



NOTICE

Check solvent levels

Record the VOLUME in the bottles you are using

Example: C=100mL, D=350mL

BEFORE YOU START

YOU

ARE

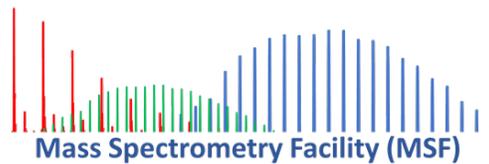
RESPONSIBLE

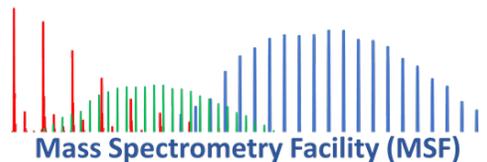
- If the solvent drops below the frit and the UPLC pump is damaged.
- Understand how much solvent your sequence will use.
- Loss of solvent prime may require instrument down-time to correct!

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



DEPARTMENT OF
CHEMISTRY &
CHEMICAL BIOLOGY





Using the Walk-Up Instrument LC/MS Waters QDa

Revision date: May 23, 2024

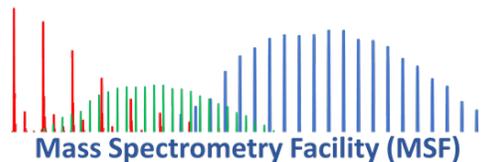
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1 Best Practices

- 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.



- 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
 - Walker-group, Walker
 - Whitten-group, Dave!
 - Gold-group, Gold!
 - Aronoff-group, Aronoff!
 - Rack-group, Rack!
 - Olivier-group, Jean!
4. QUESTIONS?
 - a. Please ask!
 - b. NOT SURE if something is 'normal' – ask!

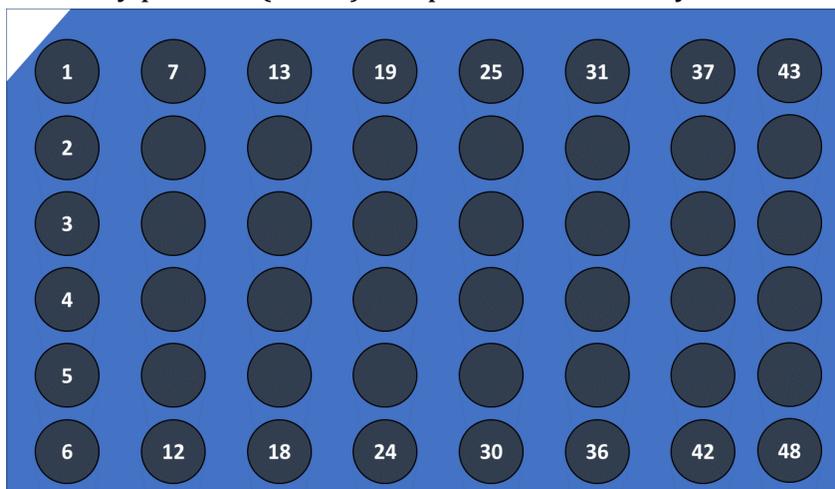
3 Running Samples

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

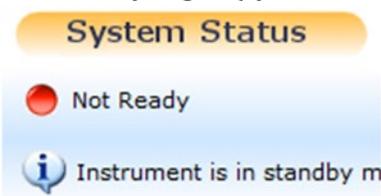
a. Start Masslynx



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)

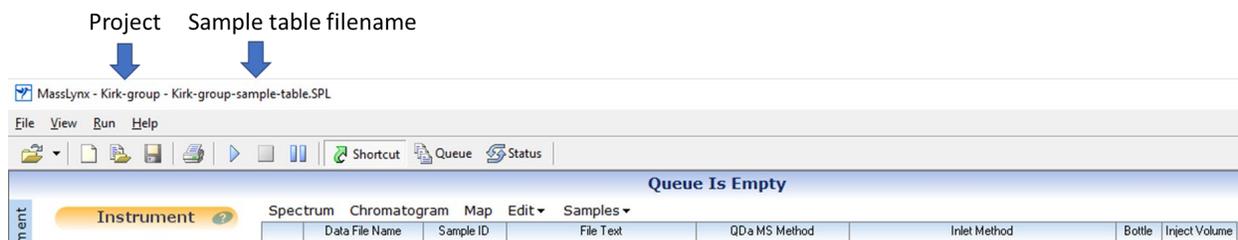


3. Open your PROJECT and sample table

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.

