NOTICE

Check solvent levels
Record the VOLUME in the bottles you are using
Example: C=100mL, D=350mL

BEFORE YOU START

YOU

ARE

RESPONSIBLE

- If the solvent drops below the frit and the UPLC pump is damaged.
- Understand how much solvent your sequence will use.
- Loss of solvent prime may require instrument down-time to correct!

Don't be the one that causes delays for yourself and everyone else.

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Using the Walk-Up Instrument LC/MS Waters QDa

Revision *date:* May 23, 2024

1 Best Practices

1) Naming of files – avoid special characters except _ and also avoid [space]
   a) Data: month day year _ run number _ sample_misc.
      i. Example: 042023_r01_stuff_1uLinj
   b) MS method: month day year, mode, m/z range, runtime
      i. Example: 042023_posesi_150_600_12min
   c) Inlet method: month day year, user, FIA/UPLC - %solvent, solventID, runtime
      i. Example: 042023_CM_uplc_gradient90AC_12min

2 Overview

1. Things to think about before starting:
   a. Will you be using FIA (flow injection analysis) or LC/MS?
      i. Typical flow-rate (0.3-0.8 mL) and maximum (2mL/min)
   b. Which ionization mode to use
      What mode has worked for similar samples in the past?
   c. Solvent system
      Your sample MUST be soluble in the mobile phase composition (ranging from 90% water to 100% acetonitrile at pH ~3)
   d. Appropriate concentrations
      Guideline is approximately 1 mg/mL
2. Create an isotope model
   a. Use software of your choice (molecular weight calculator; masslynx; other)
   b. Use your molecular formula to generate what the isotope envelope will look like – this helps you determine if you are getting the right signal.

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c. Consider Na adducts, and remember that ESI creates protonated species.

3. Computer guidelines
   a. User login – use your group login so your methods/data are stored together

Kirk-group, Kirk	Aronoff-group, Aronoff!
Walker-group, Walker	Rack-group, Rack!
Whitten-group, Dave!	Olivier-group, Jean!
Gold-group, Gold!	

4. QUESTIONS?
   a. Please ask!
   b. NOT SURE if something is 'normal' – ask!

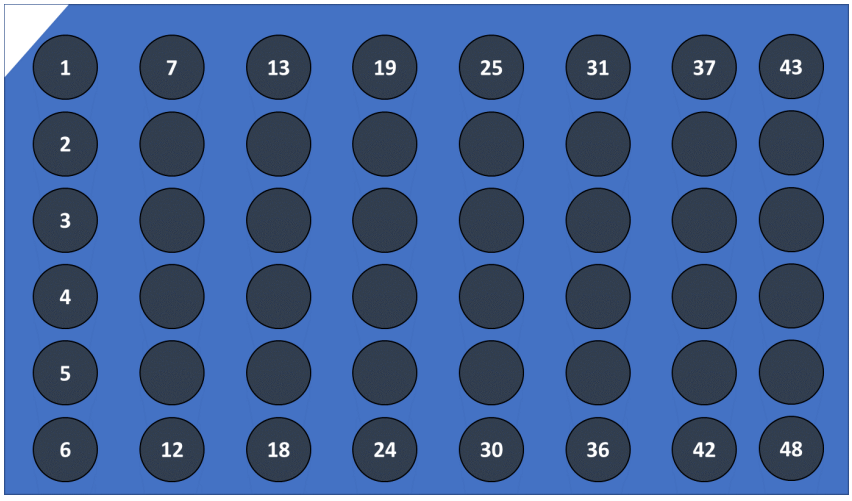
3 Running Samples

3.1 FIRST!!

1. Check solvent levels – estimate the volume you're going to use – is there enough?
   a. Ask MSF personnel if you're not sure

3.2 Load Sample and start Masslynx software

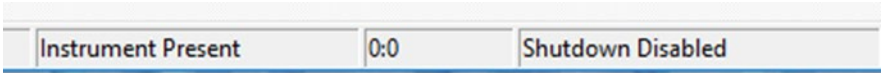
1. Load your sample
   a. Note tray position (1 or 2) and position IN the tray:



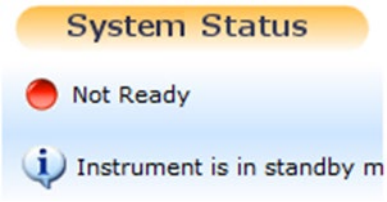
For tray position 1 and vial #1, your entry in sample table = "1:1"

