# Agilent 6545 LC-QTOF User Manual

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#### I. Introduction

The use of this instrument is allowed only by qualified users after receiving training from IMSERC personnel. Do not run this instrument without approval from IMSERC staff. Failure to do so may cause damage to the instrument, produce invalid data, and result in additional fees and/or removal of all IMSERC privileges.

This short set of instructions is meant to serve as a guide for 'routine' data collection on the instrument. Please read this standard operating procedure and acquaint yourself with the instrument. If something happens that you do not understand while using the system, please **stop** and **get help**. In any event, be completely prepared to justify your actions. The cost of even minor repairs is considerable.

The **Agilent 6545 QTOF**, or **LC-QTOF** for short, is coupled to an Agilent 1290 Infinity II UHPLC system and is a highmass resolution, accurate mass instrument (HRAM) that is mainly used for sample profiling (proteomics, metabolomics, lipidomics), LC-MS/MS, purity analysis, or any research question requiring chromatography coupled to HRAM detection. The UHPLC coupled to this instrument has **one location** where a User can install their own Column (based on their set Method) in the multicolumn thermostat and has the capability to use multiple mobile phase solvents. The autosampler (multisampler) can, in theory, have 432 vials in its 8-tray compartments. In practice, the top two trays, plates 7 and 8, are set up to accept 96-well plates, while plates 1 – 6 are set up to accept autosampler vials. The multisampler also has multi-wash capability, which utilizes three different solvent lines to wash to conduct seat back flush and needle wash before every sample injection.

The creation of a method requires IMSERC personnel to use reference standards of the analytes-of-interest or a test sample to validate the retention time(s) on the corresponding Column being used. IMSERC personnel must create these methods to evaluate for the best chromatographic separation, the highest sensitivity, and the most accurate Method for the experimental need before a User can effectively use it for their research.

### II. Safety

All users of IMSERC must review the general safety policies at http://imserc.northwestern.edu/about-policies.html.

Familiarize yourself with the location of standard safety stations, such as eye wash and shower stations, just outside of BG70. When using the computer, gloves should be removed.

### III. Data Management

Your personal data folders are created during training and are located under your supervisor's group folder (Project Folder). See a staff member if you do not have a personal folder on this instrument yet. Your personal files are located in either the Methods, Worklist, and Data subfolders and must contain your Lastname\_Firstname as the main folder name. Inside these Data and Worklist subfolders, you will create a new folder that references the date the data was collected.

#### **IV.** Software

Data Acquisition is performed on the Agilent MassHunter Data Acquisition Program, which can be accessed through the Control Panel. The Control Panel icon can be accessed on the Desktop. Depending on the data output need and experimental design, the Agilent MassHunter Qualitative Analysis or the Agilent MassHunter Quantitative Analysis Programs are used. Agilent files can also be analyzed by third-party or open-source software packages. The Agilent program suite is available for use on the data analysis computers at the ground level of IMSERC. The use of IMSERCterm also makes it possible to access these programs, but one needs permission to access them. Request access through this form or the IMSERC FAQs on the IMSERC website.



### V. Sample Preparation

Before training on this instrument, sample preparation and your overall experimental design should have been discussed in great detail with IMSERC personnel. At a bare minimum, topics such as the sample preparation, type of resuspension solvent and chromatography needs should have been discussed.

All samples should have gone through some extraction procedure, centrifugation, and/or filtration processes. If you are not certain that your sample preparation matches IMSERC-MS expectations, contact IMSERC personnel immediately to discuss.

When conducting quantitative M.S. on this instrument, the use of internal standards greatly improves the accuracy of the quantitative M.S. assay as they can normalize any variations from sample preparation and ion suppression during data collection. When creating calibration curves for a particular analyte, there will be a specific linear range of concentration that can be generated for the curve to accurately determine concentrations of unknown samples. Due to the introduction of an internal standard and other processes in sample preparation, there will be a dilution factor that will be used across calibrator and Q.C. samples, as well as the actual samples themselves.

