Agilent InfinityLab LC Series

Mass Based Fraction Collection Concept Overview

Technical Note

This Technical Note describes the concept of mass-based fraction collection.

General Information

Mass-Based Fraction Collection System Components

Mass-based fraction collection is designed for standard Prep LC systems that operates with an iQ MSD, a Flow Modulator and an additional Make-up Pump.

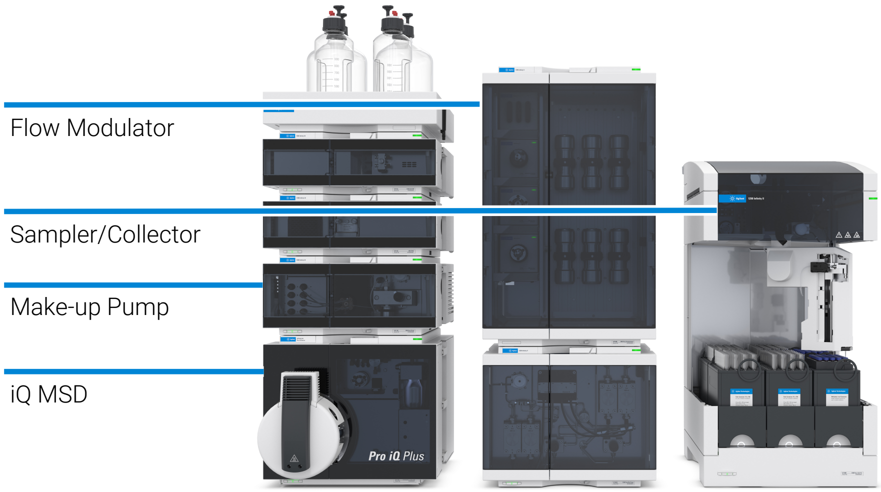


Figure 1: Mass-Based Fraction Collection System Components

The iQ MSD is used to determine the mass of the purified compounds. The Flow Modulator (FM) diverts a small part of sample to the destructive iQ MSD, and the Make-up Pump helps to direct the diverted sample to the iQ MSD.

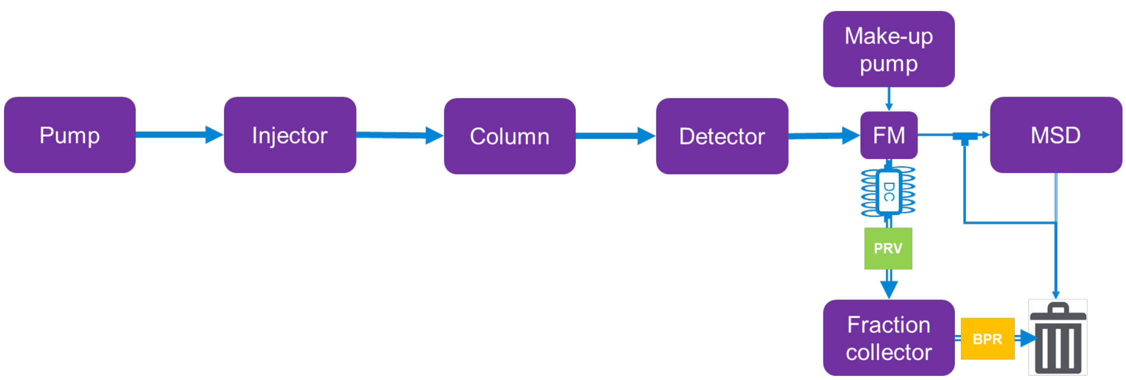


Figure 2: MBFC flow path

FM	Flow Modulator
PRV	Pressure Relieve Valve
BPR	Back Pressure Regulator

A delay coil is required to delay sample to be observed in the Fraction Collector after it has been observed by the iQ MSD. The order of the sample observation should be UV-Detector, iQ MSD, Fraction Collector, with enough time separating the iQ MSD and the Fraction Collector.

Use the following tables as a reference for all capillary connection kits and specific tubing kits used in a MBFC system.

	Flow Range 4-8 mL/min	Flow Range 15-40 mL/min	Flow Range 40-80 mL/min	Flow Range 8-200 mL/min
InfinityLab capillary kit, for 1290 Infinity II Purification Systems with UV detector	5067-7015	5067-7016	5067-7017	5067-7018
InfinityLab tubing kit, with RFID, for 1290 Infinity II Preparative Open-Bed Fraction Collector	G9321-60953	G9321-60952	G9321-60954	G9321-60951
Tubing kit for Preparative Fraction Collector	G1364-68603	G1364-68604	G1364-68605	
Capillary kit for mass-based fraction collection systems	5067-7023			

The following analytical Fraction Collector Tubing Kits can also be used in a MBFC system:

	Flow Range 1 mL/min	Flow Range 1-5 mL/min	Flow Range 4-8 mL/min
Tubing kits for Fraction Collector	G1364-68601 Tubing Kit 1 mL/min, 0.15 mm ID	G1364-68602 Tubing Kit 1 - 5 mL/min, 0.25 mm ID	G1364-68603 Tubing Kit 4 - 8 mL/min, 0.5 mm ID

NOTE

In analytical mass-based fraction collection, there are limitations on the minimum flow rate applied to the flow modulator.

Flow Path Detection Signals

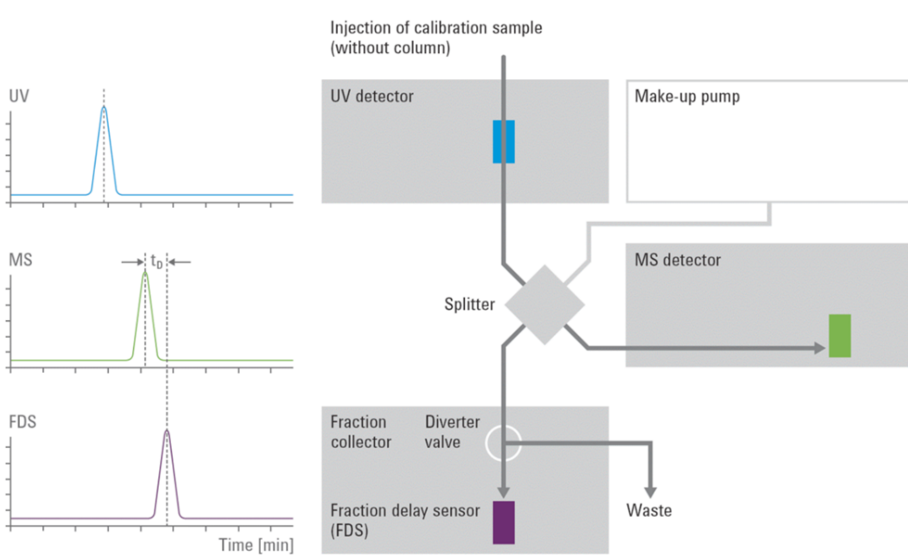


Figure 3: Signal detection in the MBFC flow path

Hardware Installation

The installation of the modules will be done by an Agilent service representative. In this chapter, only installation of user-installable options and accessories are described.