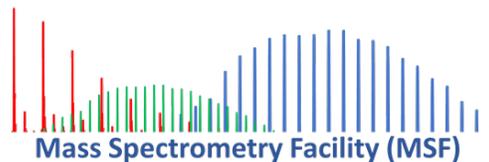
1. Example: 022423_R01_sampleinfo

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)



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



MS Console

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

The screenshot shows the MS Console interface for the QDa Detector. The status is 'QDa is not ready' with 'Stop Flow Active'. The instrument is in standby mode. The Control menu is open, showing 'Operate' and 'Reset QDa Detector' options. The main display area shows calibration and expiration information.

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

The screenshot shows the MS Console interface for the QDa Detector in 'MS Display' mode. The status is 'QDa is getting ready'. The main display area shows a diagram of the instrument with various parameters: Probe Temperature (370 °C, circled in red), ESI Capillary (0.00 kV), Cone (15 V), Source Temperature (119 °C), Ion Guide 1, Ion Guide 2, Quadrupole, Turbo Speed (100.0 %), Turbo Power (40), and Turbo Temperature (42 °C). The Detector is also shown.

2. Masslynx main window: Open 'inlet method'

Instrument ?

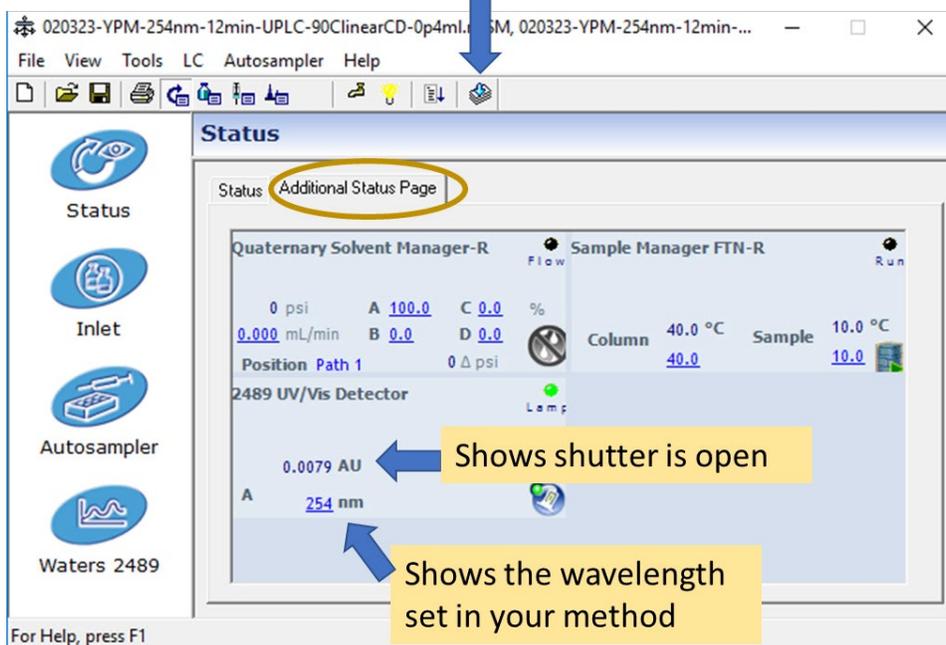


Inlet Method

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

Download to instrument



The screenshot shows the Masslynx Status window with the 'Additional Status Page' selected. The window title is '020323-YPM-254nm-12min-UPLC-90ClinearCD-0p4ml... SM, 020323-YPM-254nm-12min...'. The menu bar includes File, View, Tools, LC, Autosampler, and Help. The left sidebar has icons for Status, Inlet, Autosampler, and Waters 2489. The main content area displays the following information:

Quaternary Solvent Manager-R				Sample Manager FTN-R	
Flow	Run				
0 psi	A 100.0	C 0.0	%	Column 40.0 °C	Sample 10.0 °C
0.000 mL/min	B 0.0	D 0.0		40.0	10.0
Position Path 1	0 Δ psi				