Any questions and concerns should be directed to IMSERC personnel immediately.

Detail	Recommendation	Consideration
Vials	Agilent Vial 5182-0714, Agilent cap 5185-5865	The Autosampler picks up the vial. With the wrong vial dimensions, the injection needle could miss or hit the top of the glass vial.
Solvent	HPLC-grade water, methanol, Acetonitrile, isopropanol, dichloromethane	Listed solvents include the mobile phase solvents located on instruments. Your Sample must be soluble in your chosen solvent and compatible with a combination of the mobile phases listed. Avoid DMF and high concentrations of DMSO.
Concentration / Purity	Varies by Method	Sample(s) may be diluted with modifiers to increase signal/ionization efficiency, such as 0.1% Formic or Acetic acid, ≤ 10 mM ammonium formate or acetate Sample(s) should have gone through some extraction procedure, centrifugation, and/or filtration processes.
Volume	500 μL	If the volume available is too low, use Agilent vial spring loaded insert 5182-8872
Labeling	Name and solvent	Sample identification & safety / proper disposal

### VI. Quick Start Checklist

- a. Start NUcore Reservation
- b. Check mobile phase volumes
- c. If applicable to your Method, switch out the mobile phase bottles.
- d. If applicable, install your Column
- e. On the Desktop, click on Data Acquisition
- f. Load your Method
- g. If mobile phase solvents have been replaced, update the Software's actual solvent volumes.
- h. Turn on ALL Modules
- i. Purge Pump for 5 minutes
- j. Add pump backpressure trace in the chromatogram plot
- k. Once the Purging Pump has finished, check for leaks in the column compartment.
- Prepare your Worklist and ensure your default Worklist Parameters (scripts, default data folder location) are correct
- m. Add a wash or a Blank run after your samples have been analyzed.
- n. Extend your NUcore reservation if necessary
- o. Remove your Column once your finished or the next day if you're doing an overnight run.
- p. End your reservation in NUcore after your analysis is complete.



### VII. Initial Steps: Mobile Phase & Column Installation (if required)

1. Start your NUcore reservation on your phone or computer. You can start your reservation 5 minutes before your actual posted reservation if there isn't an existing user before yours. Note that if a reservation has not been started 10 minutes after the intended start time, Nucore automatically deletes it.

#### 2. Check Mobile Phase volume

- a. Ensure that the mobile phase bottles are installed in the appropriate solvent line channel for your Method. Mobile phase bottles can be found in the second rack of the black cart behind you.
- b. If there is not enough volume ( <200 mL) in any of the two bottles, contact IMSERC-MS staff immediately to refill the bottle(s).

#### 3. Installing the Column

a. Follow the instructions provided during your training to install your Column. Typically, the Column will be installed in Position 6, which features a Quickconnect Assembly for easy connection and disconnection.

To install the Column:

- 1. Ensure the Quickconnect latch is in the open position.
- 2. Align the inlet of your Column with the connector, ensuring you follow the flow diagram.
- 3. Gently screw the inlet by turning the blue screw (or the Column itself) until it is "finger-tight."
- 4. Close the Quickconnect latch to secure the Column.
- 5. Attach the outlet of the Column to the brown plastic fitting.
- 6. If the Column is not properly tightened, the system's backpressure will remain unchanged, potentially leading to leaks around the connections.



b. Important: Store the Column end plugs in a safe place to avoid misplacing them.