Hardware Requirements

Supported MSD Sources

Product Name	Description
G6160A: Agilent InfinityLab LC/MSD iQ	Electrospray ionization (ESI) m/z 2 - 1450 Polarity switch time < 25 ms
G6160B: Agilent InfinityLab LC/MSD iQ Pro	Electrospray ionization (ESI), Multimode ionization (MMI), Atmospheric pressure chemical ionization (APCI) m/z 2 - 1600 Polarity switch time < 15 ms
G6170A: Agilent InfinityLab LC/MSD iQ Pro Plus	Electrospray ionization (ESI), Multimode ionization (MMI), Atmospheric pressure chemical ionization (APCI), Agilent Jet Stream Source (AJS) m/z 2 - 3000 Polarity switch time < 15 ms

Required Firmware

All Infinity II/III LC modules require at least firmware version X.07.43

The MSD module requires at least SQ-MSD ESW firmware version 12.1

Compatible Firmware Versions

For further information about minimum firmware requirements, firmware compatibilities and emulation for backward compatibility with specific software environments, please check the latest Firmware Bulletin.

The firmware update tools, firmware and documentation are available from the Agilent web.

- https://www.agilent.com/en-us/firmwareDownload?whid=69761

Software Installation

Device Connection

All LC modules, including the MSD, are connected to each other via CAN cables.

The LC detector and the MSD each use a LAN cable to communicate with the software through a hub.

Software Requirements

It is expected that the used PC complies with the minimum OpenLab requirements. For more information, refer to the *OpenLab CDS Workstation Installation and Configuration (CDS_v2.8_WorkstationGuide_en.pdf, D0028022)*.

The following minimum software requirements are needed for MBFC to work properly with OpenLab CDS:

- OpenLab CDS 2.8 with Feature Pack 2
- LC driver version 3.10
- LCMS driver version 3.2

To operate properly in a network environment, the LAN interface must be configured with valid TCP/IP network parameters. Agilent recommends the following IP details:

IP address MS	192.168.254.12
IP address LC	192.168.254.11
IP address PC	192.168.254.10
Subnet Mask:	255.255.255.0

For a networked installation, your Agilent service representative will be able to assist.

Software Configuration

Before you can start to configure the instruments, you need to prepare a corresponding OpenLab CDS project. Then you can add instruments that are assigned to the new project.

For detailed information on how to create a new project and add instruments, refer to OpenLab Help and Learning Online.

Configure an Instrument

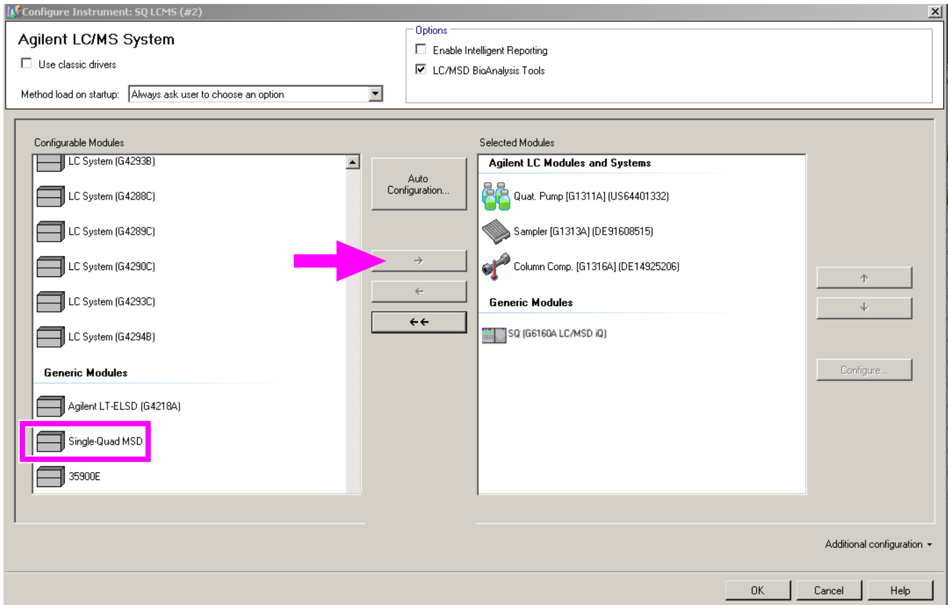
After an instrument has been added to the Control Panel, it must be configured for its specific hardware components. In order to configure an instrument, the instrument must be turned on and connected to the same network as your Agilent Instrument Controller or Workstation.

The modules and instruments of the LC system are configured through Auto Configuration, whereas, the iQ MSD must be manually added and then configured.

For detailed information on how to use Auto Configuration to configure your instrument, refer to OpenLab Help and Learning Online.

