Purity | Recovery | Throughput

Gilson Guide to Fraction Collection

GILSON®

ABOUT THIS GUIDE

This guide provides information about fraction collection techniques using TRILUTION® LC Software. If you are unable to find the information you are looking for within this guide, please contact Gilson Technical Support for assistance.

Contact Gilson Technical Support

techsupport@gilson.com

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Chapter 1

INTRODUCTION

Gilson provides high quality, dependable solutions for today's demanding liquid chromatography applications. With products that are designed for purification and engineered for scientists, Gilson offers advanced systems for fraction collection including a complete line of stand-alone and software-controlled fraction collectors ranging from small footprint to large vessel capacity models.

TRILUTION® LC Software provides complete purification system control and offers a variety of fraction collection features. Determining which function or set of software functions will work the best for each application can be done effectively with the fundamentals provided in this guide. Each feature is explained in detail including the conditions it should be used under for a full understanding of the function.

Click here to watch Gilson's *Ask a Scientist* Fraction Collection video series to learn more!

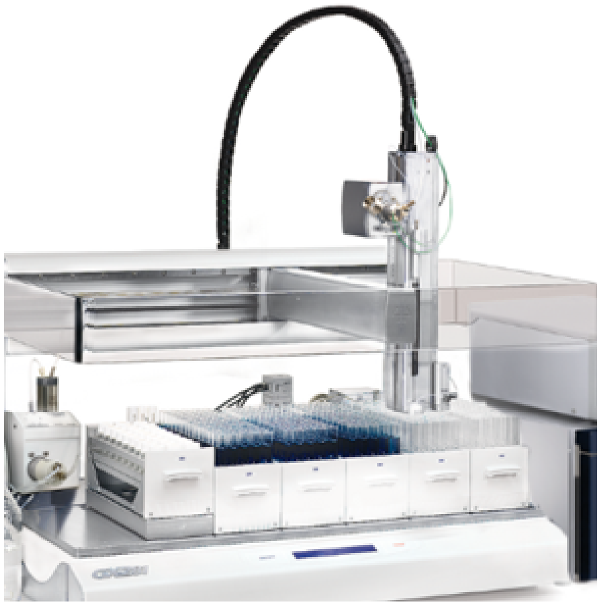


Figure 1
Fraction collection valve on GX-281 Liquid Handler

Chapter 2

FRACTION COLLECTION TECHNIQUES

The cornerstone of purification is fraction collection. Regardless of the application, sample purification requires accurate fraction collection. Fraction collection can be based on a simple function of time or volume or can be more complex based on the signal from the detector. The technique selected for a purification run depends on the goals of the purification, including the number of fractions generated for further processing and the desired purity of the collected fraction. This requires balancing the needs for fraction purity, recovery, and sample throughput when selecting the fraction collection technique and creating the method (Figure 2).

Figure 2
Purification balance

Time/Volume

Time and Volume are the most basic types of fraction collection. These types do not consider the detector signal and simply fractionate at steady intervals based on the user setting (Figure 3). Since the collection is not dependent on the detector signal, time and volume collection provide the lowest purity and generate the most fractions. These types of collections can be useful if the detector signal is weak or no detector signal is expected, allowing collection even when there are no detectable peaks.

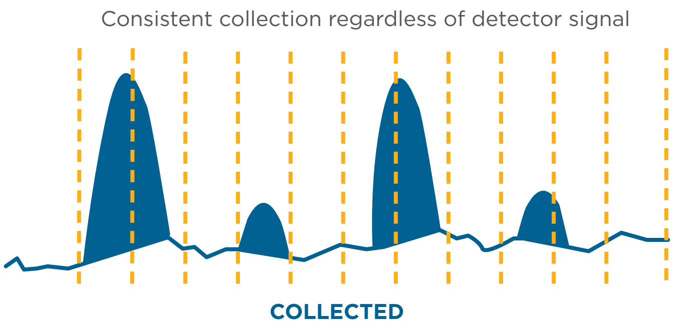


Figure 3
Time/volume collection

Level

Level, or threshold, is the most basic method for using the detector signal to determine when to collect fractions. Fraction collection begins when the detector signal passes through, or is above, the set value (threshold). Once the detector signal falls below the threshold, fraction collection stops (Figure 4). This method has two limitations. First, fraction collection may not trigger if the sample is dilute, and the detector signal does not reach the threshold. Second, any co-eluting peaks would be collected together and would require additional steps to separate. Third, if the threshold is set too close to the baseline noise level, many small fractions could be generated as the signal moves above and below the threshold.

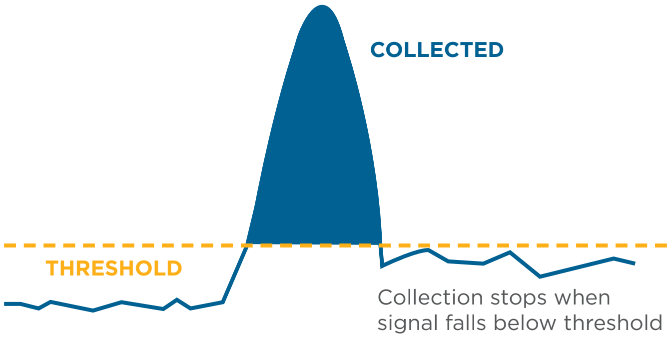


Figure 4
Level collection

Slope

Slope collection is a more advanced method for using the detector signal to determine when to collect fractions. Software calculations are used to determine where to collect on the peak based on the slope, or angle, of the peak inflection and deflection. Fraction collection begins when the peak front is seen on the detector signal and stops when the peak tail is seen on the signal (Figure 5). By using this method, peaks, including co-eluted peaks, can be collected; however, if the detector signal is noisy, unwanted fractions may be collected.