### VIII. Data Acquisition

### a. Loading Method and Updating Mobile Phase Volumes



#### Program Icons of the Control Panel (A) and Acquisition Software (B) on the Taskbar

- 1. The Acquisition Software should be open upon starting. If not, the program icon looks like the icon above.
- Load your Method as shown below. During training, a method(s) would have been created for your specific experimental need. This Method file contains the parameters for chromatography, mobile phase bottle positions, and M.S. In the Method Menu, click Open. Navigate to the Methods/PI PI\_LastName/LastName\_FirstName

Agilent MassHunter Workstation Data Acquisition							
File	View	w		Met	hod Tools Help		
 Contex	t: Acq	uisiti	on		New		
 Instrun	nent Sta	tus			Open		
Multi	sample	er	Idlo		S S		
J.			EMF		Apply Reset		
	2.00	ομι S	9		D:\MassHunter\Methods\Tobias\Position1_C18\Tob D:\MassHunter\Methods\Tobias\Position1_C18\Tob		

3. The Acquisition Software monitors mobile phase solvent usage as long as the actual volumes match the software. Right-click on the Binary Pump (A) (specifically the center blank area), and choose Bottle Fillings (B).

Instrument Status					
Multisampler	Binary Pump	Not Ready	Quat. Pump	Standby	Colui
емғ⊘ 1 2.00 µL	0 on = off	EMF©		EMF (	ل 39.99°
↓ ↓ • c [4 * c]	888	A Error Me	thod Device	•	Po D P
		Switch C	n I I I I I I I I		
0.00 / 0.00		ভার্ত্ত Switch S উন্নিট Switch S	olvent Selection	Valve B	
Chromatogram Plot		🛱 Bottle	۰		
	TIC	Preș	9 Ip		
767-		Seal	ime		
6E7 - 5E7 -					



4. Under the Fillings section, update the "Actual Volume" of the mobile phase bottle(s)s being used. Click OK to continue.

🧱 Bottle Fillings								
Solvent Bottle								
Fillings								
Actual Volume	Total Volum	e						
A1 0.88 📫 liter	1.00	liter						
A2 0.57 - liter	1.00	liter						
B1 0.51 🕂 liter	1.00	liter						
B2 0.48 🕂 liter	1.00	liter						
Actions								
Prevent analysis if level falls below	ow	0.10 ÷	iter					
✓ Turn pump off if running out of so	lvent							
Waste Bottle								
Filling								
Actual Volume	т	otal Volume						
Waste bottle: 0.00 ÷	liter	0.00	liter					
Actions								
✓ Prevent analysis if level raises above       0.10     ↓								
Turn pump off if waste volume has reached maximum limit								
	Ok	Cancel	Help					

#### b. Purging Pumps and Enabling Backpressure Trace

- 1. Click the green "On" icon to turn on all LC-MS modules (A).
- 2. The Multisampler, the binary pump, the quaternary pump, the Column Compartment, the MWD (if enabled), and the QTOF will now turn on and initialize to their starting conditions based on the **Method** selected.



- 3. The Binary pump will need to be purged of old mobile phase solvents in its lines with the new mobile phase. Right-click on the Binary Pump (specifically the center blank area) **(B)**, and choose Prepare Pump... **(C)**.
- 4. In the pop-up window, choose Purge. Set the Duration, Flow, and Composition B, as shown below. Click Start. Note: the pump will now run at full flow for 5 minutes, purging the pumps of old mobile phase solvents and pushing through the new mobile phase in the loaded Method to the waste lines. This will ensure that the mobile phase being used is filled up to the sample loop. Proceed to the next steps while the pump is purging.

		$\sim$				
📃 Pr	epare Pump			-		×
	Purge					
	Use for changing mobile phases	, drawing solver	t or for rem	oving air bu	bbles.	
0	Duration: 5.00 📫 min	Comp	osition A:	50.00 ‡	%	
	Flow: 5.000 - mL/n	in Com	osition B:	50.00 📫	%	
	Conditioning					
	Minimize the pressure ripple by	dissolving air bu	ubbles in the	e pump head	s.	
0	<ul> <li>Note: Solvents will flow thr Method parameters are app pressure.</li> </ul>	ough the LC sys blied for flow rate	tem and col e, compositi	lumn. ion and max		
	Duration: 15.00 🛟 min					
	Prime					
0	Draws solvent into (both) pump head and particles from valves.	neads for removi Flow goes to wa	ing air bubb ste.	les from the	pump	
_	Do not use the Prime function for solvent type.	filling the solve	nt lines or (	changing the		
	21					
			Start	Cancel		Help



