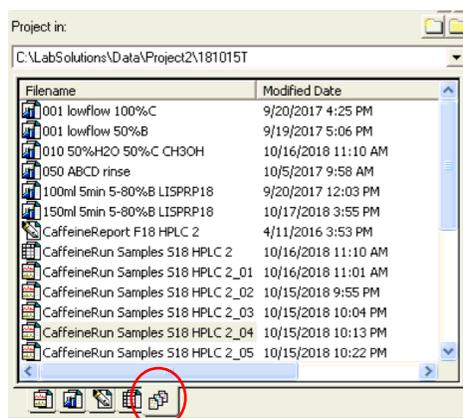


Instructions for processing data on the HPLC using LC Solution software.

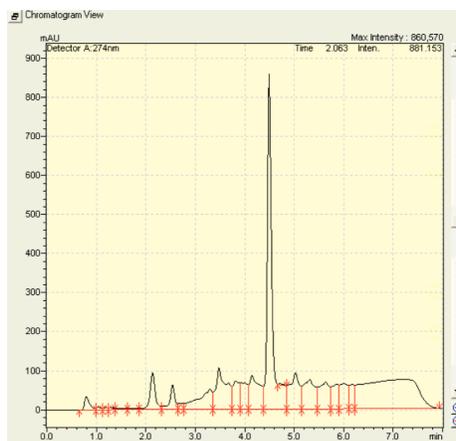
1. Open two **Postrun** windows, one for the caffeine report and one for data analysis (instructor will do this ahead of time).



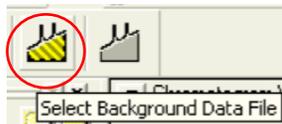
2. At the bottom left window, select the All Files tab at the bottom (check this button often, to be sure all the file types are shown in the window)



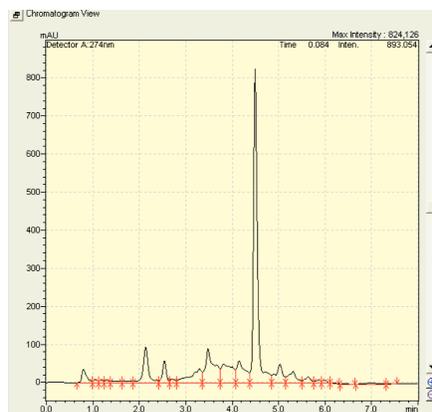
3. Double-click on the filename that corresponds to your sample - check with your instructor and consult the sign-up list. The file should open and display something similar to this:



4. Subtract the blank sample. Click on the **Select Background Data File** button (with yellow stripes) and select the filename that corresponds to the "blank sample" (check with your instructor and/or consult the sign-up sheet)



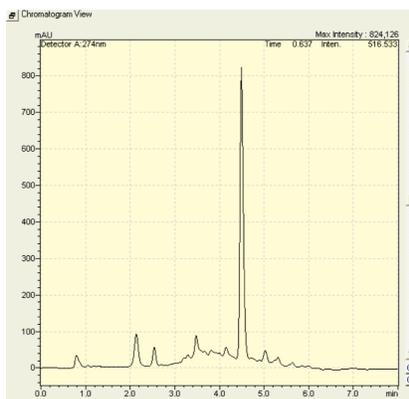
The resulting chromatogram should look like this - the baseline should appear more level:



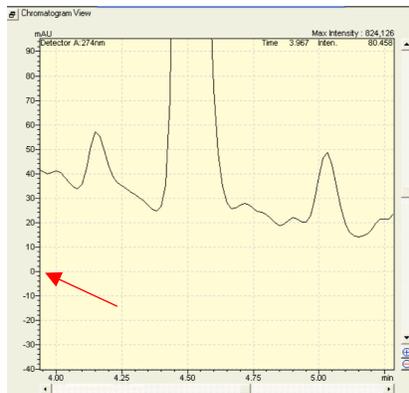
5. If not already visible, on the **View menu**, select **Manual Integration Bar**, and select the **Reject Peaks** button (8th button from the left).