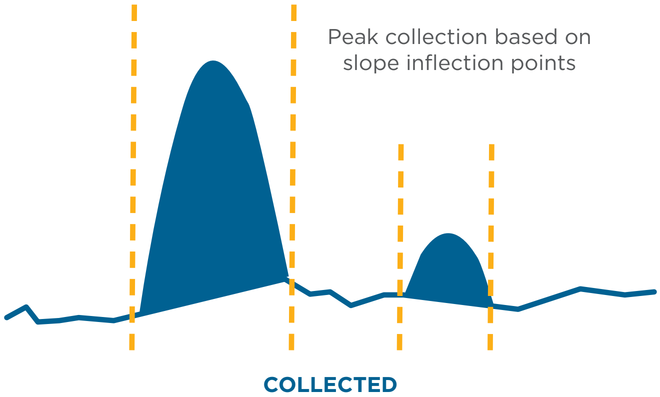


Figure 5
Slope collection

Chapter 3

HOW TO COLLECT FRACTIONS WITH TRILUTION® LC

TRILUTION LC is capable of collecting fractions by slope, level, time, and volume and is also able to combine techniques. TRILUTION LC includes two main tasks that set how to collect fractions, Fraction Collection Settings and Conditional Fraction Collection. Fraction Collection is dependent on the monitored data channels.

Detector Settings

The detector channel set as the primary channel in the method configuration will be used for fraction collection (Figure 6). Any additional detector channels can be used as secondary channels when combining fraction techniques using the Conditional Fraction Collection task.

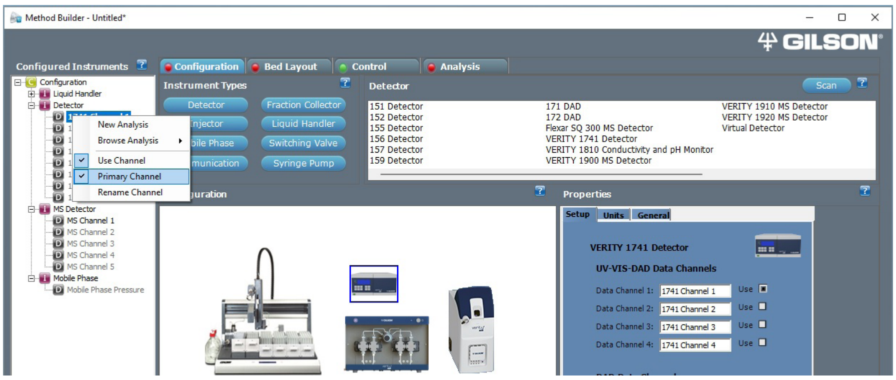


Figure 6
Setting detector channel as the primary channel

Start / Stop Fraction Collection

The Start Fraction Collection and Stop Fraction Collection tasks must be included in the method to indicate when collection should occur. Regardless of fraction settings, no collection will occur prior to the Start Fraction Collection task and no collection will occur after the Stop Fraction Collection task. The Start Fraction Collection task is typically scheduled after the injection task in the method.

Fraction Collection Settings Task

The Fraction Collection Settings task can be used to handle most basic fraction collection needs. This task should be used to collect fractions by Slope, Level, Time, or Volume based on the primary data channel (Figure 7). Add this task to the method prior to the Start Fraction Collection task to set the fraction collection parameters.

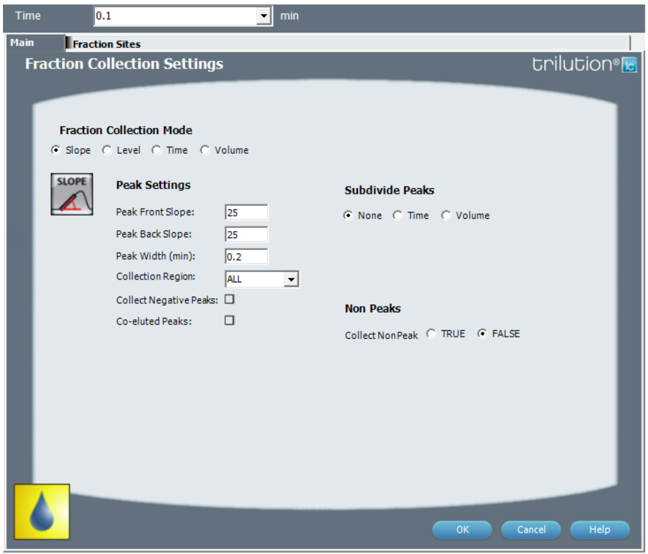


Figure 7
Fraction Collection Settings task

Time/Volume Collection

Volume and Time are the most basic types of fraction collection in TRILUTION LC. In the Fraction Collection Settings task, select the Time or Volume option and enter the desired time or volume interval. Be sure to keep the tube capacity in mind when selecting the time/ volume to prevent overfilling fraction tubes.

Slope Collection

Slope collection allows setting parameters for Peak Front Slope, Peak Back Slope, and Peak Width (Figure 8). When collecting fractions by slope, the front slope setting determines where peak collection will begin on the front of a peak. Consider the angle of the peak's ascending edge when selecting a slope value. Higher front slope values will start collection later on a peak, where the slope is rising quickly, while lower front slope values will start collection earlier on a peak.

The back slope setting determines where peak collection will end on the tail of a peak. Higher back slope values will end collection earlier on the tail of a peak. Lower back slope values will end collection later on the tail of a peak. This parameter can be used to confirm the tail of a peak is collected or that a tail on a peak is discarded. Note that if the back slope is set too high, TRILUTION LC will have difficulty ending collection on short tailing peaks.