2. Login to computer and prepare sample table
    a. Start Masslynx

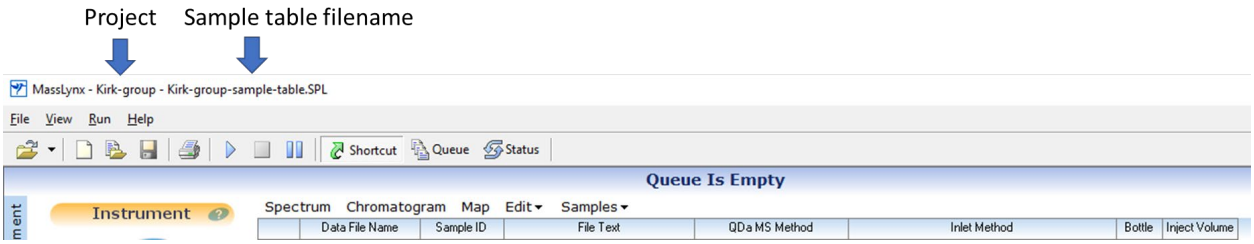
    b. Message at bottom of Masslynx window should show “Connecting to instrument” and then “instrument present” for normal operation.



    c. System Status Message (lower left) should show ‘instrument is in standby mode’ and red ‘not ready’ light (system status lower left)



3. Open your PROJECT and sample table
    a. Masslynx window, File, Open Project, select your project (****.PRO).
    b. D:\UNM-QDA-2023\CCB*your-group-name*
    c. File, Open, to load your group (or personal) sample table (****group.SPL)



    d. Add or create entries for your samples in the sample table
        i. Best practice: **filename** has date and run number at beginning.
            1. Example: 022423_R01_sampleinfo
            2. Note!! No special characters. _ is ok
        ii. File text - all characters are fine (this is a comment field)
        iii. Verify QDa MS and Inlet methods, vial location, and injection volume
            1. There is a 40-character length limitation on the names of MassLynx MS methods (.exp files)

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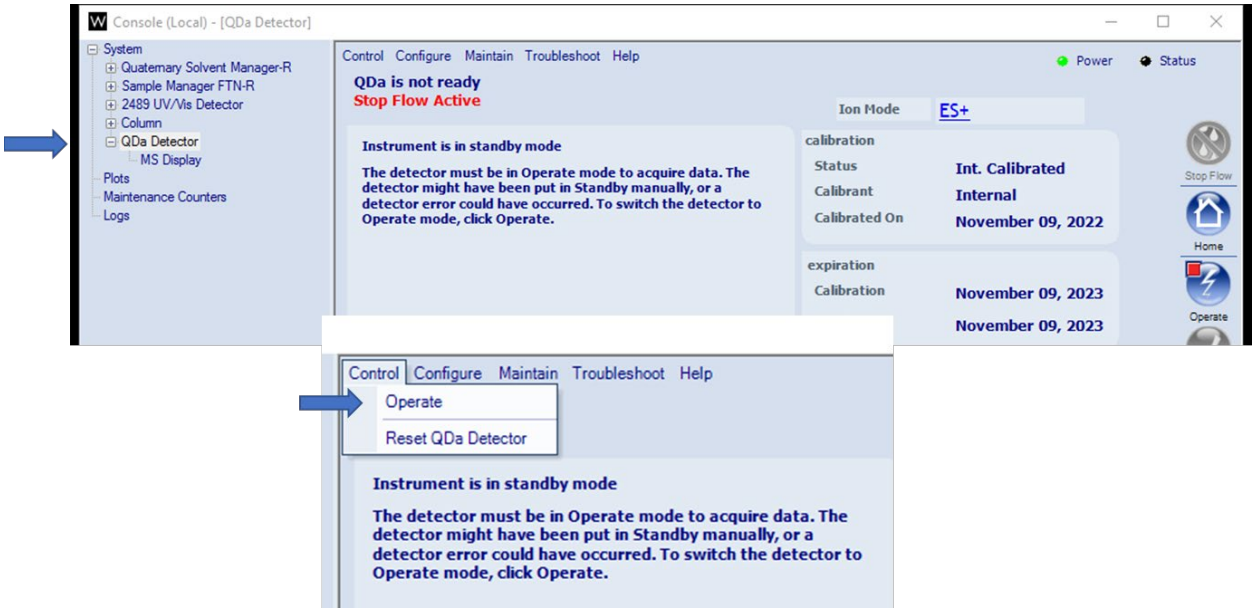
2. There is a 55-character length limit for function names in MS Method

