Agilent 1260 Bio-LC Series Master Class

Rotating Session 1: Ultra-sensitive analysis of released n-Glycans with Fluorescence detection

In this practical session you will:

- Learn about critical workflow steps to enable confident detection and quantification of low-abundance IgG glycans with UHPLC instrumentation
- Asses FLD detection performance for critical high-resolution glycan separations such as sensitivity, repeatability, and peak shape.
- Explore Injector programming as a tool for automated sample preparation workflows such as dilution series

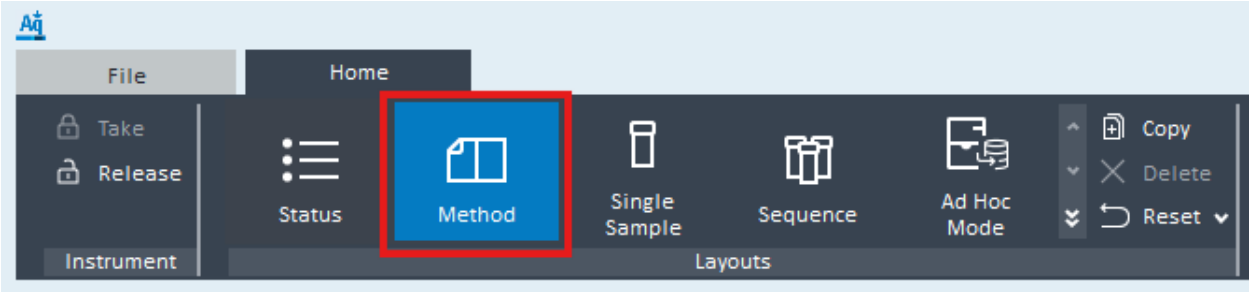
System preparation:

Make sure:

- All modules are switched on and ready
- The Agilent AdvanceBio Glycan Map Rapid Resolution HD 2.1x100mm, 1.8um is attached and well equilibrated with mobile phase in starting conditions.
- Mobile phases are connected (A = 50 mM ammonium formate in water, pH 4.4, B = Acetonitrile, Wash = water)
- IPC HuIgG N-Glycan Library sample (2x dilution), and appropriate trays are placed in the autosampler
- OpenLab CDS Acquisition is running with all the modules ready
- Fluorescence signal is selected in the Online Plot
- OpenLab CDS Data Analysis is launched

PART 1. Building the LC/FLD Method for Analysis:

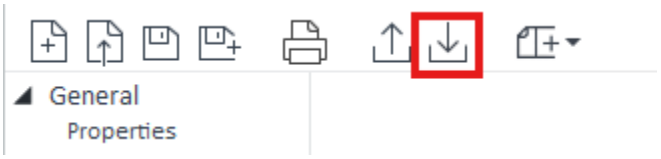
- In Openlab CDS Acquisition window, select "Method" in the top ribbon.



- Load Method "**MC_Glycans 1290FLD.amx"** and review module settings. Make sure method follows as below for each module.
-

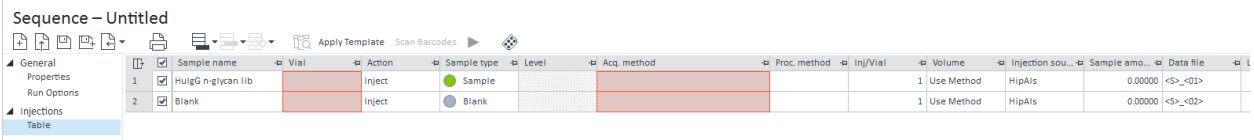
Parameter	Value
Column	Agilent AdvanceBio Glycan Map Rapid Resolution HD 2.1 × 100 mm, 1.8 µm (p/n 858700-913)
Column temperature	60.0 °C
Injection volume	1 µL
Mobile phase	A) 50 mM ammonium formate in water, pH 4.4 B) Acetonitrile
Flow rate	0.5 mL/min
Gradient	Time %B 0 75 1 75 21 71 28 40 29 40 30 70
Stop time	30 minutes
Post time	10 minutes
FLD	Excitation) 285 nm Emission) 345 nm

- Once the method is complete. Save again and “send method to instrument” to begin conditioning.



