located at {New volume (D)>2024>GPC_STD_Methods_DATA_600mm_column>Methods} to ensure these parameters match with those used for GPC standards to guarantee reliable and reproducible results for sample analysis.
   Parameters/settings:
   Sample run time: 22 minutes
   Pump A flow rate: 1.00 mL/min
   Pump B flow rate: 0 mL/min
10. Prepare the sample by dissolving 0.5-1 mg of the compound in 1 mL of THF or Water (dependent on the mobile phase), sonicate

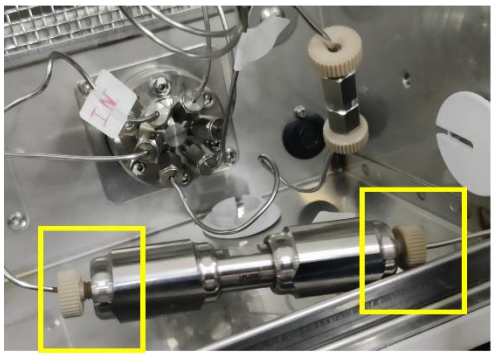
and filter through a 0.45/0.25 micron syringe filter. Place the sample in the sample chamber.

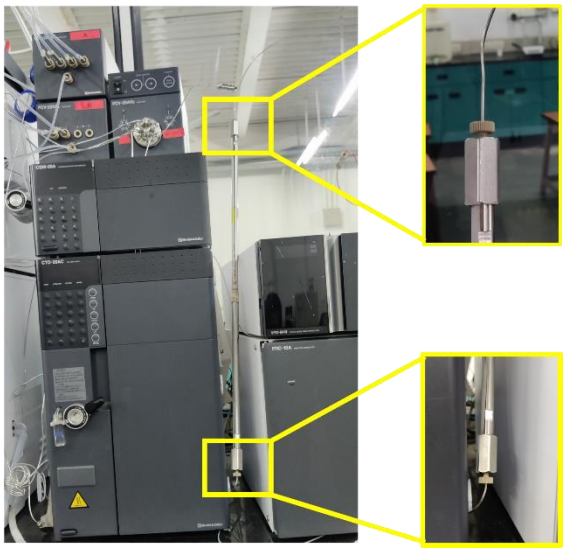
11. Create a batch file, enter sample details like sample name, injection amount, and method file, and then start the sample run.
12. After the sample run is complete, carefully remove the column, place stoppers on both ends and store it in the designated drawer. Then, connect the joint line.
13. Switch the mobile phase to IPA, purge the line for 5-10 minutes, and run the mobile phase for at least 30 minutes through the system. Store the previously used mobile phase in a capped bottle with the date labelled.
14. To turn off the instrument, Turn off all instrument switches and the nitrogen generator switch.
15. To determine the sample's molecular weight via GPC post-run analysis:
    - Open the sample data file in GPC postrun.
    - Load the calibration method file 'GPC-Org-THF_Calibration_600mm column' from the location: {New volume (D)>2024>GPC_STD_Methods_DATA_600mm_column>Methods}.
    - Proceed with molecular weight analysis using the loaded calibration method
16. Finally, shut down the computer and dispose of waste solvent in the waste drum and cap the waste bottle to prevent solvent vapor accumulation in the room.

Do’s and Don’ts before you begin:

1. Always fill in the logbook kept near the GPC with the appropriate comments.
2. Always write the date on which a new THF bottle (with stabilizer) was opened with a marker.
3. Do not inject high volumes (> 10 μL), as it might saturate the detector (especially the sensitive ELSD).
4. Do not operate ELSD detector beyond 50 °C.
5. The mobile phase should be sonicated to remove air bubbles.