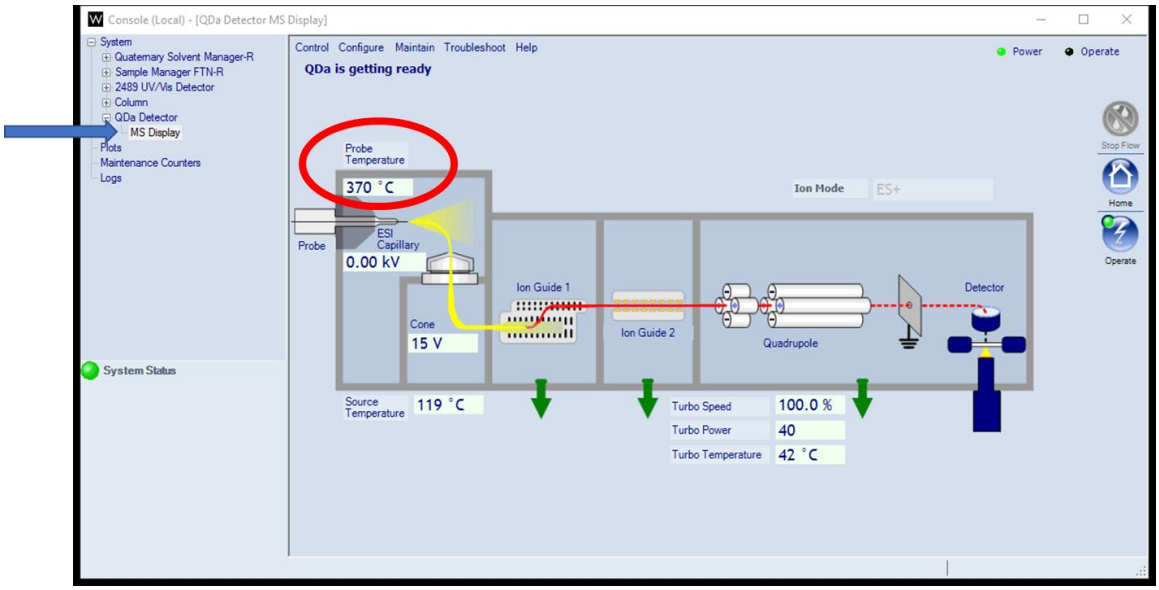
3.3 Prepare Instrument for operation

1. Instrument tab: Open MS console

a. Select QDa detector, Control menu, 'operate'

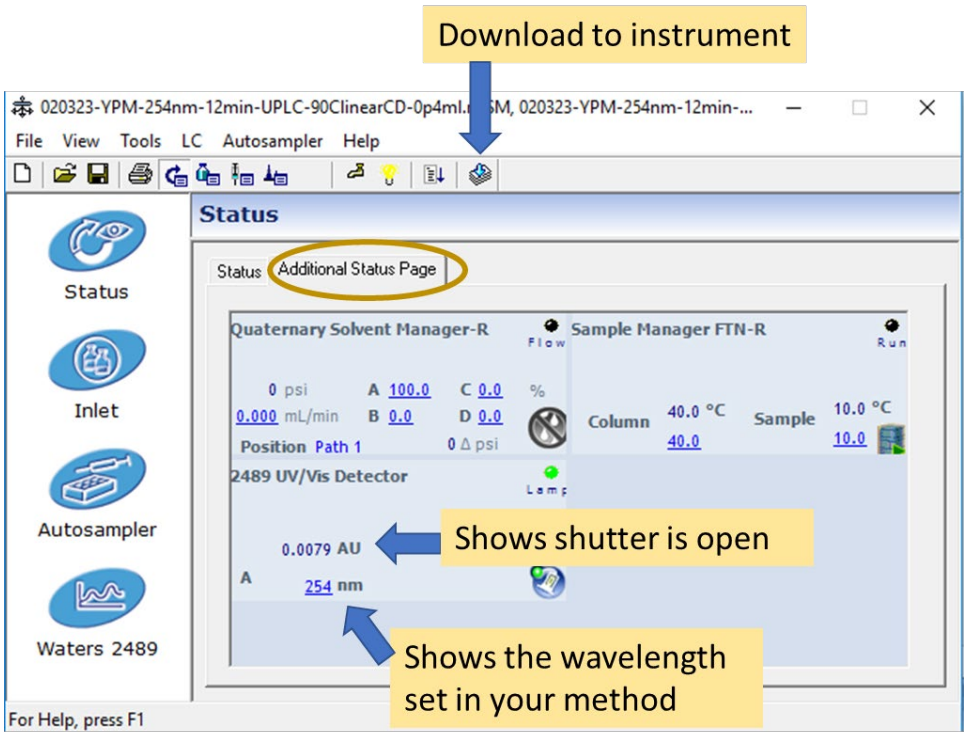


b. Select "MS display" – probe temperature should increase – it will go to 600°C.



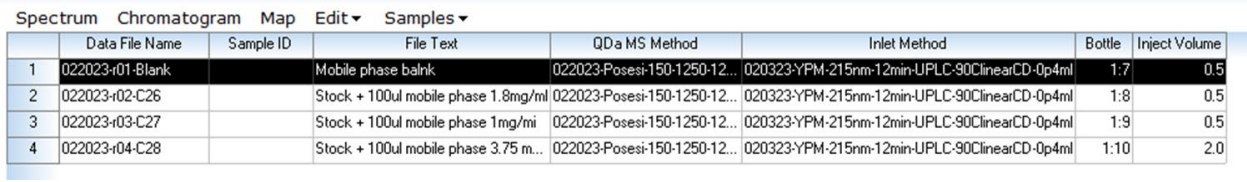
2. Masslynx main window: Open 'inlet method'

   a. Inlet method window
      i. File, Open your method
      ii. Download to the instrument
      iii. Go to "additional status" page
         1. Record flow, pressure, etc. in log book
      iv. Confirm 'lamp' indicator on UV/VIS detector is green