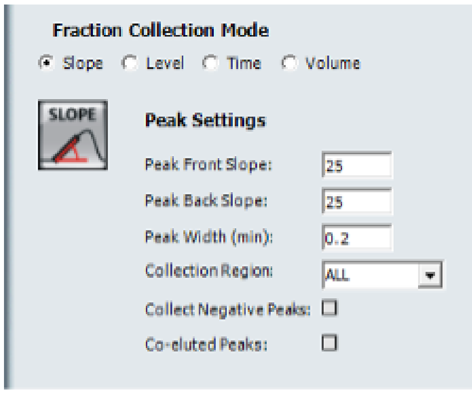


Figure 8
Slope collection parameters

The example in Figure 10 shows the back slope value incrementally increased to demonstrate how changing the value affects the amount of a peak that is collected (full peak shown in Figure 9). Front slope and peak width values were held constant in this example.

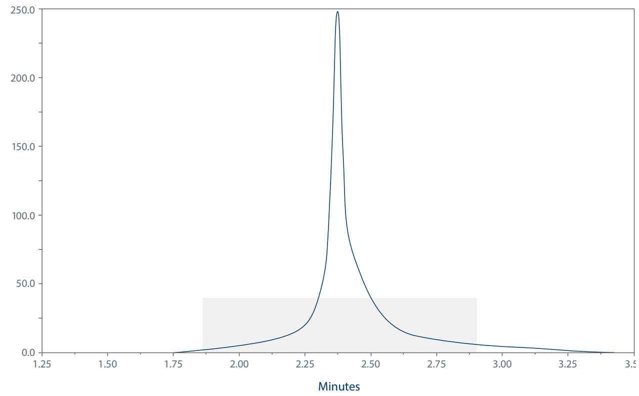


Figure 9
Full peak

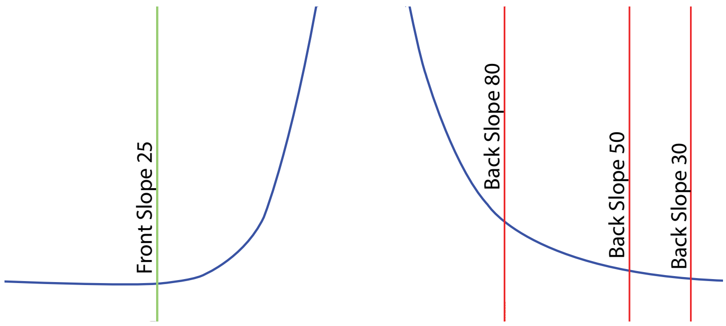
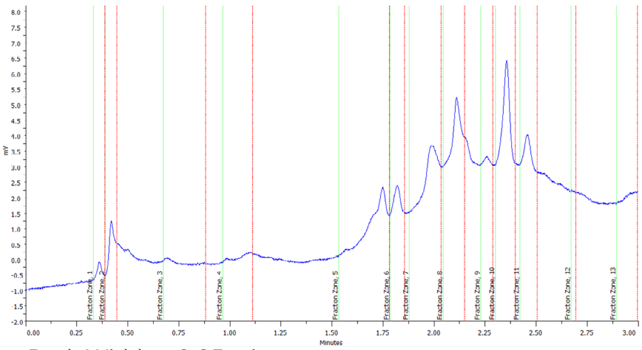


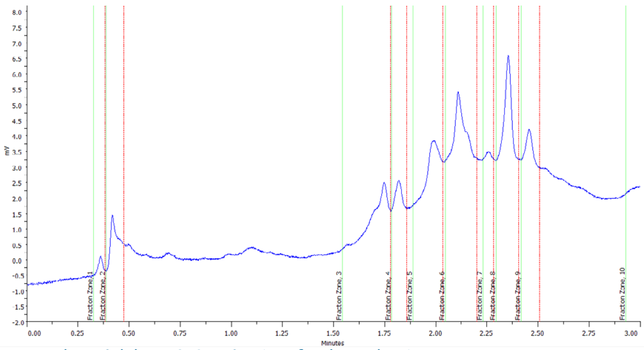
Figure 10
Impact of varying back slope values

The peak width value is a time window over which front and back slopes are calculated. Increasing the peak width value will decrease the number of small or narrow peaks that are collected, thus serving to filter out baseline noise. The default (0.2 min) is acceptable for most chromatograms, and the typical range of values is about 0.2 to 0.5 min (although values outside this range are valid settings).

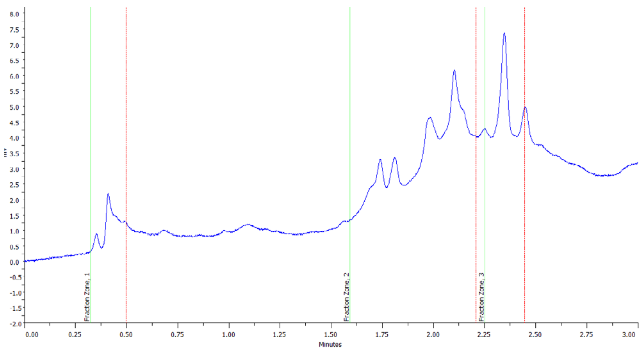
The following examples demonstrate the impact on fraction collection with varying peak width settings (Figure 11). By increasing the peak width, fewer fractions are collected; however, increasing too much can result in combining multiple peaks into a single fraction.



Peak Width = 0.05 min
13 Fractions Collected



Peak Width = 0.2 min (Default Value)
10 Fractions Collected



Peak Width = 0.8 min
3 Fractions Collected

Figure 11
Impact of varying peak width, front and back slope held constant at 25

Collection Region (Front to APEX, APEX and APEX to Tail)

Slope collection also allows for fractions to be collected based on specific regions of the peaks. When collecting by slope, a peak can be collected or subdivided based on a peak's APEX by selecting Front to APEX, APEX, or APEX to Tail (Figure 12). With the Collection Region set to ALL (software default), the full peak is collected.

Front to APEX is useful when a chromatogram has poorly resolved peaks and a high preparative flow rate is used. This enables collection of a nearly pure fraction, while still maintaining a reasonably high recovery.

APEX to Tail is commonly used when collecting poorly resolved peaks or peaks that have significant tailing but will result in reduced recovery.