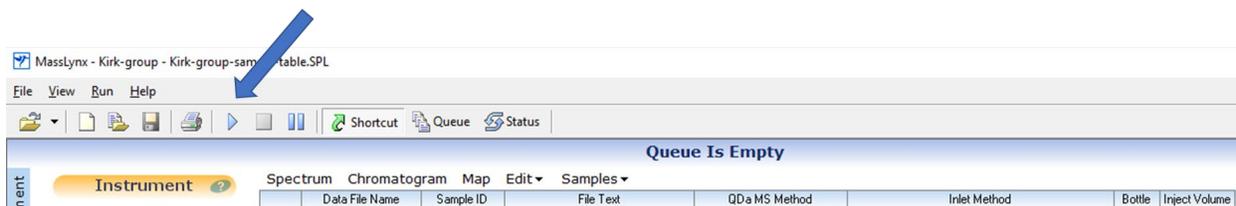
Below the solvent manager, it shows '2489 UV/Vis Detector' with a green lamp indicator and the text 'Shows shutter is open'. The absorbance reading is '0.0079 AU' and the wavelength is '254 nm', with a note 'Shows the wavelength set in your method'. The bottom of the window says 'For Help, press F1'.

3.4 Run Sample(s)

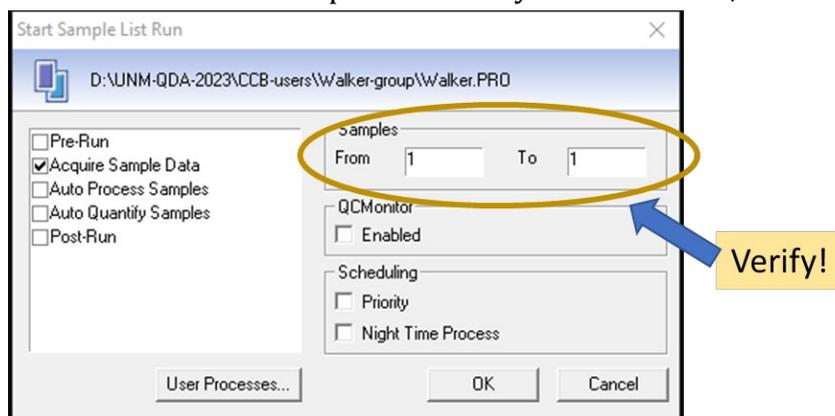
1. Masslynx main window
 - a. Select rows for injection

	Data File Name	Sample ID	File Text	QDa MS Method	Inlet Method	Bottle	Inject Volume
1	022023-r01-Blank		Mobile phase balnk	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:7	0.5
2	022023-r02-C26		Stock + 100ul mobile phase 1.8mg/ml	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:8	0.5
3	022023-r03-C27		Stock + 100ul mobile phase 1mg/mi	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:9	0.5
4	022023-r04-C28		Stock + 100ul mobile phase 3.75 m...	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:10	2.0

- 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



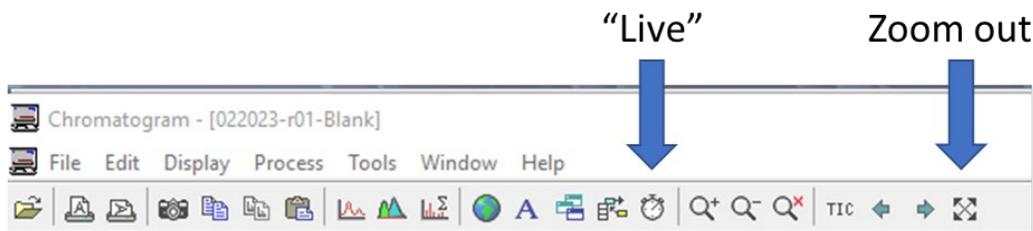
3.5 Display data while running

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

	Data File Name	Sample ID	File Text	QDa MS Method	Inlet Method	Bottle	Inject Volume
1	022023-r01-Blank		Mobile phase balnk	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:7	0.5
2	022023-r02-C26		Stock + 100ul mobile phase 1.8mg/ml	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:8	0.5
3	022023-r03-C27		Stock + 100ul mobile phase 1mg/mi	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:9	0.5
4	022023-r04-C28		Stock + 100ul mobile phase 3.75 m...	022023-Posesi-150-1250-12...	020323-YPM-215nm-12min-UPLC-90ClnearCD-0p4ml	1:10	2.0