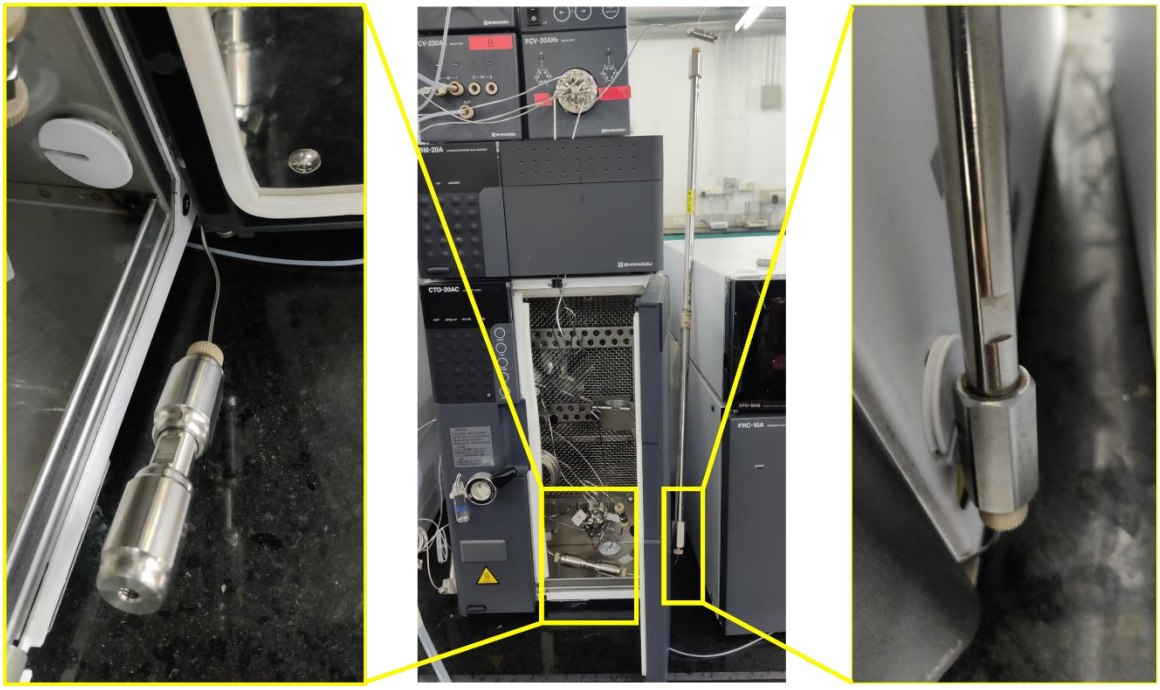
6. Sample preparation protocol: Weigh 0.5 – 1 mg of sample (do not add approximately), add HPLC grade THF (with stabilizer), sonicate the sample vial and filter it through 0.45/0.25 micron syringe filter into another clean HPLC vial and then perform analysis. Please ensure that the sample is fully dissolved at the operating temperature (20 °C) for at least 30 minutes.
7. Always check the level of solvent in the amber coloured bottle and also the level in waste bottle before you begin.
8. When utilizing a 300 mm column, it fits snugly within the designated column compartment. In the event of a leak at the column connection point, the compartment's indicator displays a warning signal, illuminated in red. Yellow boxes in image inculcates possible leakage point.
9. Due to size constraints, the 600 mm column cannot be housed within the compartment and must be installed externally. Ensure the compartment door is closed before initiating a sample run; otherwise, the process will not start. Since the column is outside the compartment, the automatic leak detection system is inoperable. Therefore, manual checks are essential. Verify all potential leakage points (illustrated below) before starting the sample run.





10. To attach the column, first install the guard column inside the compartment, then connect a small tube to it, ensuring the door can close properly, and finally attach the main column outside the compartment to this connection tube. Check below image for

reference.



Upon noticing leakage, inspect ferrule threads by removing it. If undamaged, reattach and tighten firmly to eliminate leakage. *Do not use a wrench/spanner for tightening the guard or normal column*.

For more details, please go through Agilent column user guide.