The APEX option collects the Front to APEX portion and the APEX to Tail portion of the peak into separate tubes. APEX collection is useful when running chiral compounds that are poorly resolved. This provides the same purity capabilities and potentially more recovery than Front to APEX, but also generates more fractions that need further processing. This mode could also slightly lower recovery compared to collecting the whole peak due to the fraction valve switch at the most concentrated part of the peak.

NOTE	**Peak subdivision by time or volume is also possible with slope collection (see page 8).**

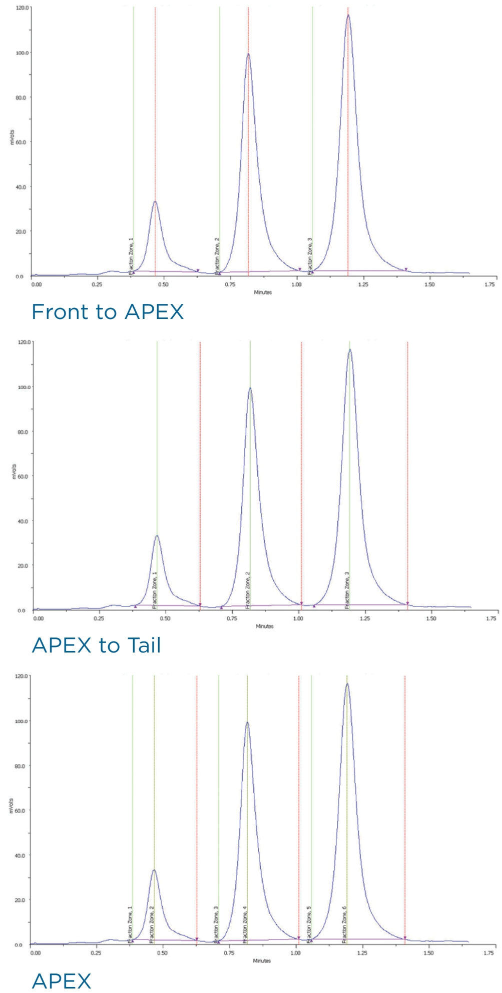


Figure 12
Impact of varying collection region

Level Collection

Fraction collection by level is based on a user-set value, or threshold (Figure 13). When the detector signal exceeds the set level, collection begins. When the signal goes below the level, collection ends.

- Level can collect positive and/or negative peaks.
- Peak subdivision (see below) by time or volume is also possible with level collection.

Peak Subdivision

When collecting by either slope or level, peaks can be further subdivided by either time or volume (Figure 14). Subdivision will ensure that collection tubes will not overfill when collecting wide peaks or when pumping at high flow rates. Subdividing, or slicing, peaks also allows the peak to be cut into smaller sections for additional quality checks or to confirm if peaks are co-eluting with additional processing.

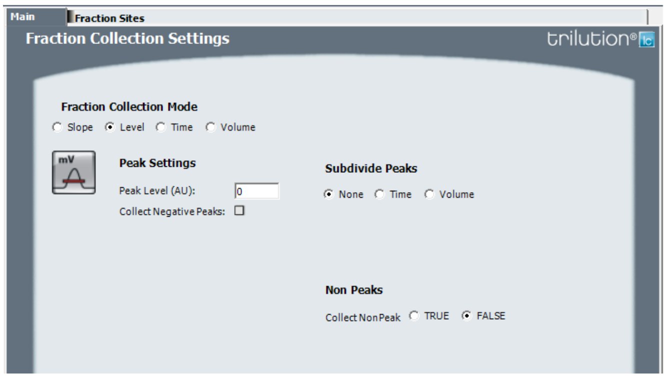


Figure 13
Fraction Collection Settings task with Level option selected

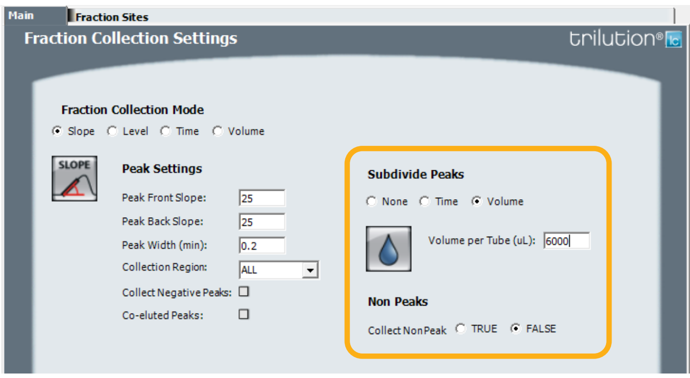


Figure 14
Fraction Collection Settings task, selecting to subdivide peaks by volume

Non-peak Collection

Non-peak collection can be activated during level or slope collection. When active, fractions are collected during the entire collection window. Peak and non-peak regions are separated based on the settings for level or slope. Non-peak regions can be subdivided by time or volume (Figure 15).

Setting non-peak collection is useful to ensure that no compounds are missed but will generate the largest number of fractions to process.

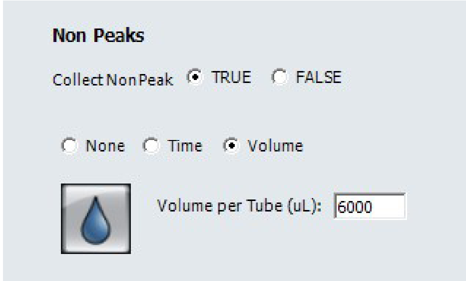


Figure 15
Non-Peak subdivision parameters

Collection and Travel Depths

The ability to set collection and travel depths is a useful feature when collecting fractions through the probe. This is often required when collecting into sealed vials, when collecting into vessels with a small diameter, such as microplates or 2 mL vials, or when performing low volume fraction collection that requires contact between the probe and receiving well or liquid surface.