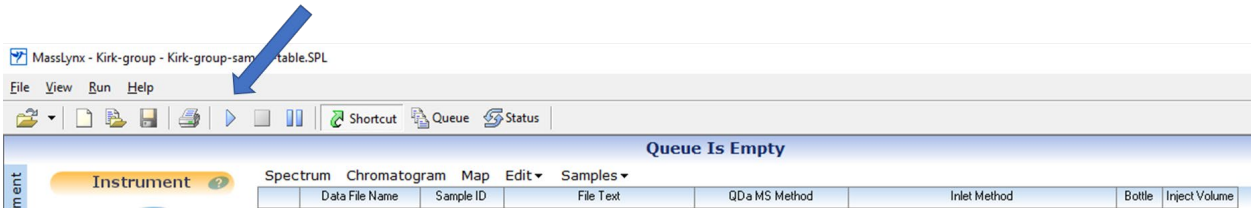


3.4 Run Sample(s)

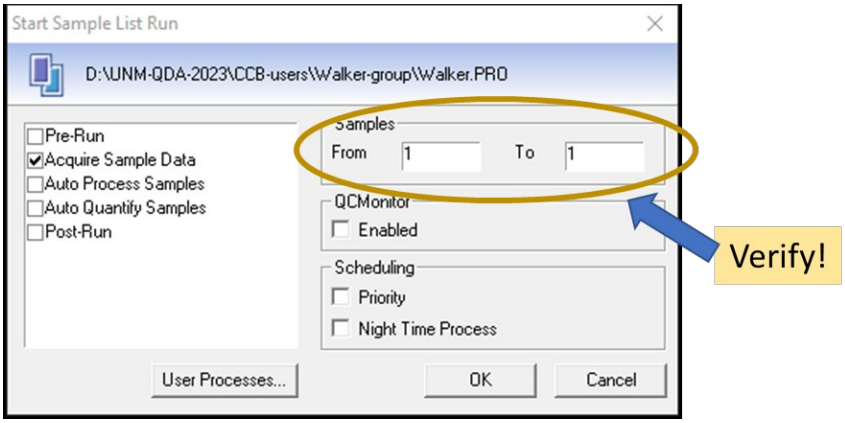
1. Masslynx main window
    a. Select rows for injection



    b. Click on 'arrow' to start

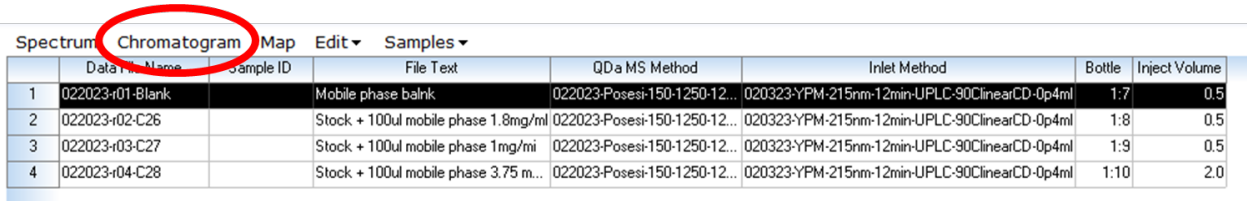


    c. "save changes to sample table" – answer YES
    d. confirm rows in the sample table that you want to run, click ok



