**HPLC Start-up procedure**

1. Turn on detector
2. Turn on pump if not powered on
3. OPEN Logger Lite 1.6.1
4. Change time duration to 3000 (or as needed) seconds
   1. Click ‘Experiment’ > Click ‘Data Collection’ > Change DURATION from 30 seconds to 3000 seconds > Done
5. Zero Detector on machine
6. Zero detector on computer:
   1. Click ‘Experiment’ > Click ‘Calibrate’ > Select ‘Go!Link 1: Instrumentation Amplifier’
   2. Check ‘One Point Calibration’ > Click ‘Calibrate Now’ > Enter Value in Data Points: 0 mV > Click ‘Keep’ > Done
7. Check that reagent is 20% acetonitrile (ACN)
8. Run 20% ACN through column:
   1. Check that FLOW RATE is ≤ 0.20 mL/min
   2. Check that PURGE VALVE is closed
   3. START pump (***NOTE****: You should notice the pressure increases)*
   4. Slowly INCREASE FLOW RATE to 1.00 mL/min
   5. Allow column to rinse for 10 minutes
   6. Slowly DECREASE FLOW RATE to < 0.1 mL/min
   7. STOP pump
9. Purge lines:
   1. OPEN purge valve
   2. SWITCH reagents from 20% ACN to Mobile Phase (0.10 M NH4OH2, 3% ACN)
   3. START pump
   4. Slowly INCREASE FLOW RATE to 1.50 mL/min
   5. Allow pump to run for 7 minutes
   6. Slowly DECREASE FLOW RATE to < 0.10 mL/min
   7. STOP pump
   8. CLOSE purge valve
10. Prepare for sample injections:
    1. START pump
    2. Slowly INCREASE FLOW RATE to 1.00 mL/min
    3. Allow Mobile Phase (0.10 M NH4OH2, 3% ACN) to run through column for 10 minutes
11. Proceed to Sample Injection procedure

**HPLC Sample Injection Procedure**

1. Check that injection valve is in the LOAD position. If not turn into the LOAD position.
2. Rinse LOAD SYRINGE three times with water
3. Rinse INJECTION VALVE three times with water
4. Zero detector on computer:
   1. Click Experiment > Click Calibrate > Select Go!Link 1: Instrumentation Amplifier
   2. Check ‘One Point Calibration’ > Click ‘Calibrate Now’ > Enter Value in Data Points: 0 mV > Click ‘Keep’ > Done
5. Dilute time points with 50-µl H2O
6. Take up 25-µl of first sample into load syringe
7. LOAD 25-µl sample into injection valve
8. Quickly turn injection valve to INJECT position
9. Click on ‘COLLECT’ in Logger Lite 1.6.1
10. When peak returns to base line STOP data collection

***NOTE:*** *Make sure to record each injection on injection log.*

1. Remove and RINSE load syringe three times with H2O *(****NOTE:*** *Only rinse load syringe in between sample injections, NOT injection valve.)*
2. Return INJECTION VALVE back to LOAD position.
3. Repeat steps 6-11 for next sample, however after you click ‘COLLECT’ you will be asked to ‘STORE LASTEST RUN’.

**HPLC Shutdown procedure**

1. Turn off detector
2. Slowly DECREASE FLOW RATE to < 0.1 mL/min
3. STOP pump
4. Purge lines:
   1. OPEN purge valve
   2. SWITCH reagents from Mobile Phase (0.10 M NH4OH2, 3% ACN) to 20% CAN
   3. START pump
   4. Slowly INCREASE FLOW RATE to 1.50 mL/min
   5. Allow pump to run for 7 minutes
   6. Slowly DECREASE FLOW RATE to < 0.20 mL/min
   7. STOP pump
   8. CLOSE purge valve
5. Run 20% ACN through column:
   1. START pump (***NOTE****: You should notice the pressure increases)*
   2. Slowly INCREASE FLOW RATE to 1.00 mL/min
   3. Allow column to rinse for 10 minutes
   4. Slowly DECREASE FLOW RATE to < 0.1 mL/min
   5. STOP pump