5. By default, MassHunter Acquisition displays only the TIC signal over time in the Chromatogram Plot Panel. Within the Chromatogram Plot panel, right-click on the white space (A) and click on Change... (B)



6. In the pop-up window, in the Available Signals box (6a) and select Binary Pump: Pressure to add to the Selected Signals box (6b). Select Predictable Range in the middle section of the window and change the range from 0 to 1000 bar. (6c) You can now visualize the Pump Pressure over your usage, which will be important when assessing potential pressure increases due to clogs.



7. While the pumps are purging, click the Worklist tab to fill out your sample table. (A)

#### c. Preparing the Worklist

- 1. In the Worklist Panel, there are several ways to fill out the sample queue: (1) load an existing Worklist, which contains the default locations and post-analysis scripts, and "Save As" as a new worklist, or (2) start from scratch. All Worklist files should be saved in the Worklist Folder under your name, with the format yearmonthday\_AnalysisName. If starting from scratch, ensure that the Method location and Worklist scripts are enabled (step 3) and the default file location is set up correctly (step 2).
- 2. Starting from a previous Worklist:
  - a. In the Worklist Panel, click the "Open Worklist" icon, and choose the previously used Worklist to open.
  - Rename this file as a new Worklist by clicking the "Save As Worklist" icon. All Worklist files should be saved in the Worklist Folder under your name, with the format yearmonthday\_AnalysisName.
  - c. To change the Root Data Folder, click on the upper-left-hand corner of the Worklist table **(A)** and choose **Worklist Run Parameters.. (B)** in the Drop-down menu.
  - d. In the pop-up window, go to the Data File Settings Tab (C) and change the Root File Folder location by changing the Date on the folder location. You do not need to click the ellipsis icon to make a New Folder. Click OK to continue (D).

Worklist	Worklist Run Parameters	×
	Run Parameters Data File Settings Additional Parameters	
: 🗋   🍸 🔲 🎑   🕨 🔲       🛃   20220812_L	Operator Information	
Sample Nam	Operator name	
Add Multiple Samples	Plan Informatio Method part te on and DA V Synchronous DA	
A Add Sample	Bun Tune Standard Stat	
Add Script		
Copy Row(s) Add Copied Row(s)	Method Paths Method: D:\MassHunter\Methods\Vickkon'\deLaCodre_Hotense Overside DA: D:\MassHunter\Methods	
Delete Row(s)	Scripts	
Add Column(s)	Pre-worklat	
Show/Hide/Order Columns	Post-worklist SCP_PumpsAliandMSRefOff(){MH_Acq_Scripts.exe}	
Tast Size	cleanup	
lext Size	Post-Analysis	
Add Worklist		
Worklist Run Parameters	Disk space Information (GB)	
	54 Available Disk space 10 Free Disk space th	reshold
Impo klist	_ Run Settings	
Wra R fOff	Overlapped injection     Wait Time for Ready: 10	(min)
Trace st Run	Clear sample after run	
	ОК	Cancel

3. Before closing the Worklist Run Parameters Window, ensure that the correct Method Path shows your Method Folder (D:\MassHunter\Methods\YourPI'sLastname\YourLastName\_YourFirstName) and Post-worklist Script is enabled, as shown below. Click OK to continue.

Method Paths	
Method:	D:\MassHunter\Methods\Tobias\Lipidomics
Override DA:	D:\MassHunter\Methods
Scripts	
Post-worklis	st SCP_PumpsAllOff()(MH_Acq_Scripts.exe)
Clean-up	
Post-Analys	is



4. For each session, adhere to the following guidelines to maintain consistency across sample injections, to maintain instrument cleanliness and uptime, and to ensure correct file-saving locations.