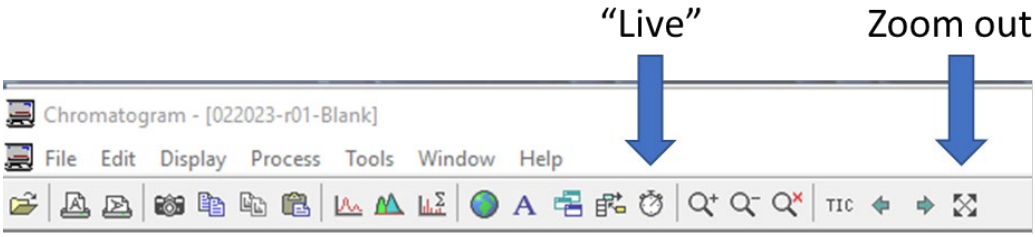
3.5 Display data while running

1. Select sample in run table
2. Click on 'chromatogram'



3. Right click on TIC data to display MS data

4. Select 'live' clock icon if data is not updating

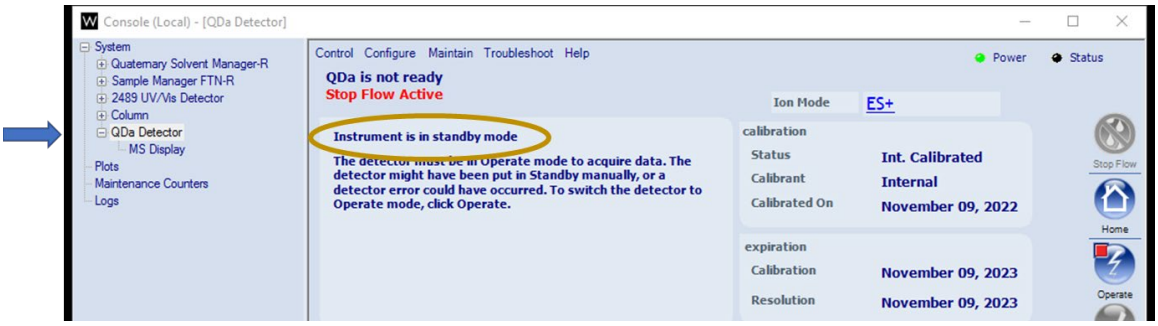


4 Auto Shutdown / cleanup

The instrument is set to perform 'auto' shutdown after each batch.

This process may take **up to 2 minutes**.

Wait for the MS console to show that the QDa detector is in standby mode:



IMPORTANT:

1. Close the Console window
2. Close Masslynx
3. Please take your samples back to your lab.
4. Please 'sign out' of the computer

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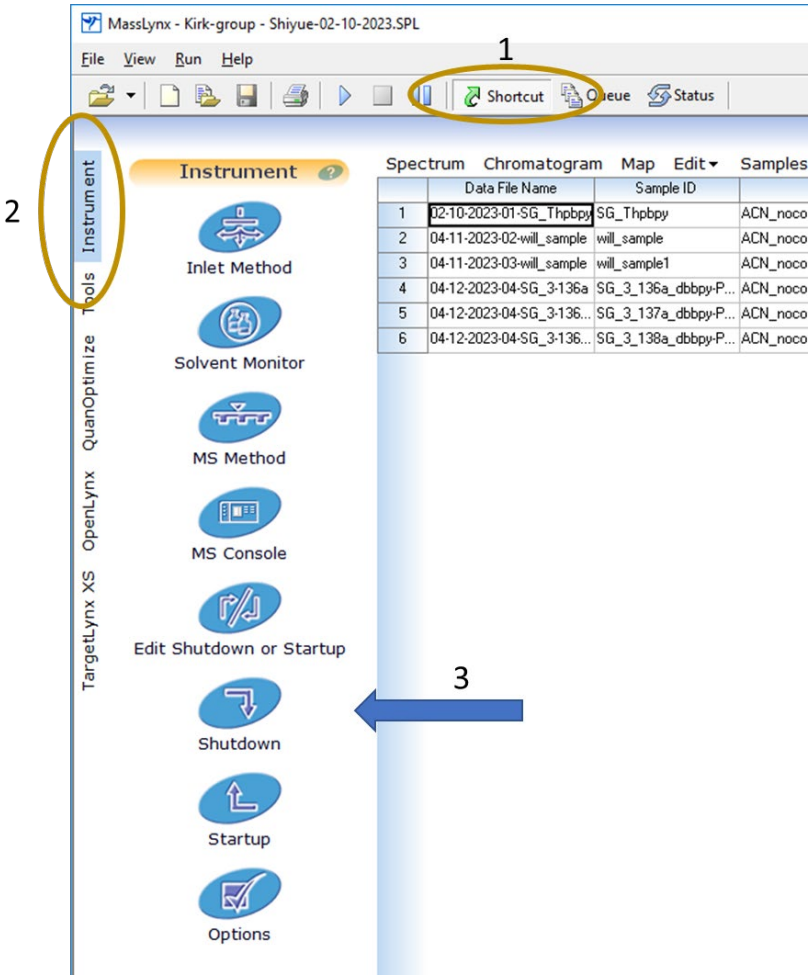
5 Manual Shutdown / cleanup

IF the auto-shutdown was not set – follow this procedure.

In main Masslynx window, select "shortcut" menu, click on "instrument" tab, **click** on "shutdown", this process may take **up to 2 minutes**.