> **NOTE** **Collecting fractions through the probe requires the appropriate high mount fraction collection kit for the liquid handler.**

The Fraction Collection Depth is the Z-value (measured up from the tray) where the probe will descend to before fraction collection begins. After collecting, the probe is raised to the Travel Depth and moves horizontally to the next available fraction well (Figure 16).

Example: When working at a 125 mm clamp height, with deep well microplates at 108 mm, you could establish a travel height of 110 mm that would allow the probe to clear the top of the plate. A collection height of 100 mm would then make the probe descend 10 mm into each well before beginning to collect.

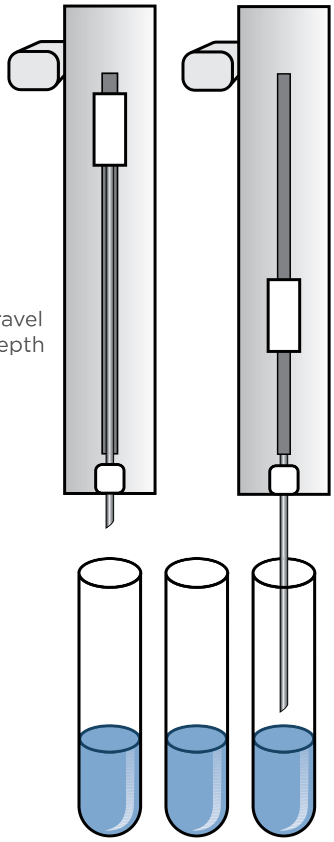


Figure 16
Collection and Travel Depths

Collection and Travel Depths are set within the Fraction Sites tab. To activate, click the Set Collection and Travel Depths option and set the depths according to the configuration (Figure 17). By default, the Collection Depth and Travel Depth are set to -1, which is a bypass setting.

These two parameters may be used independently. For example, the user may only want to set and lock in a Travel Depth. In this case, the desired value is entered for Travel Depth, and the Collection Depth is set to -1, which is the bypass setting. When choosing Collection and Travel Depths, the user has the freedom to select any value within the Z range of the instrument, and therefore must be aware of other racks and tubes on the bed. Optimizing Collection and Travel Heights minimizes the motion of the probe. Because the fraction collection valve is in the divert (waste) position during these movements, it is very important to use these parameters correctly to maximize fraction recovery.

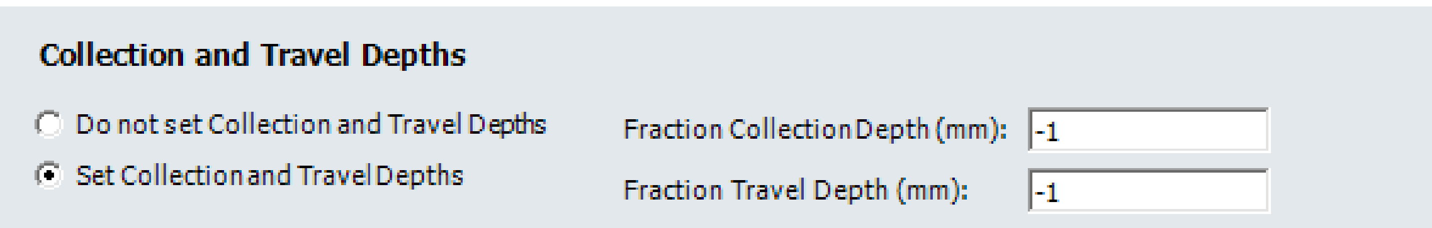


Figure 17
Fraction Collection Settings task, Collection and Travel Depth options

Configuring Fraction Locations

In the Fraction Sites tab, a zone and a starting well can be specified to be used for fraction collection (Figure 18). The zone indicates the area where the fraction is collected on the available rack space of the fraction collector/liquid handler. The well indicates the starting collection tube number within the specified zone. Use the supplied variable (#Fraction Well) to set the starting tube at run time. A value of 0 indicates continuous collection, where fractions are always collected in the next available tube within a run.

There are four Fraction Sites that can be entered. This allows multiple fraction collectors to be added for increased bed capacity and can be used in conjunction with a Fraction Collection System (see page 19). If only one instrument is being used for fraction collection, only one Fraction Site needs to be specified.

