c. **After a few minutes, the instrument will be done initializing, and then Open Vasudevan Lab (Online) by clicking the icon on the desktop.**

d. **Check solvent and waste bottles.**

i. Check the four solvent bottles on top of HPLC and make sure there is enough solvent to run your samples.

1. If you fill the solvent bottles, adjust the volume levels in the program. The instrument will shut down if the solvent bottles get below a set point.

a. Left click on the "Bottle" icon and enter new volumes.

b. Click OK.

c. Be sure there are no bubbles on the filter. If there are, open the purge valve (black knob located on front of the pump module—turn it left to loosen but do not turn it all the way) and pump the mobile phase at 5ml/min. until the bubbles have been removed. Stop the pump and close the purge valve. (See Section "b" for how to purge the system).

ii. Check the waste bottle on the floor. If full, change bottle and bring the full bottle to the stockroom for disposal, following proper protocol.

e. **Prepare buffer and place buffer line in buffer solvent bottle.**

i. **Filter buffer solution.**

ii. Remove buffer line from methanol solvent bottle and thoroughly rinse it with DI water. This will avoid contaminating the buffer solution.

iii. Use Kim-Wipe to gently remove any excess water from solvent line and glass frit.

iv. Place line in buffer solvent bottle.

v. Check for air bubbles in the solvent line and glass frit. If bubbles are present, make sure they are removed when you purge the line.

b. **Load purge method and flush out buffer line** (File > Load > Method).

i. **Open purge valve** (black knob located on front of pump module).

ii. Load purge method - c:\Chem32\1\methods\purge.m.

iii. Verify the flow rate is set to 0.00 mL/min (Right click on the pump section> Method to adjust the flow).

iv. Turn on pumps (Right click on the pump section>Control>On).

v. Set the buffer pump to 100 % and the flow rate to 5.00 mL/min.

vi. Click OK.

vii. Flush line for a couple minutes. Make sure all the air bubbles are out of the line.

viii. Set the flow rate back to 0.00 mL/min.

i. This method will stabilize the column with the solvent mixture used in the analysis (70% buffer/30% ACN).

e. **Load rampup sequence** (File > Load >Sequence

2. Draw speed and eject speed can range from 10ul/min to 1000ul/min. **Typically 500ul/min is used.** Default is 100ul/min. More viscous samples should be injected more slowly.

iii. DAD Signals

1. Signals
    a. Check the boxes to activate one (or more) of the signals (A-E).
    b. Enter the wavelength at which the absorbance of the sample will be measured.
    c. Enter a bandwidth (determines the wavelength range over which the absorbance is measured).
    d. Reference is the wavelength at which a reference absorbance is measured.
    e. BW is the bandwidth of the reference wavelength.
2. Spectrum
    a. Defines at which point on a signal a spectra will be taken and saved.
        i. Select “All” - spectra will be taken continuously.
3. Stoptime
    a. Time at which the DAD stops an analysis. Set this to “As Pump/Injector” so it will stop when the pump stops. This will not turn off the detector.
4. Required Lamps
    a. Select both UV and Vis.
5. Autobalance
    a. Baseline is reset to zero either before or after a run. Select

1. Not necessary to check any of these unless you want more curves displayed on your chromatogram.

viii. Run Time Checklist.

1. Select Data Acquisition and Standard Data Analysis.

d. **Save method** (File > Save As > Method). **Always save to METHOD and not your subdirectory.**

i. Name your method with your initials before the method name, e.g. BSPYRIDINE

4. Create/Edit Sequence

Note: To run just a single sample, see the FAQ section for instructions.

a. **The Offline Mode program should be in <u>Method and Run Control</u> view for the following steps.**

i. Open "Vasudevan Lab (Offline)" by clicking the desktop icon.

ii. Go to View > Method and Run Control.

b. **Select Sequence Task**  **icon.**

c. **Load sequence** (Sequence > Load Sequence Template).

i. Or create a new sequence (Sequence > New Sequence Template).

d. **Edit Sequence Table** (Sequence > Sequence Table).

i. If you have several samples to enter, use the Insert/FillDown Wizard. If you have only a few samples, skip to step ii and enter information manually.

1. Click Insert/FillDown Wizard button at the bottom of the Sequence Table window.
2. Select "Insert", enter the number of lines to insert, starting location of the vials, method name, and injection/location. In step ii, fill in any information the wizard did not do automatically.
3. In "List of Detected Ranges", select the one that is present.
4. Click OK.

ii. Enter information for your first vial in the sample table.

1. Location – location of sample in autosampler tray.
2. Sample Name – name of sample.
3. Method Name – select method.
4. Inj/Volume – number of injections per vial.
5. Sample Type – Sample/Calibration/Control -

3. Click OK.

g. **Run sequence** (Run Control > Run Sequence).

i.

iii. Place buffer line in methanol solvent bottle.
iv. Check for air bubbles in the solvent line and glass frit. If bubbles are present, make sure they are removed when you purge the line.

h. **Load purge method and flush out buffer line with methanol** (File > Load > Method).
i. Turn off pumps (Right click in the pump section>Control>Off).
ii. Load purge method (c:\Chem32\1\methods\purge.m).
iii. Verify the flow rate is 0.00 mL/min (Right click in pump section>Method).
iv. **Open purge valve**.
v. Turn on pumps.
vi. O 1 153.26 587.86 T4-BT

HPLC 1100 Frequently Asked Questions

1. How do I run a single sample?

a. **Program should be in <u>Method and Run Control</u> view for the following steps.**
   i. Go to View > Method and Run Control.
b. **Follow the instructions, but skip Create/Edit Sequence and replace the Start Sequence section with the following steps**.
c. **Enter Sample Information** (RunControl > Sample Info).
   i. Enter Operator Name, Subdirectory (where the data will be stored), Filename, Location (where the vial is in the autosampler), Sample Name, and Comments. Then click "OK" or "Run Method" **OR Run sample** (RunControl > Run Method).

2. How do I change the sequence table while the sequence is running?

b. **Program should be in <u>Method and Run Control</u> view for the following steps.**
   i. Go to View > Method and Run Control.
c. **Pause the sequence** (RunControl > Pause Sequence). The sequence will pause once the current method is complete.
d. **Edit sequence table, click OK**.
e. **Resume the sequence** (RunControl > Resume Sequence).

3. How do I configure the screen layout so all necessary windows and options are present?

a. **Program should be in <u>Method and Run Control</u> view for the following steps.**
   i. Go to View > Method and Run Control.
b. **View** > **Short Menu** ("Short Menu" should be displayed, meaning Full Menu is selected).
c. **View** > **Sampling Diagram** (ALS tray).
d. **View** > **System Diagram** (system layout, online plot, and LC parameters).
e.
f. **go to View > Online Spectra.**
g. **Program should be in <u>Data Analysis</u> view for the following steps.**
   i. Go to View > Data Analysis.
h. **View > Command Line** (allows you to enter macros).

4. How do I find lambda max for a spectrum?

a. **Program should be in <u>Data Analysis</u> view for the following steps.**
   i. Go to View > Data Analysis.
b. **There is a macro that will find lambda max of the spectrum that is displayed**.
c. **Select Spectral Tlq0.000009 2 0 6 2 reW*n3 1 ln 9 reW*nBTCs(stio)8ns)]TJETQq0.000009 2 0 6 2 reW*nB**

i.

a. **When you start OpenLAB, there is an initialization process that initializes the instrument modules**