6. Position the vertical cursor on the far left of the chromatogram (near 0.0 min) and left-click once, which will produce a blue vertical cursor, then left-click the left mouse button once near the far right side of the chromatogram. The result should be that all of the red integration markings are gone, repeat if necessary to remove any remaining red marks, then finally click the **Reject Peaks** button again (to turn it off) and proceed to step 7:



7. Zoom in around the base of the peak of interest - in this example the one around 4.5 min. Do this by moving the cross-hair cursor just to the left and above the base of the peak of interest, then hold and drag the cursor to the right and just below the zero (0) baseline - note that zero is visible on the vertical axis (red arrow):

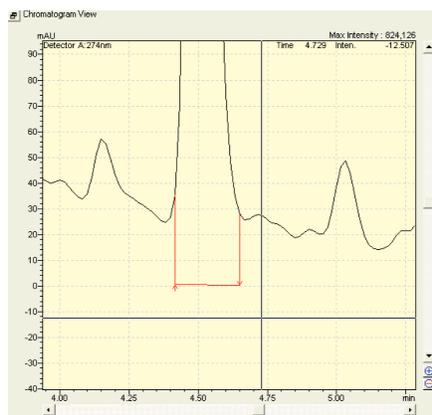


If the zoom box is incorrect, then right-click the mouse in the chromatogram window and select **Initialize Zoom** and start over.

8. On the **Manual Integration Bar**, select the **Insert Peak Start/End** button (4th from the left).



Position the cursor on the left so that the horizontal cross-hair lines up with 0.0 and that the vertical cross-hair lines up with the left-most side of the peak of interest, then left-click the mouse once. Move the cursor to the right side of the peak and while the horizontal cross-hair lines is on zero (0) and the vertical cross-hair lines up with the right-most side of the peak of interest, then left-click the mouse once. The result should look like what's below:

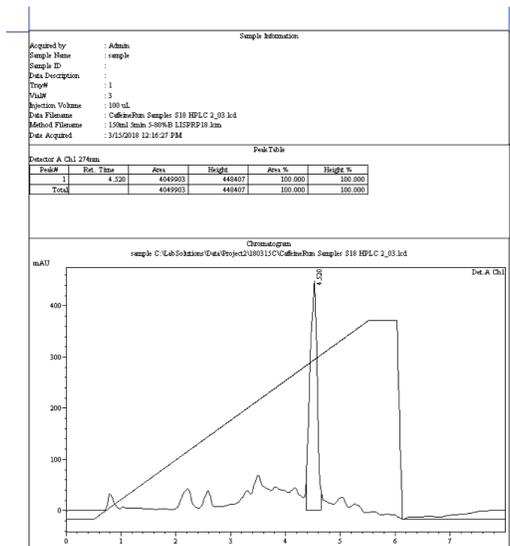
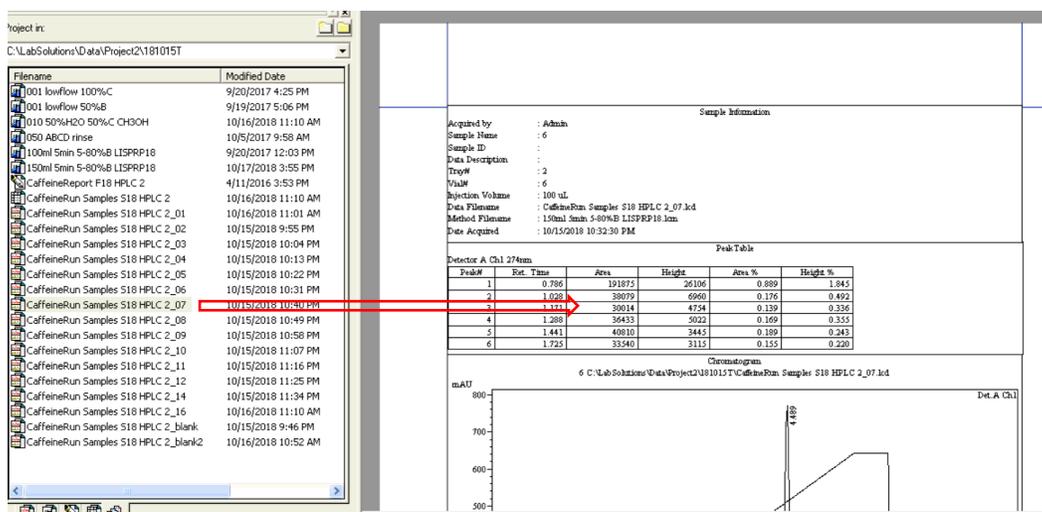


If the resulting integration doesn't look correct, then use the **Reject Peaks** button as before (steps 5 and 6) and then try step 8 again.

9. Save the file by clicking on the **Save** button, second button from the left.



10. In the second post run window, find your newly saved file in the file list and using the mouse, drag (don't double-click) the file into the report window at the right. Your file should now be shown in the window with the integration (should only be one peak) and all of the file information at the top.



11. Print the report by clicking on the Print button - make two copies if a partner needs one.