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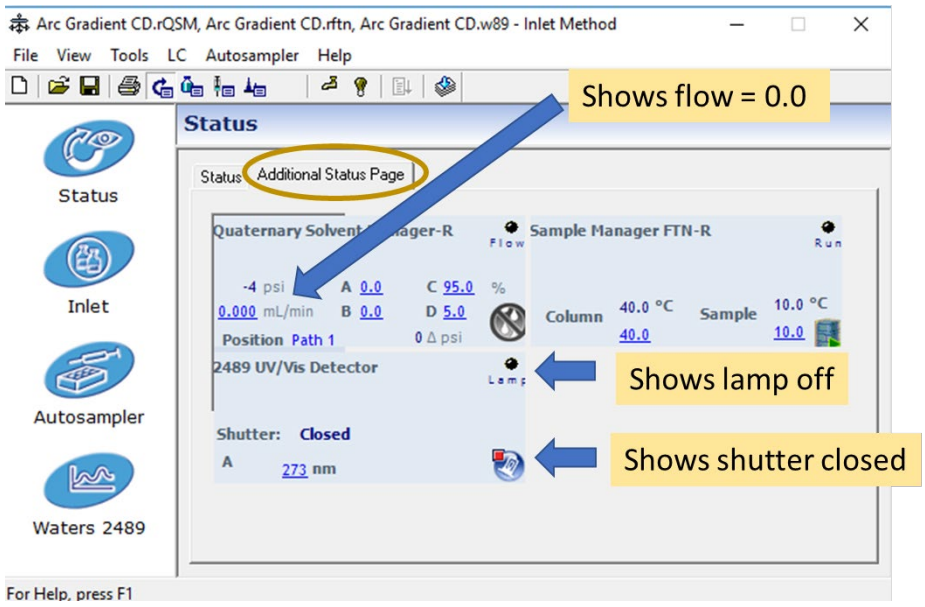
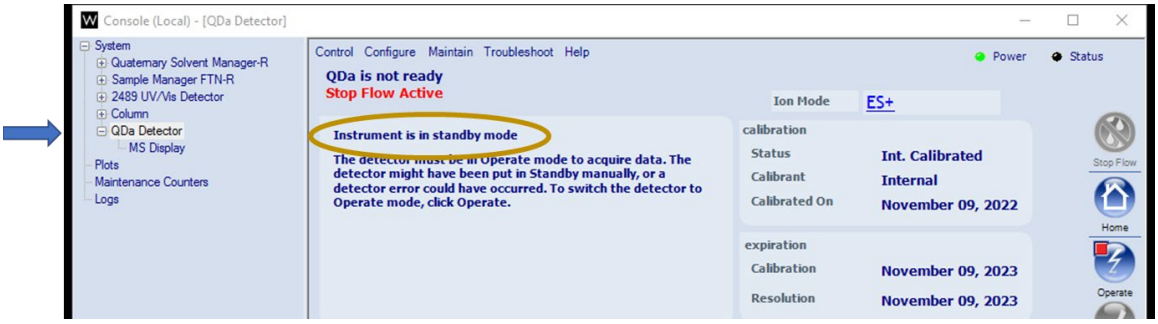
In the inlet method window, **verify** that lamp turned off and flow goes to zero



Verify in MS console that the QDa detector is now in standby mode:



IMPORTANT:

1. Close the Console
2. Close Masslynx
3. Please take your samples back to your lab.
4. Please 'sign out' of the computer

6 Modifying UV/Vis Wavelength in Inlet method

If you think you need a different LC method please consult the MSF staff.

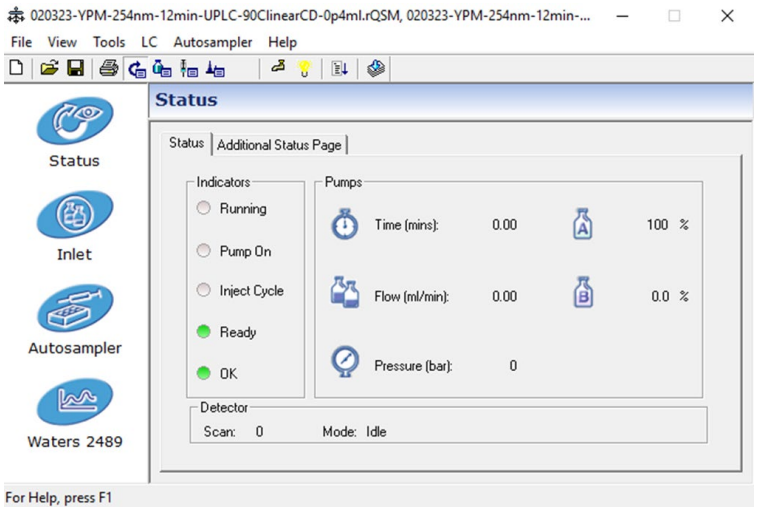
--YOU CAN DAMAGE THE INSTRUMENT IF YOU MAKE LC METHOD CHANGES—

If you only need to change the optimal wavelength for your sample -> proceed.