3. Right click on TIC data to display MS data

- Select 'live' clock icon if data is not updating

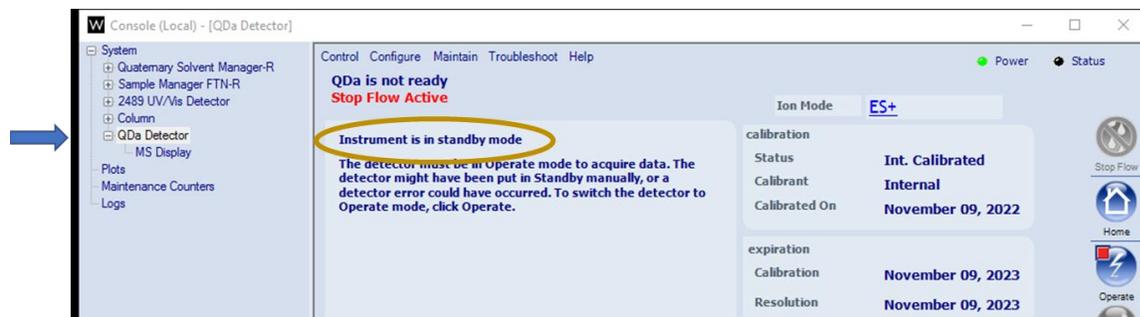


4 Auto Shutdown / cleanup

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:



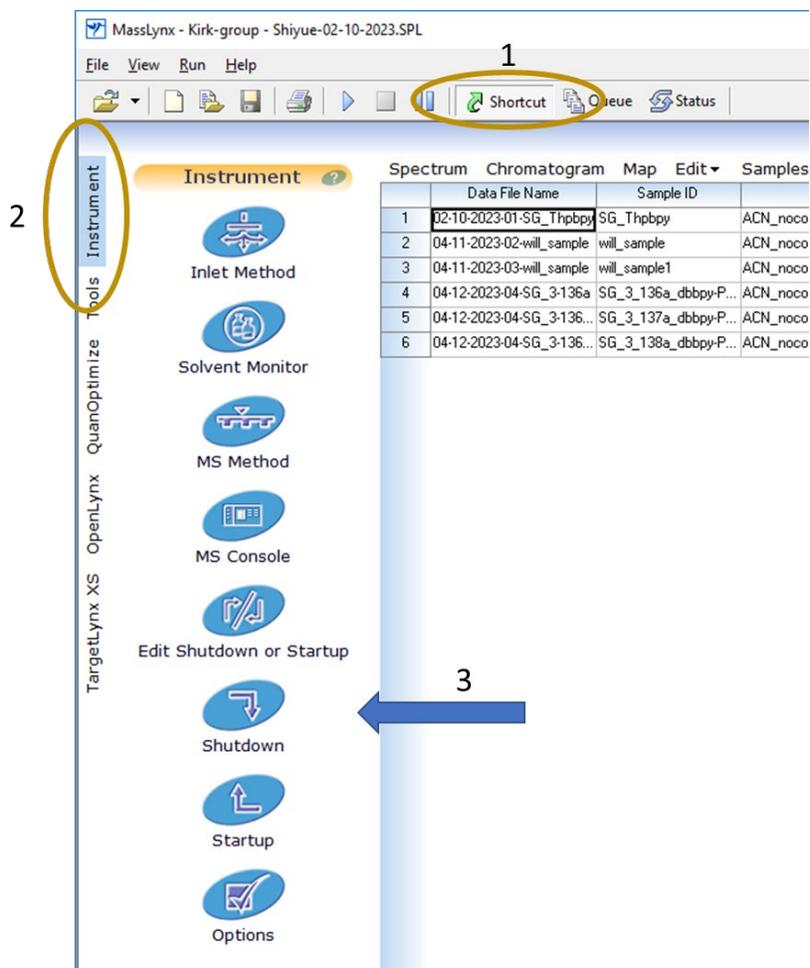
IMPORTANT:

- Close the Console window
- Close Masslynx
- Please take your samples back to your lab.
- Please 'sign out' of the computer

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**.