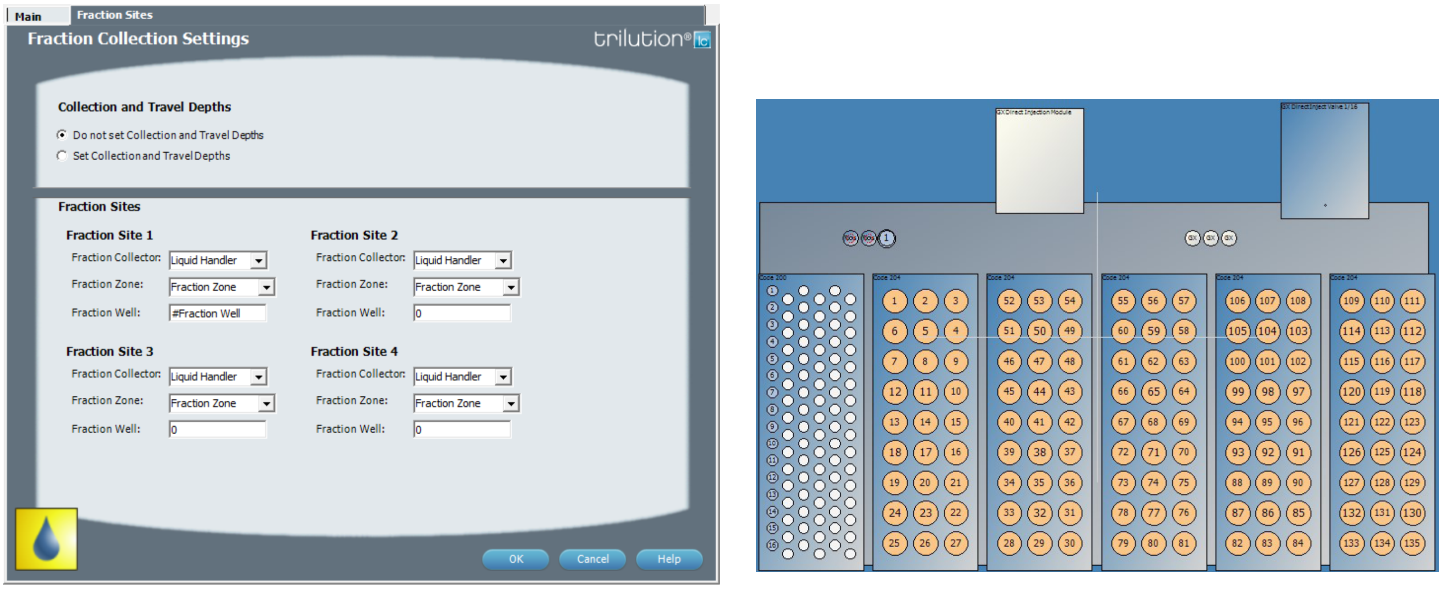


Figure 18
Fraction Collection Settings task (Fraction Sites option) and bed layout with fraction zone highlighted

Conditional Fraction Collection Task

The other main task to set how to collect fractions is the Conditional Fraction Collection task, which can be used to perform more advanced fraction collection compared to the Fraction Collection Settings task. With this task, logic conditions can be set to combine modes of fraction collection (i.e., slope/level). Conditional logic can be applied to either a single channel or multiple channels.

The conditional statement can be set to “AND”, “OR”, or “AND/OR” to optimize fraction collection.

AND: All conditions need to be met to start collection

OR: Any one condition needs to be met to start collection

AND/OR: The primary condition and any secondary condition needs to be met to start collection

Configuring Conditions

The Conditional Fraction Collection task requires three inputs: selecting the primary condition, selecting the conditional expression, and selecting secondary conditions (Figure 19). Up to seven secondary conditions can be set.

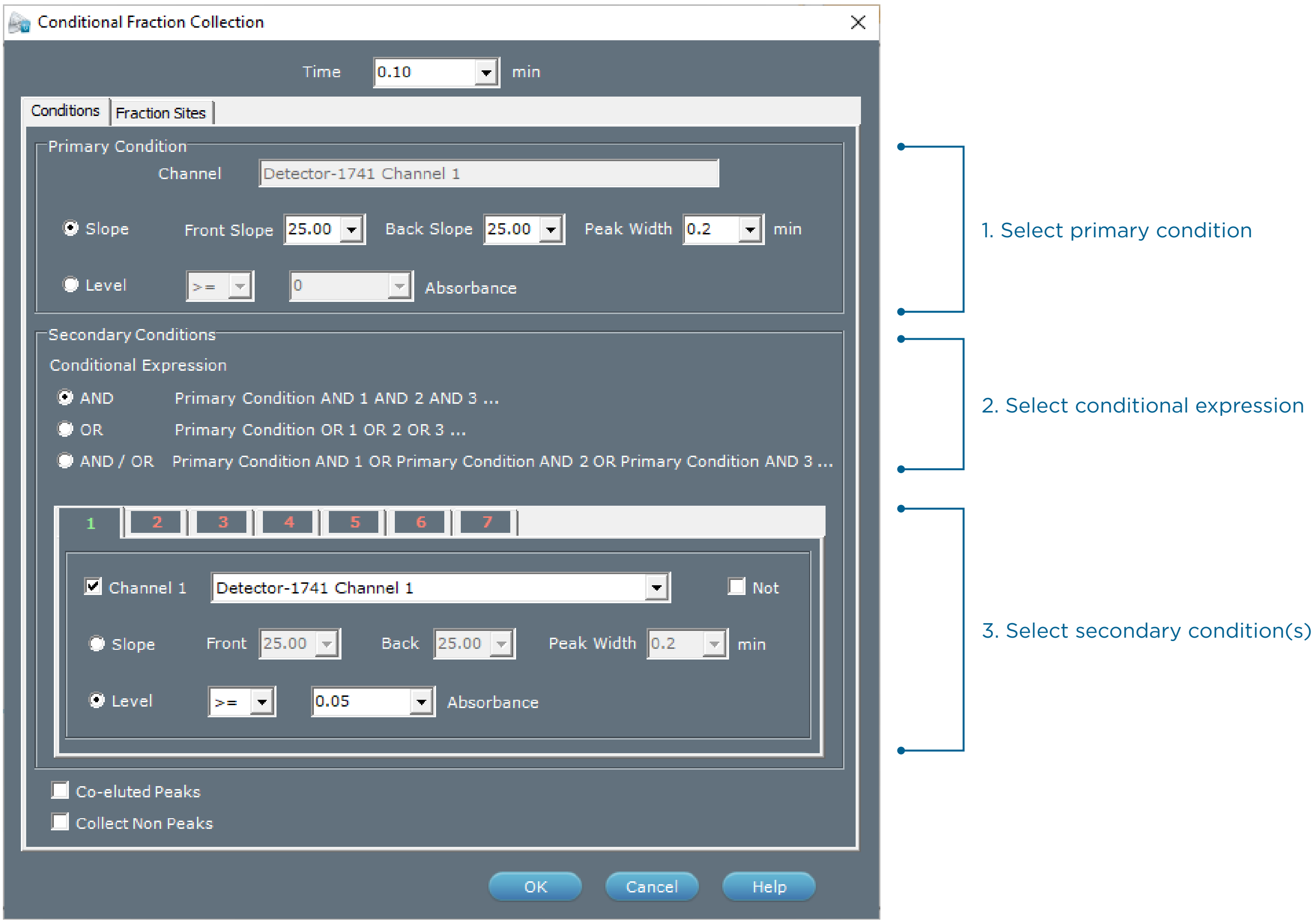


Figure 19
Conditional Fraction Collection task

Using a Single Data Channel

Using data from a single data channel, the Conditional Fraction Collection task can be used to combine slope and level collection to optimize fraction collection. In this example, a combination of slope and level is used to collect only the pure center portion of a peak (Figure 20). The same data channel is used as both the primary and secondary channel using the "AND" expression. In this example, both slope and level criteria must be met for collection to begin.