PART 2. Create the sequence and inject the sample (Glycan Library):

- Create a sequence for analysis of the sample. Be sure to select the correct location for the sample and blank and correct acquisition method.

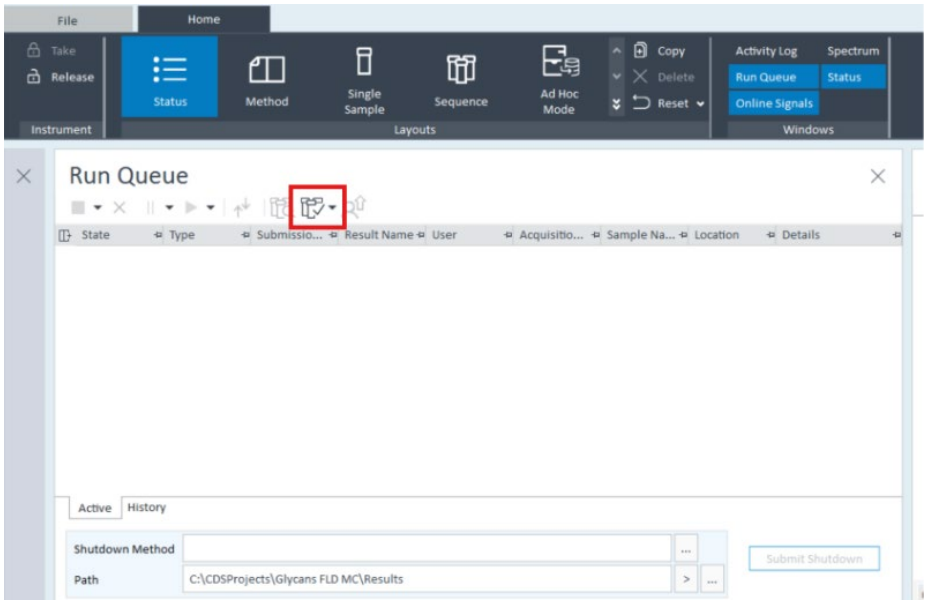


- Save the sequence and run the sequence. A shutdown method can be applied if desired.

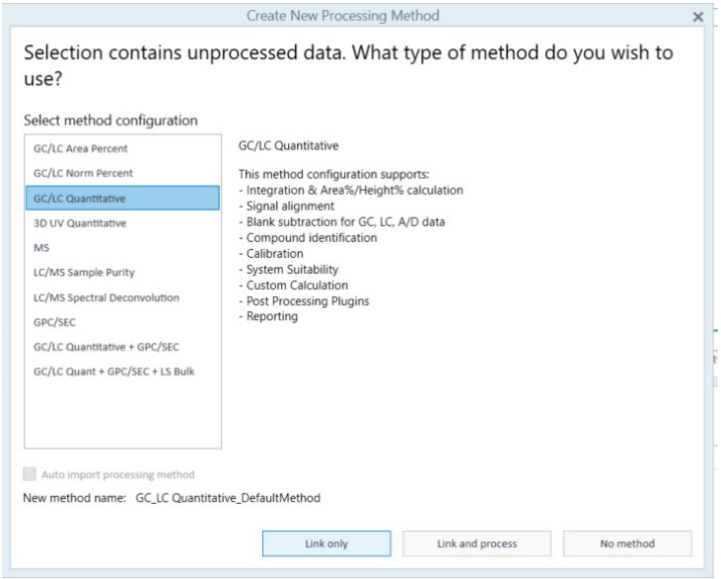
PART 3. Analyze previously collected data:

While your current sample is running, load a previously collected sequence and build a processing method.