Masslynx - Kirk-group - Shiyue-02-10-2023.SPL

File View Run Help

Shortcut Queue Status

1

2

Instrument

Inlet Method

Solvent Monitor

MS Method

MS Console

Edit Shutdown or Startup

Shutdown

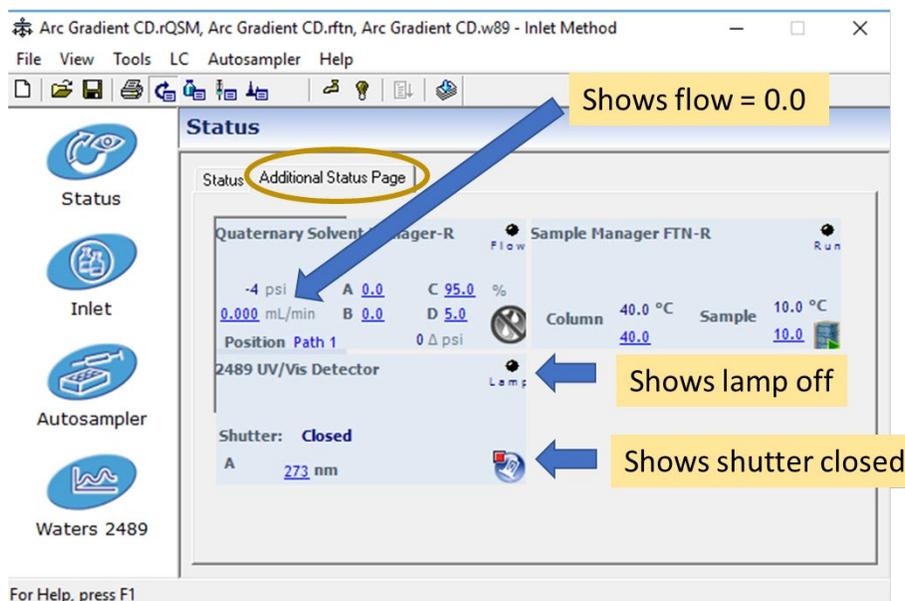
Startup

Options

3

	Data File Name	Sample ID	Samples
1	02-10-2023-01-SG_Thpbpy	SG_Thpbpy	ACN_noco
2	04-11-2023-02-will_sample	will_sample	ACN_noco
3	04-11-2023-03-will_sample	will_sample1	ACN_noco
4	04-12-2023-04-SG_3-136a	SG_3_136a_dbbpy-P...	ACN_noco
5	04-12-2023-04-SG_3-136...	SG_3_137a_dbbpy-P...	ACN_noco
6	04-12-2023-04-SG_3-136...	SG_3_138a_dbbpy-P...	ACN_noco

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



Arc Gradient CD.r.QSM, Arc Gradient CD.r.ftn, Arc Gradient CD.w89 - Inlet Method

File View Tools LC Autosampler Help

Status

Additional Status Page

Quaternary Solvent Manager-R

-4 psi A 0.0 C 95.0 %
0.000 mL/min B 0.0 D 5.0

Position Path 1 0 Δ psi

2489 UV/Vis Detector

Lamp

Shutter: Closed

A 273 nm

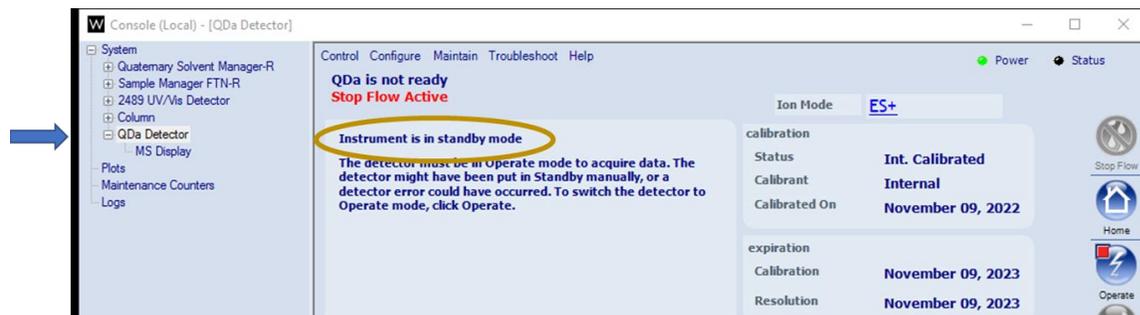
Shows flow = 0.0

Shows lamp off

Shows shutter closed

For Help, press F1

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



Console (Local) - [QDa Detector]