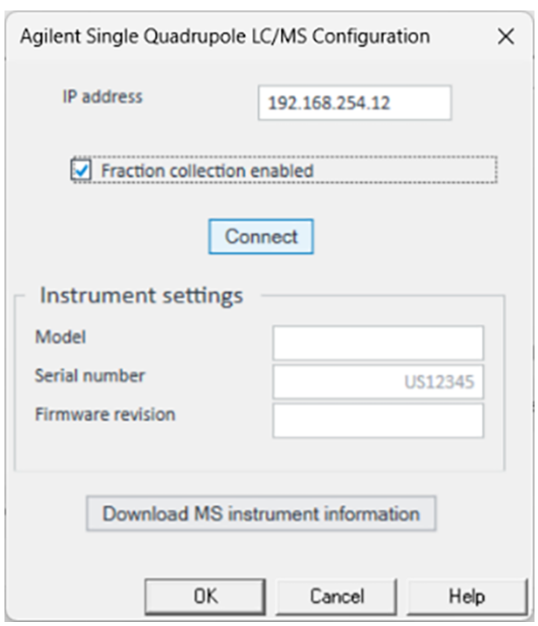
Configure the MSD

1 In the **Configurable Modules** list, select the MSD to move it to the **Selected Modules** list.



2 Double-click the module.

3 In the dialog, enter the MSD **IP address** and select **Fraction collection enabled**.

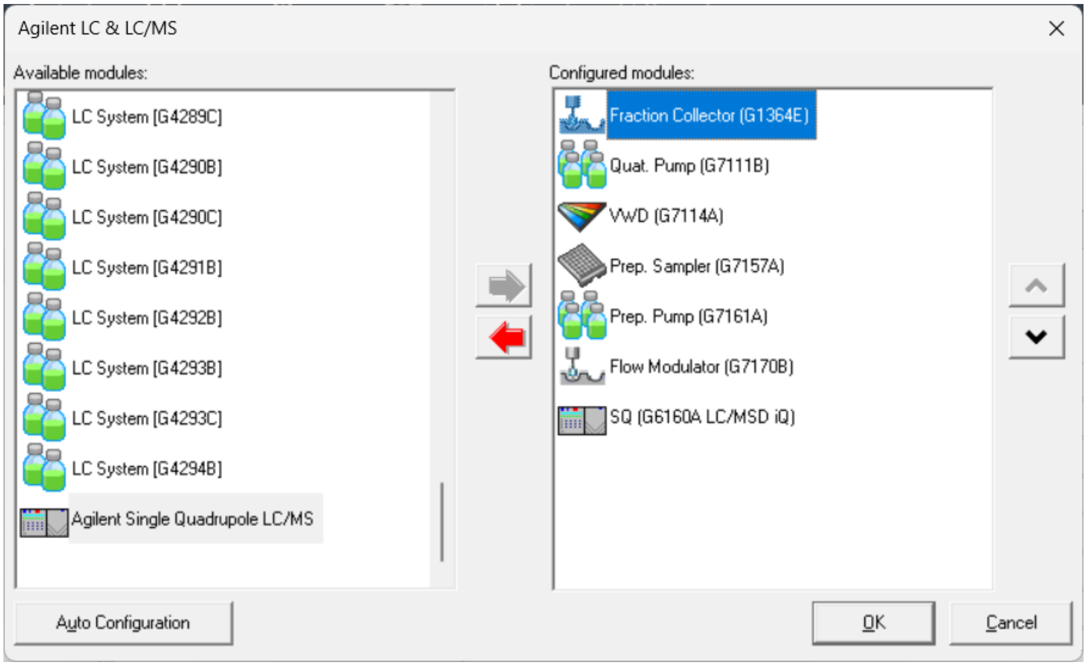


4 Click **Connect**

✓ The instrument information (Model, Serial number, Firmware revision) is filled in automatically.

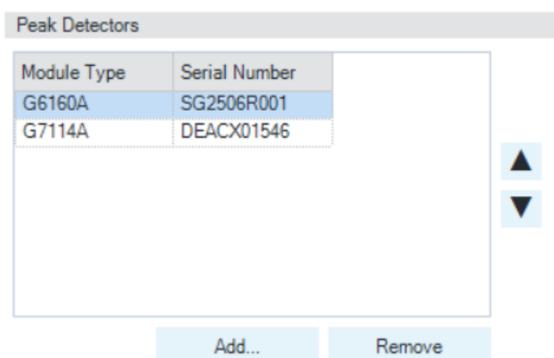
Confirm the MSD for the Fraction Collector as a Peak Detector

1 In the LC system configuration, double-click the Fraction Collector module, and verify that the MSD has been added as a Peak Detector.

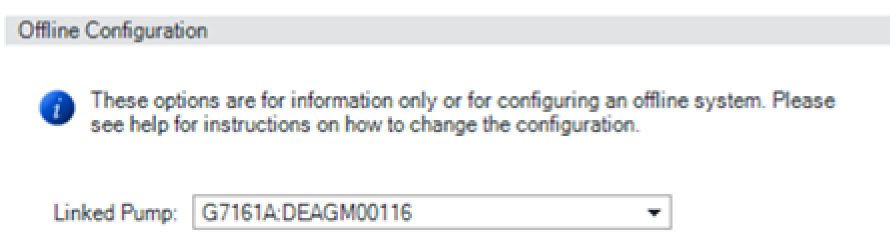


2 If the MSD is not shown in the **Peak Detector** list, add the MSD manually.

a Select **Add...** and enter the Module Type and Serial Number.



3 Make sure that a **Linked Pump** has been selected.
This is necessary for the system to apply the delay calibration values during fraction collection.



4 Select **OK** to confirm your settings.

5. Run a Delay Calibration to finish the installation/configuration. For more information on the Delay Calibration procedure, see Delay Calibration Procedure and Result Review on page 15.

Method Creation in OpenLab CDS 2.x

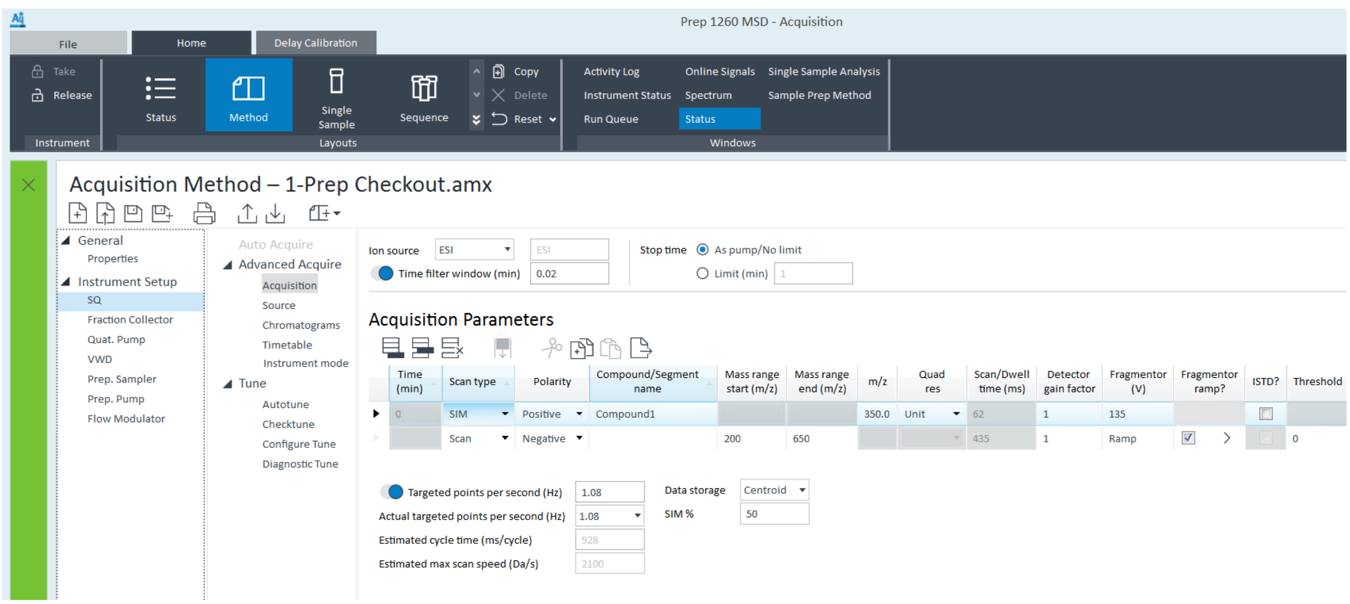
In OpenLab CDS Acquisition, you create methods for each of the LC system modules.