#### a. For all LC-MS runs:

- i. **IMPORTANT:** Each Sample Name must be unique in the Worklist, as the entire Sample Name column will be copied and pasted into the Data File column.
- ii. Begin your Worklist with at least two blank runs (either as a "No Injection" or with solvent injection) using your Method. This will clean and equilibrate the Column with the mobile phase solvents.
- iii. Verify that the Sample Position is accurately assigned. Below is a map of each tray (P1, P2, etc.) and vial position.



- iv. Add a WASH run to store the Column in the organic mobile phase post-analysis, prolonging the column lifespan.
- b. For LC-MS/MS runs (using Iterative MS/MS or conventional Auto MS/MS):
  - i. Follow all points listed above.
  - ii. Confirm that you designated injections for Iterative MS/MS and have applied the correct Auto MS/MS method, and also ensuring adequate sample volume.
  - iii. For Positive and Negative Ion analyses on the same samples, set the Method to the appropriate polarity.
  - iv. If required, add Q.C. injections evenly throughout the Worklist for system suitability monitoring

#### c. For LC-UV-(MS) runs:

i. Use the appropriate Method set up for you by IMSERC, which either shuts off the QTOF or diverts the L.C. stream to waste post-UV-Vis detection. Failing to do so can lead to QTOF fouling, requiring cleaning.

#### d. For quantitative LC-MS runs:

- i. Ensure you have the appropriate internal standards added to your samples, including calibrators, Q.C. samples, and actual test samples.
- ii. Start with 2–3 "Solvent" blanks at the beginning of the Worklist.
- iii. Include one "Matrix + internal standard" blank.
- iv. Add calibration curve (Cal) samples in order from lowest to highest concentration.
- v. Insert quality control (Q.C.) samples in descending order, starting with the highest concentration.
- vi. Include one "No Injection" blank.
- vii. For duplicate or triplicate injections, repeat steps (iii) to (vi).
- viii. After Cal and Q.C. samples, add your samples in a randomized order to minimize bias.
- ix. For Cal and Q.C. samples, input a number in the **Level Name** column, starting with the lowest concentration and moving to the highest concentration. **This will enable easy integration with the Quantitative Analysis Software.**
- x. For QC sample injection, input a "HQC" for the highest Q.C., followed by "MQC" for the middle Q.C., and "LQC" for the lowest Q.C. sample in the **Level Name** column.
- xi. **IMPORTANT: ADD** the WASH method at the end of the Worklist so that the Column can be stored with the appropriate solvent to ensure a longer column life.
- 5. After setting up the Worklist, click Save.
- 6. Click Run Worklist.
- 7. Check your reservation in Nucore for adjustments. To estimate the required time, multiply the number of injections by your Method's run time.

### IX. Column (if required) and Sample Vials Removal

- 8. For long or overnight runs, column removal is expected to be done as soon as you can.
- 9. Before removing your Column, ensure that the instrument is not running (not collecting data).
- 10. Carefully remove your Column from the MCT and close the MCT door carefully, making sure the door "clicks" upon closing.
- 11. All files will be available for access in your Group Folder. Data analysis should be done on the data analysis computers on the ground floor of IMSERC or by using the IMSERC terminal. IMSERC terminal access can be requested through the <u>FAQs section</u> of the IMSERC website.