System

- Quaternary Solvent Manager-R
- Sample Manager FTN-R
- 2489 UV/Vis Detector
- Column
- QDa Detector
- MS Display
- Plots
- Maintenance Counters
- Logs

Control Configure Maintain Troubleshoot Help

Power Status

QDa is not ready
Stop Flow Active

Ion Mode ES+

calibration

Status Int. Calibrated

Calibrant Internal

Calibrated On November 09, 2022

expiration

Calibration November 09, 2023

Resolution November 09, 2023

Instrument is in standby mode

The detector must be in Operate mode to acquire data. The detector might have been put in Standby manually, or a detector error could have occurred. To switch the detector to Operate mode, click Operate.

Stop Flow

Home

Operate

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.

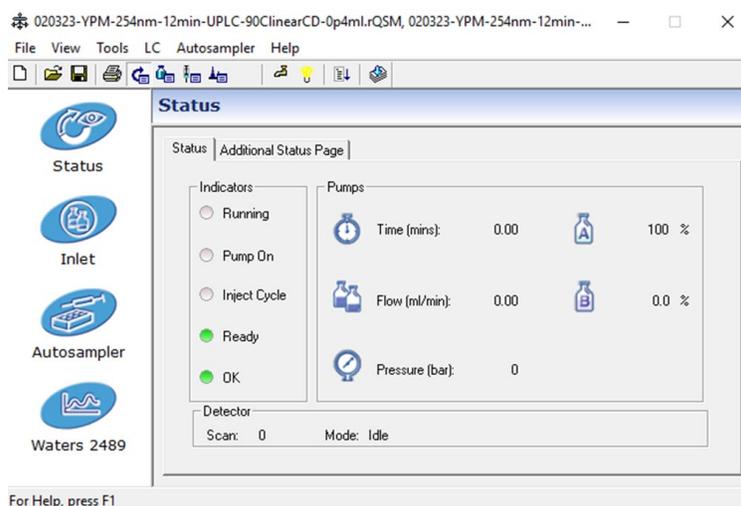
--YOU CAN DAMAGE THE INSTRUMENT IF YOU MAKE LC METHOD CHANGES--