While the Flow Modulator requires input for the Main flow, Make-up flow and Mode settings, this Technical Note only describes the method settings that are required for the iQ MSD and the Fraction Collector.

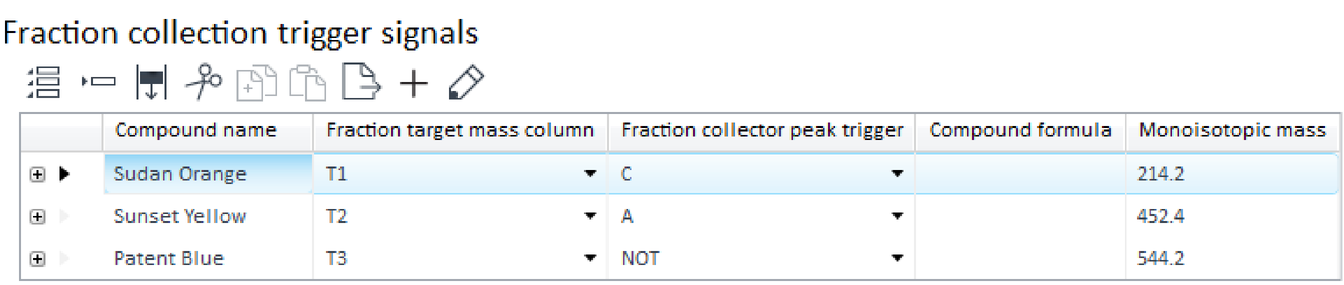
For details on the method creation and acquisition parameters, see OpenLab Help and Learning Online.

MSD Method Setup

In the acquisition method for the MSD, you must set up the parameters for monitoring as for an analyses.



For the MBFC it is additionally important to define parameters that will be used for triggering Fraction Collection. In section **Fraction collection trigger signals**, you specify details of the adducts that trigger the Fraction Collector to collect or explicitly not collect fractions.

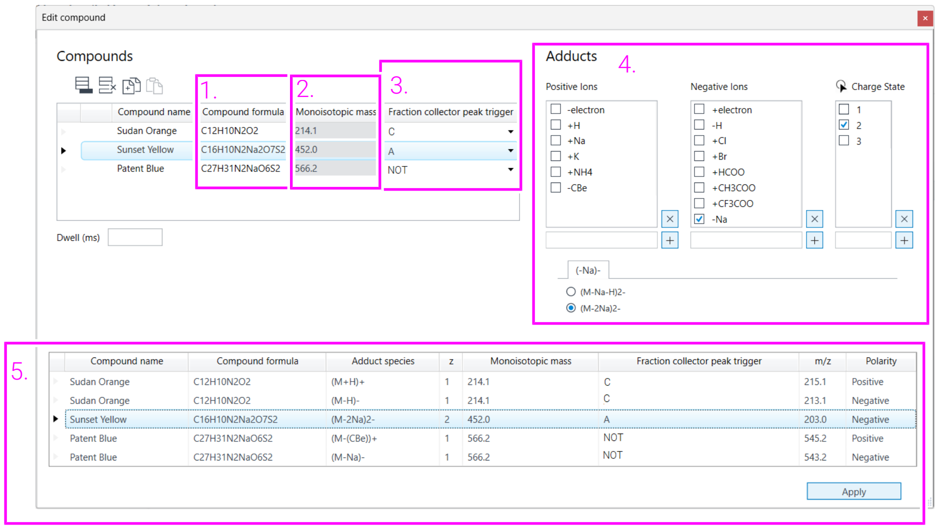


Fraction collection trigger signals

Compound name	Fraction target mass column	Fraction collector peak trigger	Compound formula	Monoisotopic mass
Sudan Orange	T1	C		214.2
Sunset Yellow	T2	A		452.4
Patent Blue	T3	NOT		544.2

Define Fraction Collection Trigger Signals

1 From the toolbar above the table, select + to create a new compound table, or select ✎ to edit the displayed compounds.



2 In column **Compound formula** (1.), enter the formula of the compound. In column **Monoisotopic mass** (2.), the corresponding mass is calculated automatically by the formula, or can be entered manually instead of the monoisotopic mass.

3 In column **Fraction collector peak trigger** (3.), specify the corresponding signal **A**, **B**, **C**, **D**, or **NOT**. This information is used as a trigger for the fraction collector and is applied to the Fraction Collector method (see Figure 4 on page 13).

4 In section **Adducts** (4.), specify the adducts for the compound:

   a Select the desired compound in the table.

   b Choose the most abundant adducts from the list. Here, the most common ones are already listed.

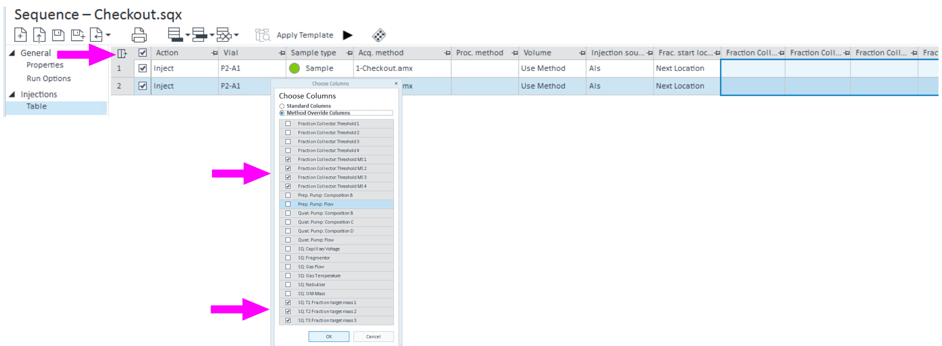
   c To create and add new adducts, enter the name in the field below, and select +. From the list **Charge State**, specify the charge-state to create particular adducts.

   The table at the bottom area (5.) lists a summary of what you have created, including the resulting adduct mass.