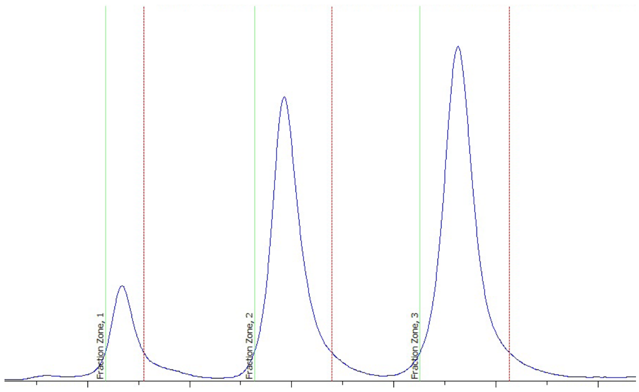


Figure 20
Conditional Fraction Collection with slope AND level on a single channel

Using Multiple Data Channels

Conditional Fraction Collection can also be used to collect fractions based on signals from multiple detector sources, such as ultraviolet (UV), diode array (DAD), mass spec (MS), evaporative light scattering (ELS) and others brought in through the analog/digital interface (506C).

A common application is to perform fraction collection based on a combination of UV and MS data. In this example, the UV is selected as the primary channel (collection by slope) while the MS data signal is selected as the secondary channel (collection by level), using the "AND" expression. Collection only occurs when both conditions are met, allowing targeted fraction collection of the single peak of interest (Figure 21).

The "AND/OR" expression can also be useful with a combination of UV and MS data with multiple MS data channels. In this example, the UV is selected as the primary channel (collection by slope), while multiple MS SIM data channels are selected as secondary channels (collection by level), using the "AND/OR" expression. Fraction collection occurs when the UV condition is met (blue channel) along with any of the secondary MS conditions (orange, light green, aqua, and red channels), allowing targeted fraction collection by mass (Figure 22). One peak is not collected as it does not meet the criteria.

Secondary data can also be collected from an ELS detector. In this example, a UV signal is selected as the primary channel (collection by slope) and the ELS data signal is selected as the secondary channel (collection by slope) using an "OR" expression. In this case, fraction collection is triggered as the peaks are detected on either the UV signal or the ELS signal. This can be a useful combination because peaks detected in the ELS signal contain compounds that are lacking chromaphores and thus do not give a UV response. As shown in Figure 23, only one peak is seen on the UV trace (blue), however the ELS signal (orange) allows for identification and purification of many additional compounds.

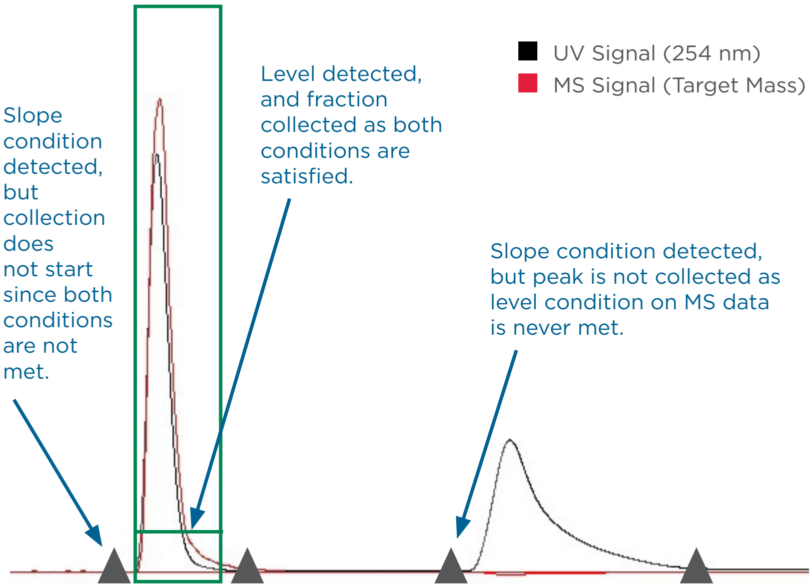


Figure 21
Conditional Fraction Collection with multiple data channels, "AND" expression

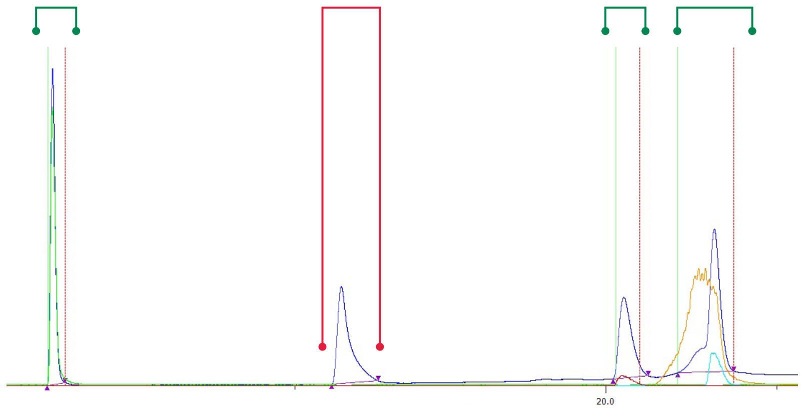


Figure 22
Conditional Fraction Collection with multiple data channels, "AND/OR" expression