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

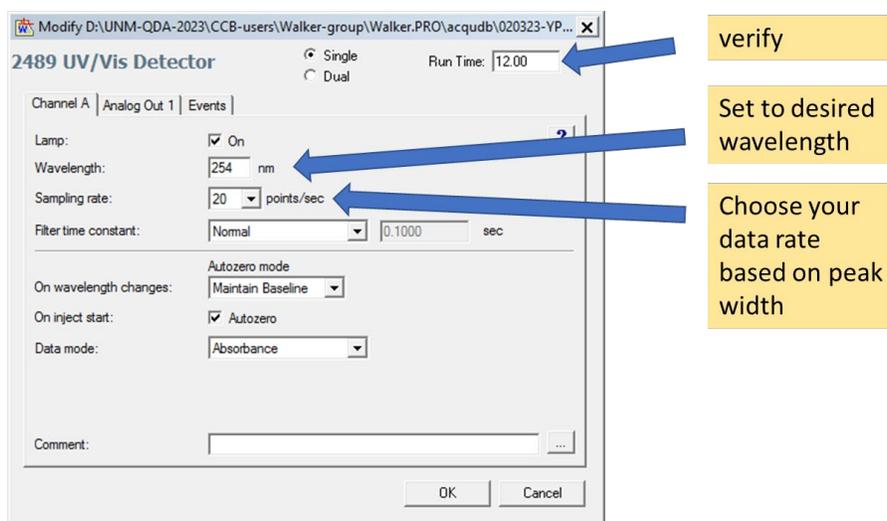
6.1 Changing UV/Vis detector wavelength

Open the inlet method window,

File, Open your method file



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



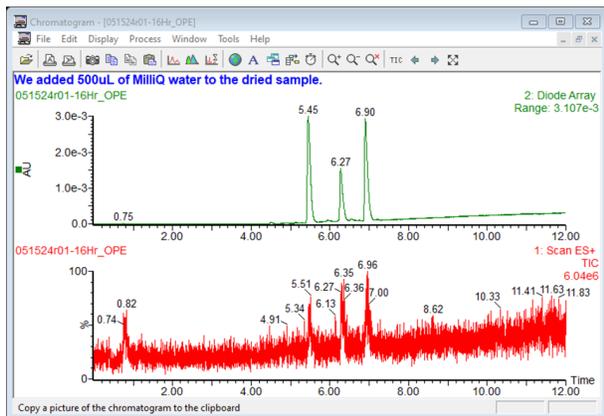
Verify that the ‘run time’ matches your autosampler and LC solvent runtimes.



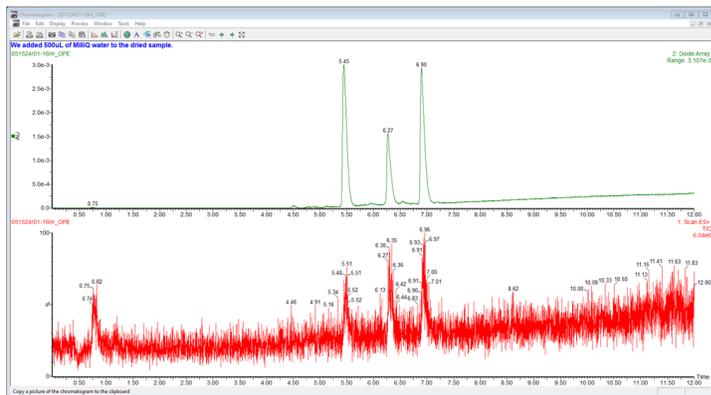
7 Tips and tricks for data viewing or capture

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

Screenshot from 'small' window

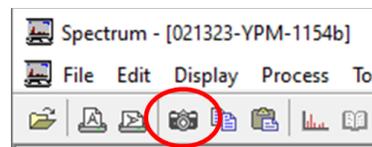
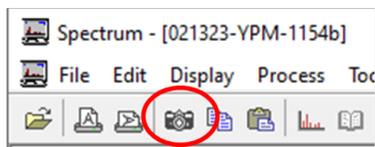


Screenshot from 'fullscreen' window

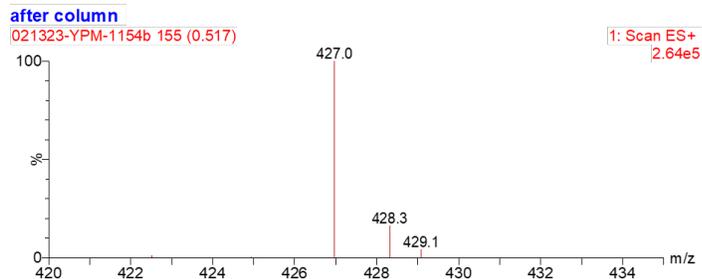


A small window gives better text/graphic ratio – easier to read

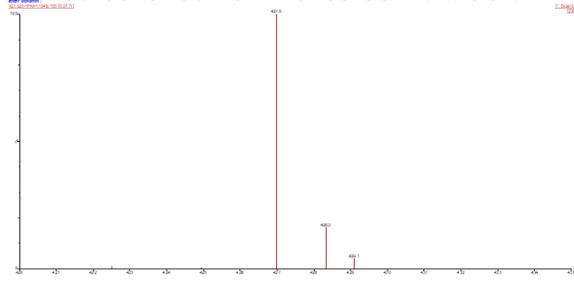
Using masslynx image capture button.