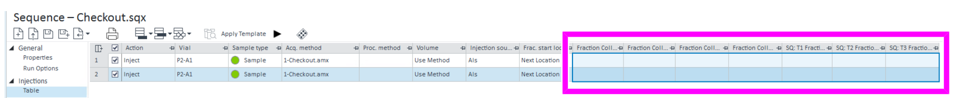
✓ Select **Apply**, to apply the MBFC parameters to the acquisition method.

Create Fraction Target Mass and Fraction Collector Threshold Parameters as Method Override Columns

1 In the Sequence table, add the Fraction target mass columns and Fraction Collector Threshold columns as **Method Override Columns** to the Sequence table.

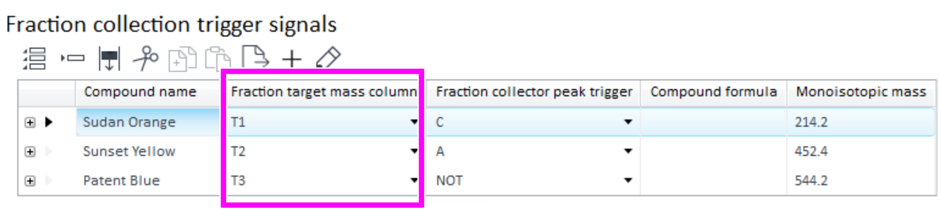


The columns are added to the sequence table.



2 Enter a mass and threshold in the newly created columns.

3 Navigate to the **Fraction collection trigger signals** table. In the **Fraction target mass column**, select the desired column (T1, T2, T3) and respective mass to be applied to the compound.



Fraction collection trigger signals

Compound name	Fraction target mass column	Fraction collector peak trigger	Compound formula	Monoisotopic mass
Sudan Orange	T1	C		214.2
Sunset Yellow	T2	A		452.4
Patent Blue	T3	NOT		544.2

✓ The mass in the sequence table is applied to the defined adducts of the fraction collection parameters. This enables you to run a method with particular adducts set up, but applied to the new masses.

Define Chromatograms to be Displayed in the Online Plot

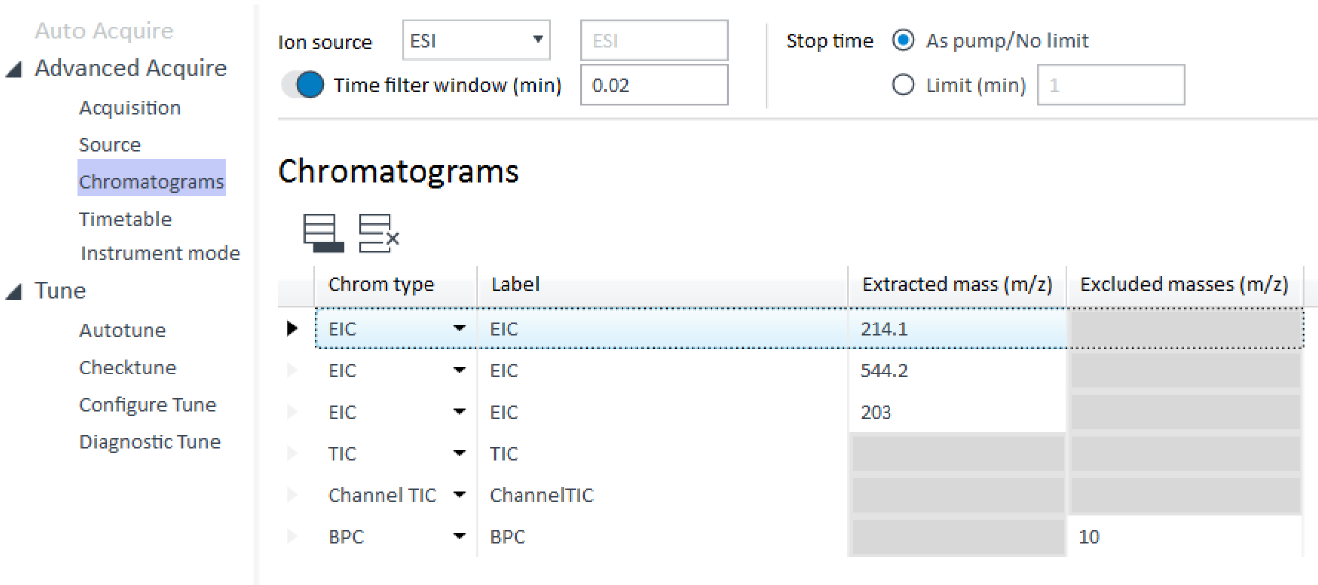
In the Chromatograms section, you define the Chromatograms that can be shown in the Online Plot. As standard, you can specify the EICs for the compounds you have collected.

NOTE The Fraction collector peak triggers are automatically added to the Online Plot.

The following Chrom types are also available:

TIC	The Total Ion Chromatogram is the overlap of all of the scan segment TICs in one trace
Channel TIC	A Channel TIC is a TIC per scan segment. TIC_1 is the TIC for scan segment 1, TIC_2 is the TIC for scan segment 2…

EIC	An Extracted Ion Chromatogram is a chromatogram for only the specified *m/z* or *m/z* range
BPC	A Base Peak Chromatogram. You can specify masses or mass ranges to exclude from this chromatogram



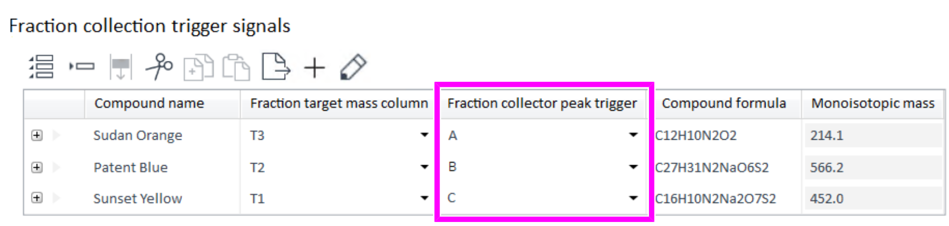
For more information, refer to the OpenLab Help and Learning Online.

Fraction Collector Method Setup

The method for triggering the Fraction Collector with an iQ MSD is similar to that of a Detector. Instead of wavelength signals, the monitored adducts by the MSD are used as signals.

Define the Peak Trigger for the Fraction Collector

1 In the MSD aquisition method, select a signal for the compound to be used as trigger for the Fraction Collector:



In the Fraction Collector method, the defined signals for peak triggers are shown under **Used Signal**.