### X. Basic Data Analysis in Qualitative Analysis (to check raw data)

### a. Using the Find By Formula Function in Qualitative Analysis

- 1. Compounds can be identified by their chemical formula and the dominant ion species generated during ionization (+H, +Na, +NH<sub>4</sub>, -H,).
- 2. The Find By Formula Workflow requires a formula or a .csv table to identify chemical compounds in each run. The formula is used to screen for the calculated m/z values of the chosen ions throughout the injection file, scoring them by their accurate mass, isotope abudance, and isotope spacing. This enables the user to screen for compounds without prior knowledge of retention time. Note to keep in mind the chromatography used (C18,. HILIC, no chromatography) and structure diversity of your sample if isomer separation and detection is possible.
- 3. To set up the Find by Formula Settings, Go to Compounds View:

📑 Agilent MassHunter Qualitative Analysis 10.0 - tobias_qual.m		
File Edit View Find Identify Method Configuration Tools Help		
☞ 🖬 🖻 🦪 🤊 • ୯ • 🛷 🍯 🖻 🛆 🕼 🏟 🗰 🍓 🐼 &	Navigator View 🔛 Compounds	View
Sample Table: GInA_2uL_04.d		
🏥 🕟 🐻 🏭		
Results	Workflow	
Result Summary File Name Last Run Method Saved Results Method Flags	Workflow Target Source Sa	ample N

4. In the Method Editor panel, go to Target/Suspect Screening and type/choose your .csv table:

Method Editor: Find by Form	ula						
🚹 🕼 🖬 🌠 🧐 • (* •	• Find by	y Formula 🔻					
Method Automation	4	EIC Smoothing	EIC Integration	EIC Pe	eak Filters	Pe	ak Spectrum (MS)
Workflow		Peak Filters (MS)	Charge State	Fragment A	Annotation	Frag	ment Peak Filter
	-	A Target Source	Formula Matching	Ion Species	Scoring	Results	Molecular Confirma
Additional Chromatograms		Target source (*.c	db, *.csv, *.cef, form	ulas, neutral m	asses)		
Reports		C15H21N706				1 <b>A</b>	
Export							
Chromatograms		Values to match					
Spectra		Require RT n compound.	natch if database co	ntains an RT f	for the targ	jet	
Target/Suspect Screening							
Find by Formula	٨	Matches per form	ula (Isomer detection	)			
Sample Purity	۵	(will autoincrease	r of chromatographi e for formulas with r	c components nultiple isome	reported rs in datat	per formul base)	a
Compound Discovery		2					
Identification	<b>A</b>						
Export							

- 5. If you have not made a .csv table, consult IMSERC-MS staff. Otherwise, type your chemical formulas and separate them by a comma.
- 6. The tabs "Target Source," "Formula Matching", and" Ion Species" are the only settings that should be changed for most cases.
  - a. For Target source, make sure that you are using the correct .csv or have entered the chemical formulas.
  - b. For Formula Matching, ensure that the mass tolerance is at 5 ppm.



- c. For Ion Species, make sure that you have +H or -H at minimum. Feel free to add +Na or other adducts but may result in false positive I.D.s. Double-check the profile mass spectrum to make sure that the prediction isotope distribution overlaps with ion peaks that make sense.
- 7. Click "Find by Formula," and choose the files for which MassHunter to screen for your compounds.
- 8. To quickly use this Workflow, go back to Navigator View, and go to Method Editor: Workflow section.



- 9. Choose "Target/Suspect Screening" and "Find by Formula" under Workflow and Compound mining, respectively.
- 10. Choose the appropriate Target source.
- 11. Click Run Method Workflow and choose the files you wish to screen for your compounds.

# b. Making Extracted Ion Chromatograms (EIC) in MassHunter Qualitative Analysis

lons (m/z) can have their elution profile (chromatograms) shown for every file you choose.

- 1. Open the files you wish to analyze.
- 2. Under the Chromatograms tab, choose Extract Chromatograms...



3. Choose EIC, under "Type", type in the desired m/z value with four decimal places. If multiple m/z values are needed to be extracted, separate each value by a comma.

- 4. Highlight the files you wish to analyze on the left.
- 5. Under the Advanced tab, the single m/z expansion mass error should be 5 10 ppm.
- 6. If you wish to integrate the EIC, click "Integrate when extracted"
- 7. Click OK to continue.