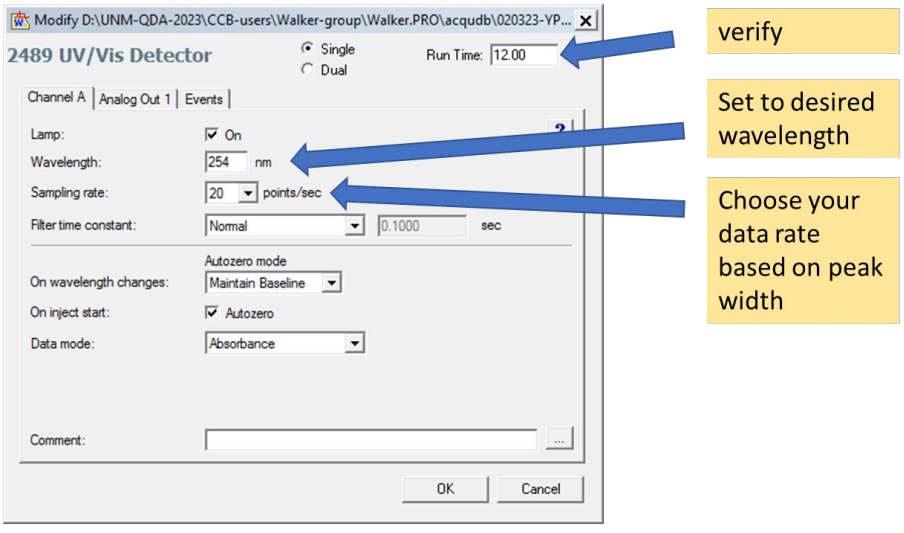
6.1 Changing UV/Vis detector wavelength

Open the inlet method window,

File, Open your method file



Click on the “waters 2489” icon to open UV/Vis detector options.

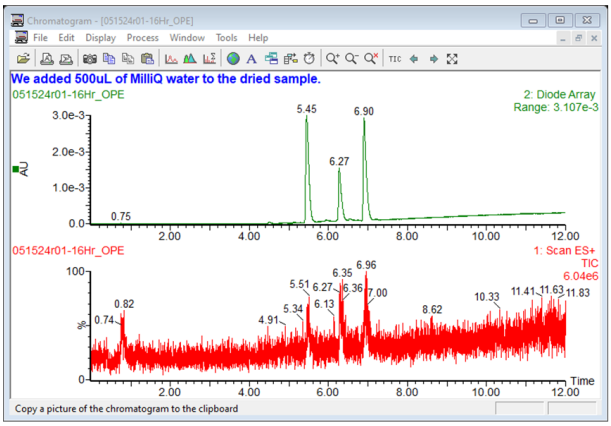


Verify that the ‘run time’ matches your autosampler and LC solvent runtimes.

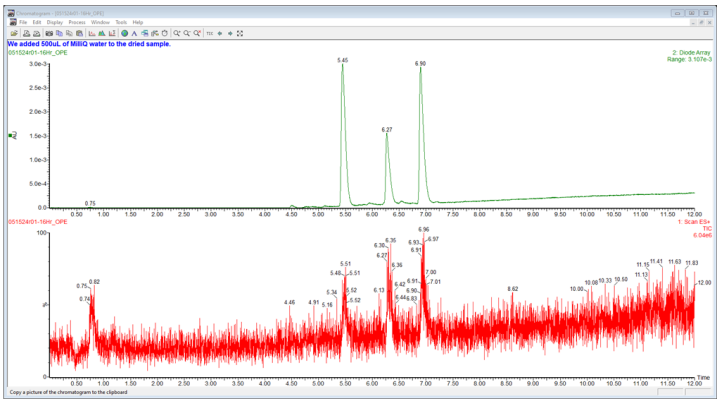
7 Tips and tricks for data viewing or capture

Using printscreen, snippit, imsert screenshot to MS product, or other 'grab'.

Screenshot from 'small' window

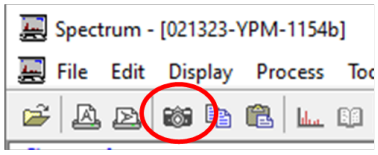


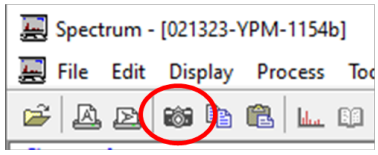
Screenshot from 'fullscreen' window



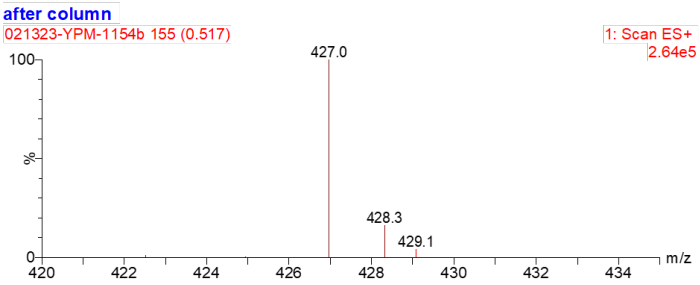
A small window gives better text/graphic ratio – easier to read

Using masslynx image capture button.