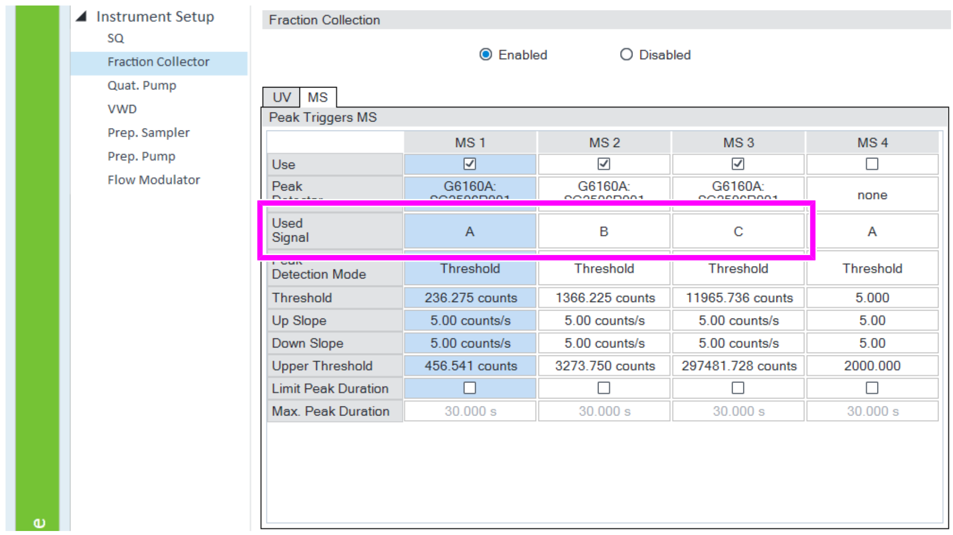


Figure 4: Peak Triggers MS parameters

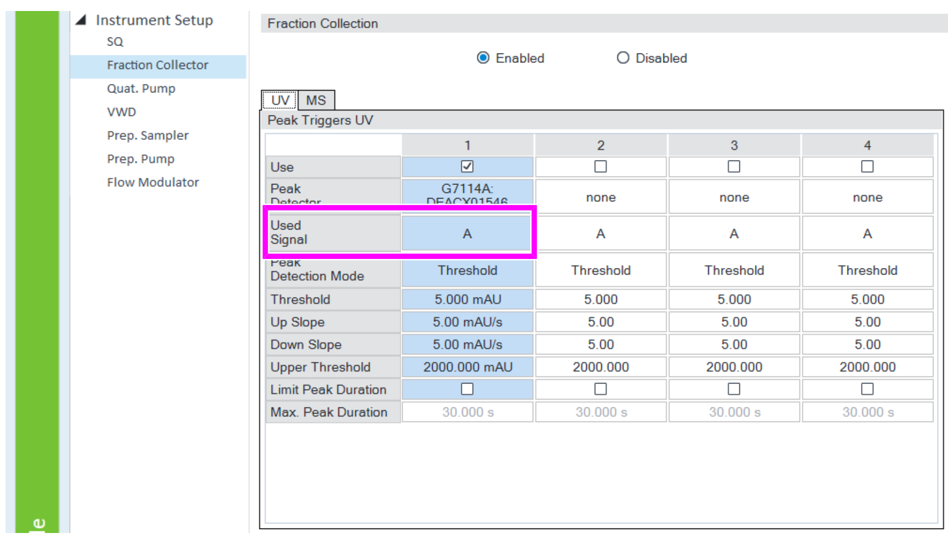


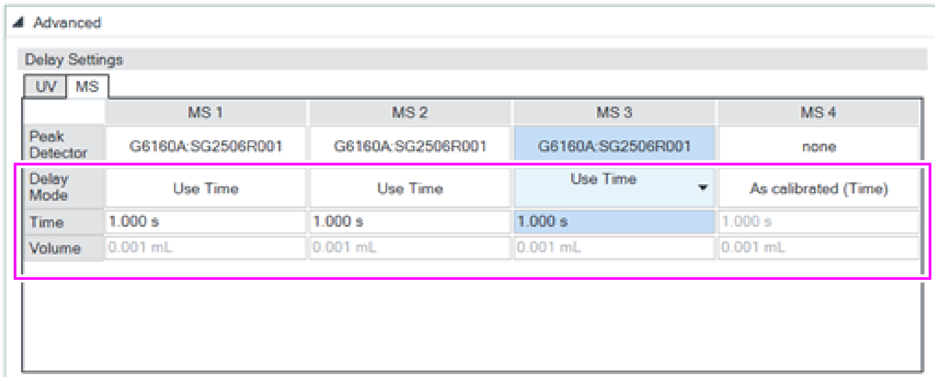
Figure 5: Peak Triggers UV parameters

2 In the table, select the option **Use** for the signal you want to enable the fraction collection.

Define the Delay Mode for the Fraction Collector

In the Fraction Collector method, you enable to override a delay time and delay volume.

1 In the MS/ UV Delay Settings, select the Delay Mode **Use Time**.



For the UV detector, this will override the Delay **Volume** or **Time**, and for the MSD, this will only affect the delay **Time** despite the option for **Volume** being available for the MSD.

Delay Calibration Procedure and Result Review

Run a Delay Calibration

Parts required

Qty.	p/n	Description
1	5190-8223	Delay Calibrant and Checkout Mix for LC The checkout mix consists of three colored components. For the delay calibration, only the second eluting component (Patent blue; blue colored) is of interest.

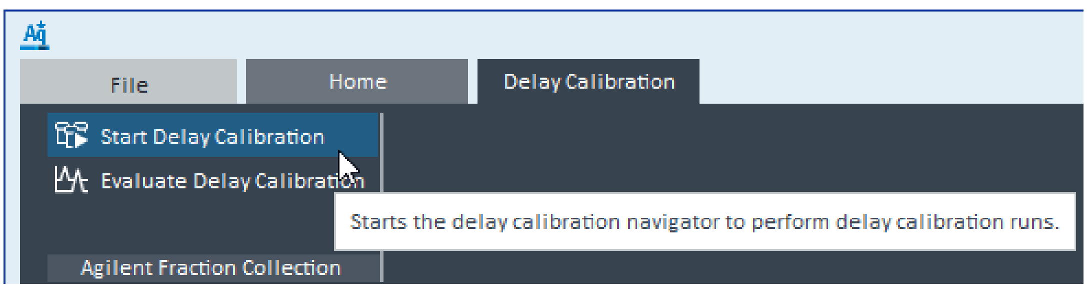
Prerequisites

- A method is already created that identifies all LC modules to include in the calibrated system. Also sample information (location, name, etc.), setup information and run for each of the modules (Sampler, Pump, Detector, etc.) must be defined. For specific details on the method for running a delay calibration, refer to the OpenLab Help and Learning Online.