8. In the Data Navigator Section, you will see the chromatogram trace of your ion under the Chromatograms tree. Uncheck the TIC scan if needed to normalize the y-axis.

9. The Peak Area of the chosen EIC or Compound can be viewed within the Integration Peak List Panel.



Data Navigator	×
Sort by Type	$\sim$
⊡ Open Data Files	
GFP_LKR049.d	
En Chromatograms	
🗌 🔨 + TIC Scan - GFP_LKR049.d	
\[         \lapha + EIC(5600.0000) Scan - GFP_LKR049.d     \]	=
Cartra	-
ground Spectra	
🗄 🖉 Compounds	
🖳 🗌 Cpd 1: C3 H7 N O3 - GFP_LKR049.d	
🚍 🔽 Cpd 2: C5 H9 N O4 - GFP_LKR049.d	
🗹 /\/\ + EIC(74.5339, 85.5248) Scan - GFP_LKR049.d	
🔽 🔟 + FBF Spectrum (rt: 1.837 min) Sub - GFP_LKR049.d	-
The second secon	×

### c. Extracting U.V. chromatograms

- 1. If your injection file has UV-Vis data, it is possible to extract the chromatogram for a specific wavelength.
- 2. Open the file that has UV-Vis data.
- 3. Under the Chromatograms tab, choose Extract Chromatograms...

Agilent MassHunter Qualitative Analysis 10.0 - QualitativeAnalysis\_QC\_Method.m. File Launch Edit View Find Identify Spectra Chromatograms Method Actions Configura Extract Peak Spectrum 🚰 🞉 🚽 🖻 🎒 🤌 - I P 📑 昰 Extract Chromatograms... 🚰 Sample Table: GFP\_LKR049.d Extract Additional natograms 🕩 🐻 🏭 Use Highlighted C atograms ۶ Results Integrate Chromatogram File Name Sample Name Sample Posit Flags Integrate and Extract Peak Spectra GFP\_LKR049.d GFP\_LKR049 P6-D4 Smooth Chromatogram serine\_500uM.d serine\_500uM P6-F2 Subtract Any Chromatogram serine\_125uM.d serine\_125uM P6-F4 Calculate Signal-to-Noise LOAS\_125uM.d LOAS\_125uM P6-E3 V Set Anchor . 1111 Clear Anchor 🚰 Data Navigator Copy to Chromatograms V S V × Clear Results Sort by Type

4. Under the Type drop-down menu, select Other Chromatograms and select the appropriate wavelength you would like to plot.

Extract Chromatograms		× 1
List of opened data files		
GLP1_15-37_20to50.d J0-2211-NorB25-T1415.d	Type: Other Chromatograms	✓ ▲ ■
	ОК Са	incel

5. After it has been plotted, any M.S. data plots would need to be inactivated to so y-axis can be normalized to the UV-Vis data in the Chromatograms Results Panel. Uncheck any active LC-MS scans in the Data Navigator Panel.

Data Navigator	
Sort by Type	
Open Data Files     JO-2211-NorB25-T1415     Oromatograms	.d
· □ / ∧ + TIC Scan - JO-	2211-NorB25-T1415.d
Spectra Background Spectra Compounds	210,4 Ref=off - JO-2211-NorB25-T1415.d



### XI. Version History

- 0.1: Initial Version 7/20/21
- 0.2: Revised 5/9/2022
- 0.3: Revised 8/16/22
- 0.4: Revised; added Binary Pump acess 12/5/2022
- 0.5: Added EIC Section 12/9/2022
- 0.6: Revised
- 0.7 Revised, added steps to turn on all L.C. modules, updated worklist creation section
- 1.0: Added step on how to update Mobile Phase Bottle Fillings, Working version 2/29/2024

2.0: Converted to new Format, added Column installation procedure, expanded Worklist Creation section, expanded data analysis 11/1/2024