Screenshot from 'small' window

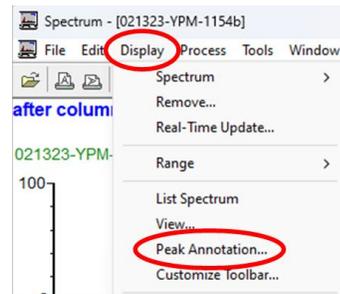
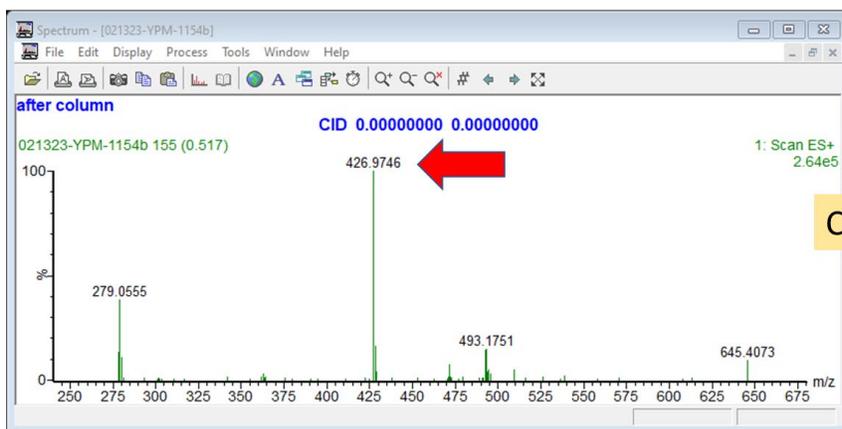


Screenshot from 'fullscreen' window

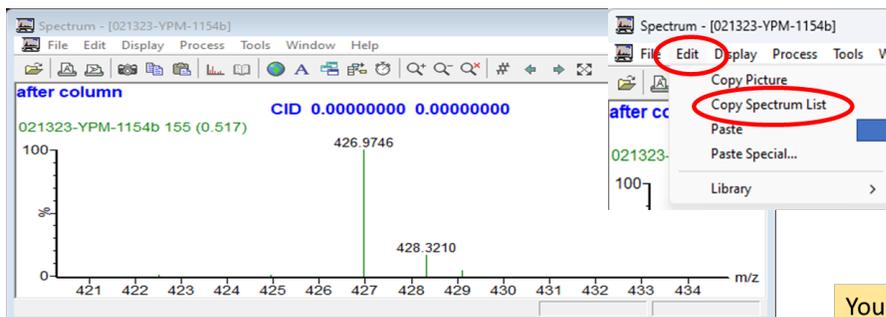
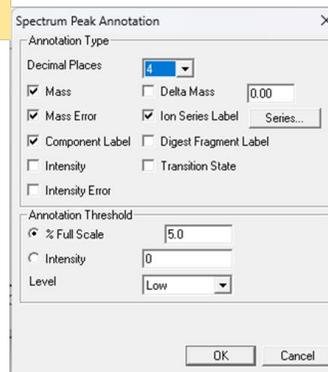


A small window gives better text/graphic ratio – easier to read

The QDa does NOT give high resolution data!!



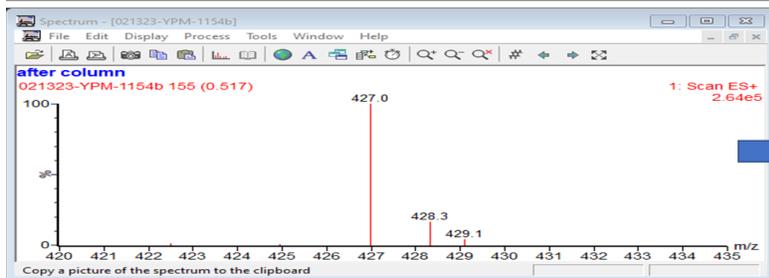
Change!



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.

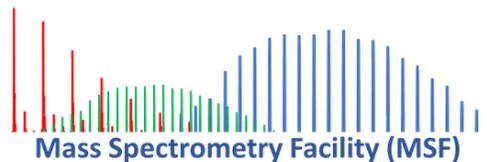


422.5 2.646e3
424.9 1.345e3
427.0 2.638e5
428.3 4.301e4
429.1 1.075e4

Text in clipboard

9 Options for Getting Your Data

- 9.1 Mestrenovo software
- 9.2 Export to *.cdf format for other software
- 9.3 Text export via “copy spectrum list” in either chromatogram or spectrum view



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