For details how to create a method for the MBFC, see Method Creation in OpenLab CDS 2.x on page 9.

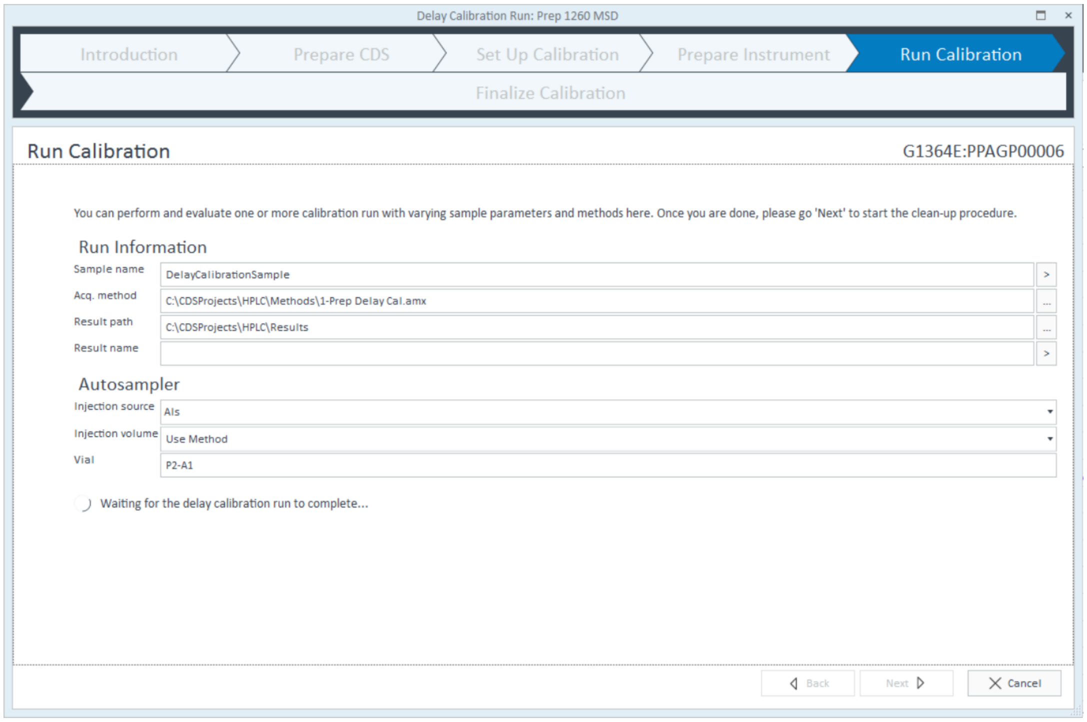
NOTE A Delay Calibration should always be carried out at the flow rate and Flow Modulator settings that is also used for purifying the samples.

1 In OpenLab CDS Acquisition, navigate to the Ribbon tab **Delay Calibration**, and select the option **Start Delay Calibration**.
NOTE: The option is only available if you have an LC Fraction Collector module configured.



A Delay Calibration navigator guides you through the calibration process in several steps.

2 In step **Run Calibration**, specify the method and sample location where the 5190-8223 (Delay Calibrant and Checkout Mix for LC) is located.



3 Once all details are provided, start the calibration.

For more information about the method creation to run the Delay Calibration procedure and to start the Delay Calibration run, refer to OpenLab Help and Learning Online.

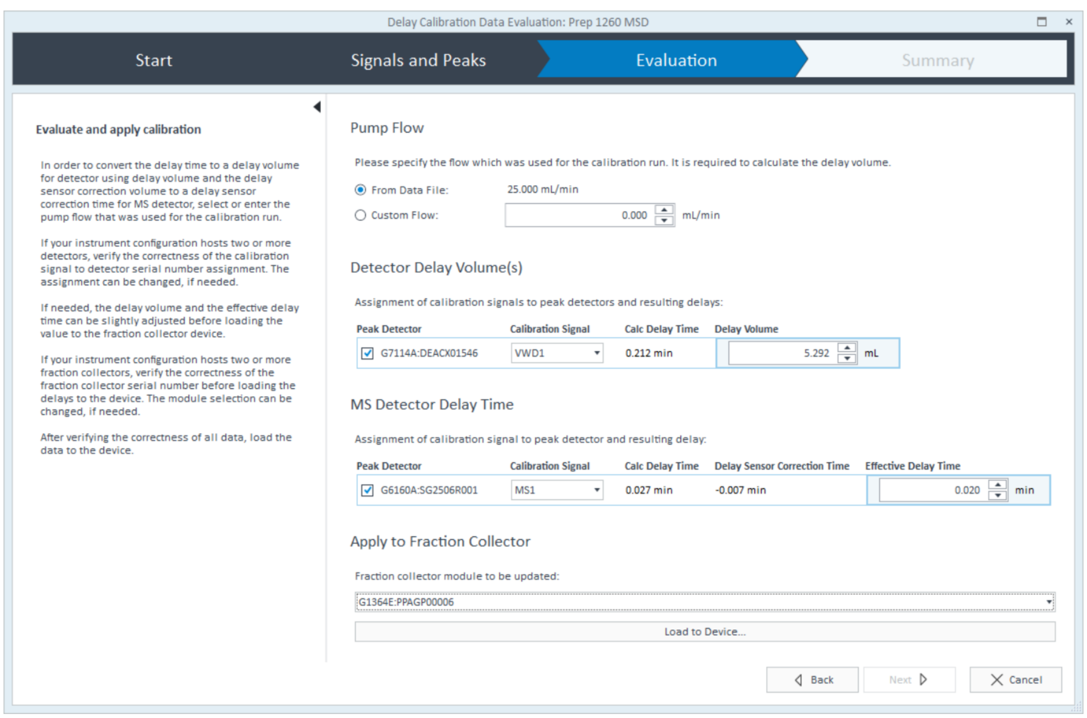
Evaluate Delay Calibration

After the delay calibration process is finished, you must review the Delay Calibration.

