



SEC-MALS HPLC cleaning protocol (9-4-2023)

Occasionally, the instrument may become contaminated due to the use of buffer/aqueous solutions. This will be obvious on the MALS, as the noise, wander, and drift will not reach their green set points even after long equilibration times. The following deep cleaning protocol should get the HPLC back into working order.

1. Prepare a 1 L solution of “magic mix”: 1:1:1:1 MeCN:MQ H₂O:Isopropanol:Methanol with 0.2% formic acid, sterile filter.
2. Disconnect any columns, remove the PEEK tubing connection going to the MALS, and cap the input port on the MALS.
3. Place a line of PEEK tubing from the outflow port on the UV VIS into the waste collection bottle.
4. Place both A and B lines into magic mix bottle
5. Purge each pump separately:
 - a. Open purge valve, set %B to 0, flow at 5 mL/min for 5 min
 - b. Stop flow, set %B to 100, flow at 5 mL/min for 5 min
6. Stop flow, close the purge valve
7. Set %B to 50, set flow to 1 mL/min
8. Flow for a minimum of 2 hours, ideally longer (4 hr to overnight)
9. Prepare a fresh 1L bottle of MQ H₂O
10. Stop flow, carefully switch both A and B lines into MQ H₂O
11. Purge both pumps as above
12. Stop flow, set %B to 0, close purge valve
13. Set flow to 1 mL/min, flow for 5 min into waste
14. Stop flow, connect output line directly to MALS
15. Resume flow at 1 mL/min, allow MALS to equilibrate, monitor noise, wander, drift to confirm successful cleaning of HPLC.

Column Cleaning protocol

1. Prepare 0.5 – 1 L Na₂SO₄ pH 3.0 (Buffer A)
2. Prepare 0.5 – 1 L 20% MeOH in 50 mM phosphate pH 7.0 (Buffer B)
3. Disconnect column from downstream detectors and guard, reconnect column only in reverse flow direction. Make sure the column is flowing directly into waste
4. Flush the column with buffer A at 0.25 mL/min for 10 CVs (~120 mL)
5. Flush the column with MQ H₂O at 0.25 ml/min for 5 CVS (~60 mL)
6. Flush the column with buffer B at at 0.25 mL/min for 10 CVs (~120 mL)
7. Flush the column with MQ H₂O at 0.25 ml/min for 5 CVS (~60 mL)
8. Stop flow, disconnect and flip the column, re-connect the guard and downstream detectors
9. Flow at 0.5 mL/min in MQ H₂O monitoring the MALS signal for improved wander/noise/drift