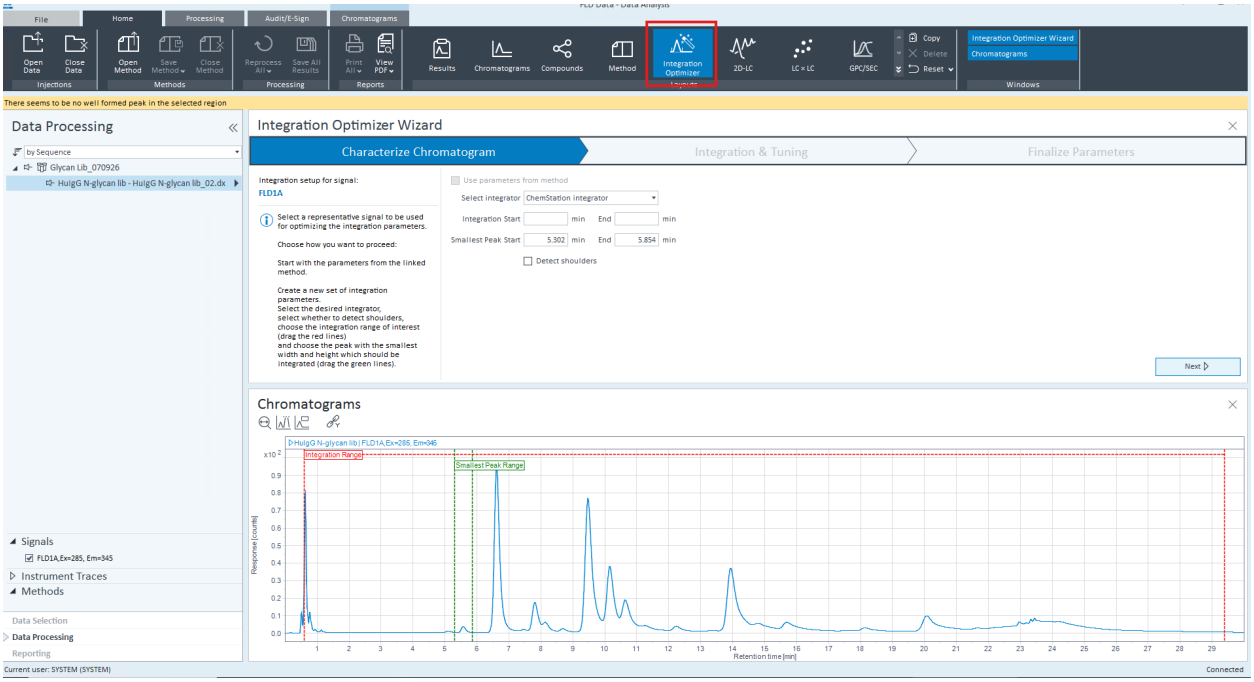
- Load the data in OpenLab CDS data analysis software. This can be done from the Control Panel after selecting your project and using the ribbon. Or from the Acquisition screen, under ribbon icon “Status” inside of the “Run Queue” as shown below.



- Select the appropriate Sequence, confirm injections are correct from the injection list, double click to open the file.
- Open a template processing method “GC/LC Quantitative”, click on Link only, once open, save this method as a new named as **GROUP NO_DATE** to begin modifying your custom processing method.



- Optimize the integration parameters using the “**Integration Optimizer**”. After applying the parameters to the method by “**Update Method-Global**”, click on “**Reprocess All”** to process your results.



- Move through Processing Method parameters on the left, assign specific Glycan peaks in Std samples as “Compounds”, create levels for calibration curve and generate a calibration curve. Reprocess as you go to update chromatograms with new parameters.
- Generate and review your calibration curve, identify the R2 that was achieved. Take note of the sensitivity of the 1290 Infinity III FLD at the lowest levels of the curve, peak shape and separation of glycans in a 30-minute run time.

PART 4. Results:

Once your diluted sample has been run, open the result in the Data Analysis Window. Apply your newly created processing method to quantify the unknown glycans in the sample of IPC HuIgG N-Glycan Library.
Discuss your results with your instructor!