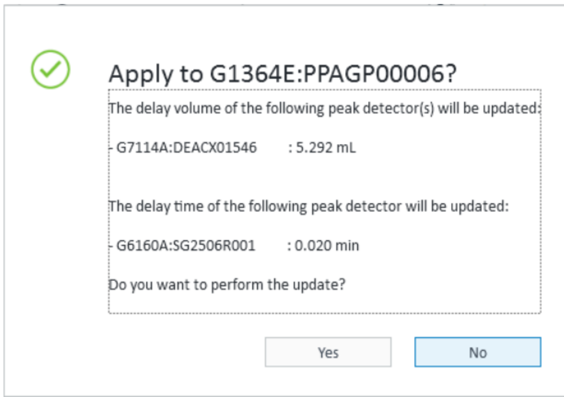
1 In OpenLab CDS Acquisition, navigate to the Ribbon tab **Delay Calibration**, and select the option **Evaluate Delay Calibration**.

2 In step **Signals and Peaks**, you modify integration events of the delay calibrant. Here, you assign the peaks in the UV, MSD and FC chromatograms.

3 In step **Evaluation**, review and apply the data for calibration.



a Select **Load to Device...** to upload the settings to the Fraction Collector.



For more information about the evaluation, refer to OpenLab Help and Learning Online.

Running the Checkout with Different Method Settings

The 5190-8223 (Delay Calibrant and Checkout Mix for LC) is run over a gradient with known conditions (column, mobile phases).

Sample	5190-8223 Delay Calibrant and Checkout Mix for LC	
Solvent A	0.1 % Formic acid in Water	
Solvent B	0.1 % Formic acid in Acetonitrile	
Gradient	0.0 min, 90 % A 0.5 min, 90 % A 4.0 min, 5 % A 6.0 min, 5 % A 6.1 min, 90 % A	10 % B 10 % B 95 % B 95 % B 10 % B
Run time	7.0 min	
Wavelength	signal A: 310 nm +/- 4 nm, 5 Hz, no reference	

Depending on the checkout column (which relates to the acquired tubing kit), different injection volumes and flow rates are used:

Column	Column ID [mm]	Injection Volume [µL]	Flow Rate Range [mL/min]
699975-302 (InfinityLab Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm), or 693975-302 (InfinityLab Poroshell 120 EC-C18, 3.0 x 150 mm, 2.7 µm)	3.0	0.5	0.4 - 0.6
695975-902 (InfinityLab Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm)	4.6	1	1.0 - 1.5
446905-802 (Agilent Prep 100Å C18, 10 x 50 mm, 5 µm)	10	10	4 - 8
446905-702 (Agilent Prep 100Å C18, 21.2 x 50 mm, 5 µm)	20	50	15 - 40
446905-302 (Agilent Prep 100Å C18, 30 x 50 mm, 5 µm)	30	100	40 - 80
446905-502 (Agilent Prep 100Å C18, 50 x 50 mm, 5 µm)	50	300	80 - 200

For the MSD, these are most abundant ions:

Compound	Color	Mass	Typical Ion Species	
Sunset Yellow	orange	452.37	M-Na (double) (neg)	203
Patent Blue	blue	566.66	M-Na (neg) M-Na+H (pos)	543 545
Sudan Orange	yellow	214.22	M+H (pos) M-H (neg)	215 213

NOTE

Due to the addition of formic acid, Sunset Yellow appears in orange and Sudan Orange appears in yellow.

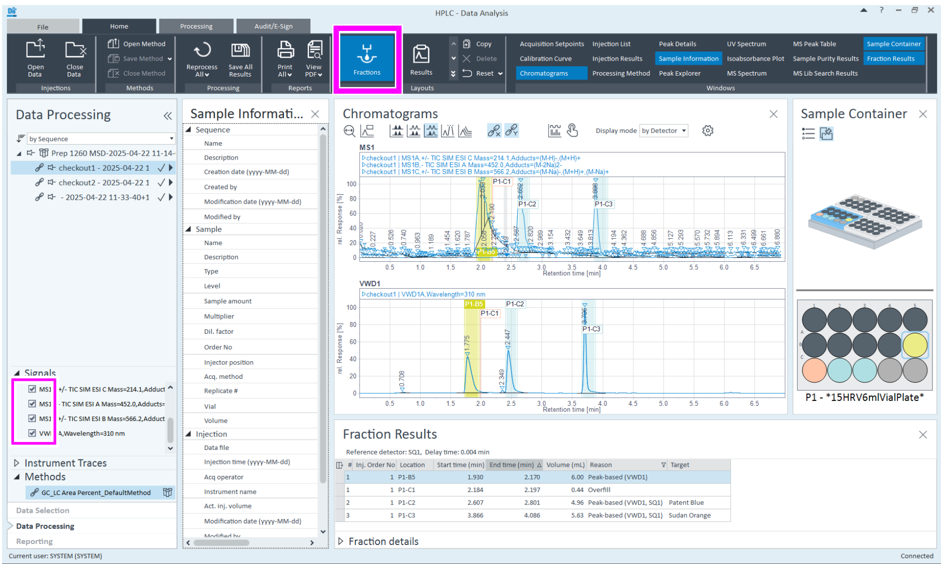
Ensure that three peaks are observed and three fractions are collected (with an orange, blue and yellow appearance). This proves that the system is working as intended; the chromatography was successful, peaks were collected and the delay time is set correctly.

Important:

- The combination of main and split flow in the calibration method needs to be exactly the same as in the target application. When a Flow Modulator (or splitter in general) is used, different flows produce different delay volumes/times, which cannot be re-calculated by the software.
- To avoid unnecessary solvent consumption, it is advised to add a Shutdown method to a sequence to turn pumps off.
- Calibration using the delay and checkout calibrant cannot be performed isocratically. Some dyes in the mixture will not elute properly from the column when a high percentage of organic solvent is used. This is a specific effect of sulfonated dyes being separated with high surface area columns, which are typical for preparative chromatography.

Reviewing Results

OpenLab CDS Data Analysis allows you to review the signals you are interested in. In the Navigation pane of **Data Processing**, select the desired data file and the signal you wish to review in the **Chromatograms** window.



The peak selection is synchronized with the data selection in other windows. If you select a specific peak in the **Chromatograms** window, the related fraction information is shown in the **Sample Information** window, and the corresponding information is also highlighted in the **Fraction Results** table as well as in the fraction location of the **Sample Container** window. Vice versa, if you select a fraction in the **Fraction Results** table, the corresponding peak will be selected in the **Chromatograms** window.

In the **Chromatograms** window, fractions are indicated by a colored box surrounding the fractionated peak. The fraction vial in the **Sample Container** window is highlighted in the same color. The color coding depends on the collected fraction type and is described in detail in the menu of Sample Container window.

The **Fraction Results** table shows information about each collected fraction, such as the location on the instrument, collection start and stop time, collected volume, reason for collection, etc.

For more information on the review of data in Data Analysis, refer to OpenLab Help and Learning Online.

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