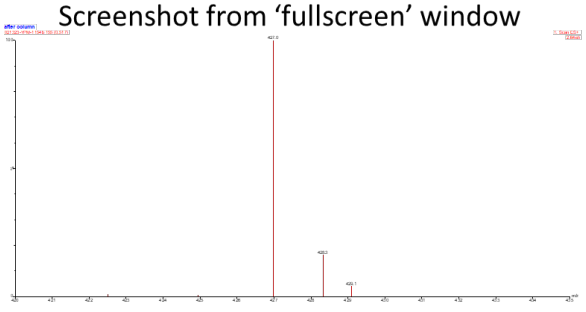




Screenshot from 'small' window



Screenshot from 'fullscreen' window



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9

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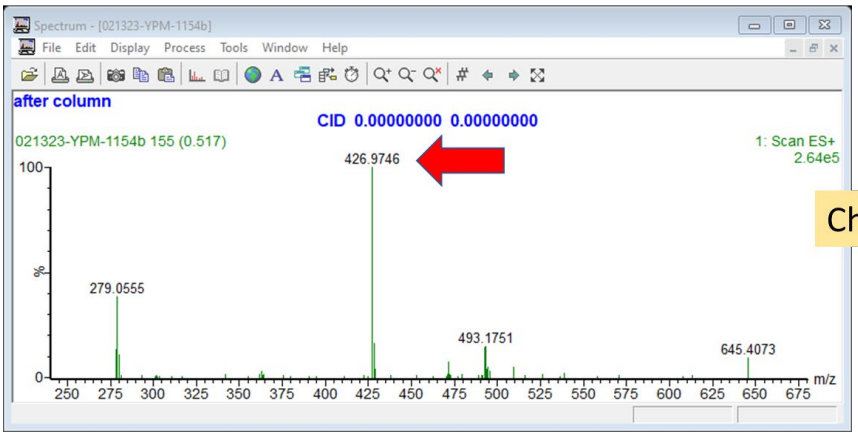
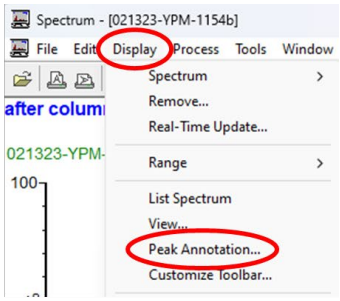
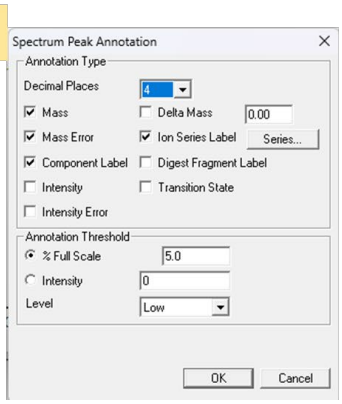
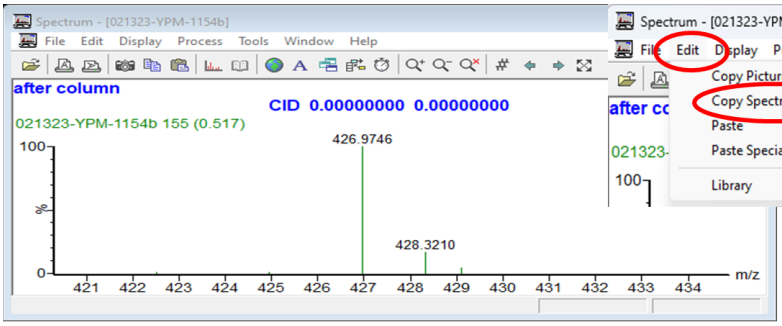
The QDa does NOT give high resolution data!!





Change!

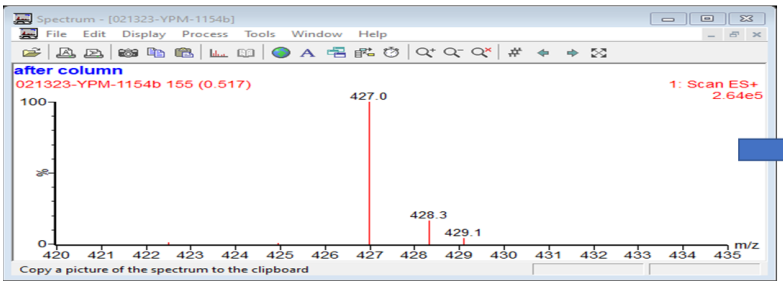




422.5123 2.646e3
424.9358 1.345e3
426.9746 2.638e5
428.3210 4.301e4
429.0903 1.075e4

Text in clipboard

You will get same decimals and m/z range as your plot.



422.5	2.646e3
424.9	1.345e3
427.0	2.638e5
428.3	4.301e4
429.1	1.075e4

Text in clipboard

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9 Options for Getting Your Data

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10 Troubleshooting

10.1 Column problems

The following issues might indicate permanent damage of the column:

1) Abnormal pressure: If the column pressure remains unusually high or low after proper cleaning and equilibration, it may indicate permanent damage.
2) Peak shape distortion: Persistent peak splitting, tailing, or fronting that doesn't improve after cleaning could suggest irreversible damage to the column packing.
3) Loss of retention: A significant and consistent decrease in retention times for known compounds, even after thorough cleaning and equilibration, may indicate permanent damage to the stationary phase.
4) Unresolved chromatography: If you're unable to achieve proper separation of known compounds that previously separated well on the column, it could be a sign of permanent damage.
5) Failure to respond to cleaning: If the column performance doesn't improve after following proper cleaning procedures, including flushing with various solvents and reversing the flow, it may be permanently damaged.
6) Physical damage: Visible cracks, bends, or other physical deformities in the column housing indicate permanent damage

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