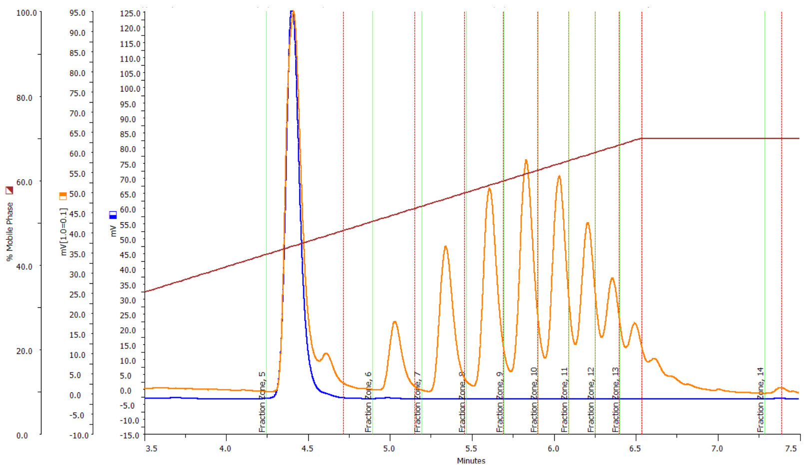


Figure 23
Conditional Fraction Collection with multiple data channels, "OR" expression

Conditional Fraction Collection “Not” Option

The Conditional Fraction Collection task also includes an option to stop collection based on a specified condition. In the following example, “Not” was selected for the aqua colored MS trace, and fraction collection stopped when that mass was detected (Figure 24).

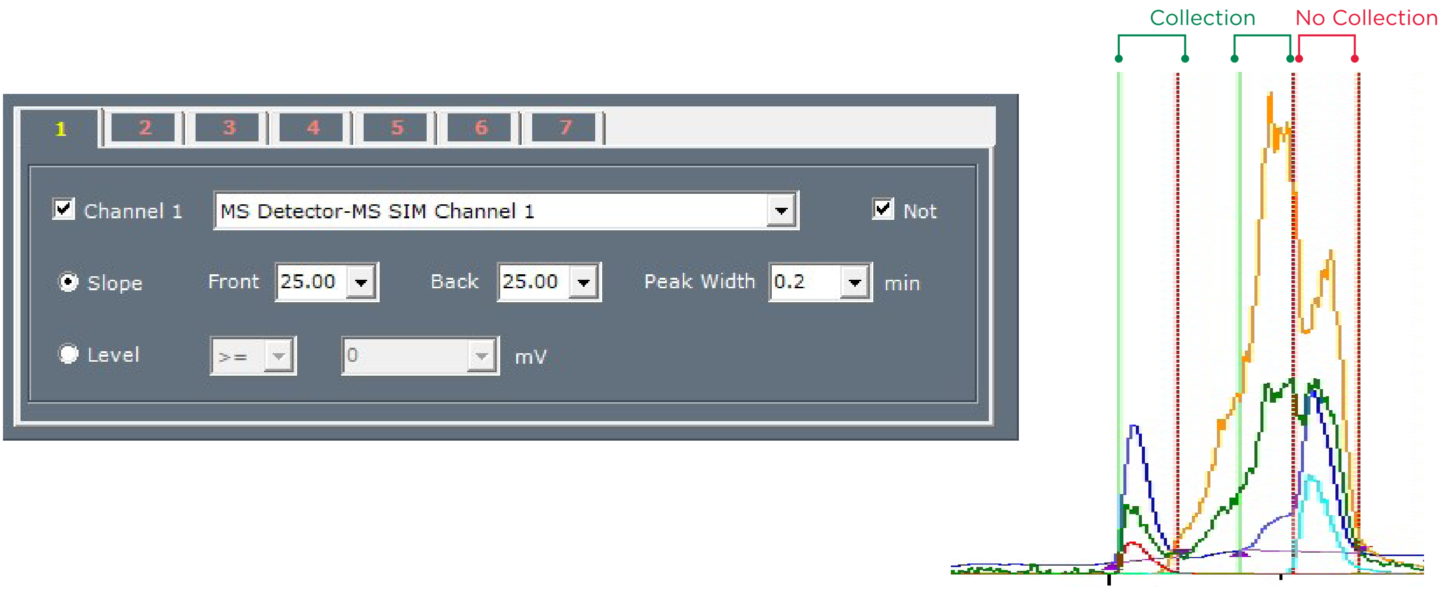


Figure 24
Conditional Fraction Collection with “Not” option, aqua peak not collected

Configuring Fraction Locations

Like the Fraction Collection Settings task, the Conditional Fraction Collection task includes a tab for configuring where to collect fractions (Figure 25). Refer to page 10 for details on settings.

Subdividing peaks when using Conditional Fraction Collection

Unlike the Fraction Collection Settings task, the Conditional Fraction Collection task does not include an option to subdivide peaks or collect non-peaks. As a result, the Set Peak Per Tube task should be used to set up peak subdivision to ensure that collection tubes will not overfill when collecting wide peaks or when pumping at high flow rates (Figure 26). Additionally, the Set Non Peak Per Tube task should be used if collecting non-peaks is required (Figure 27).

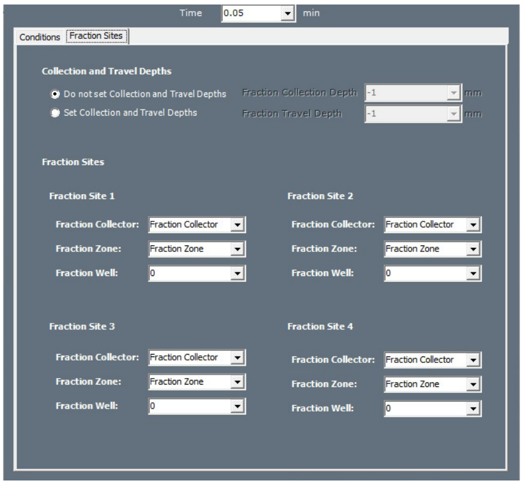


Figure 25
Conditional Fraction Collection, Fraction Sites option

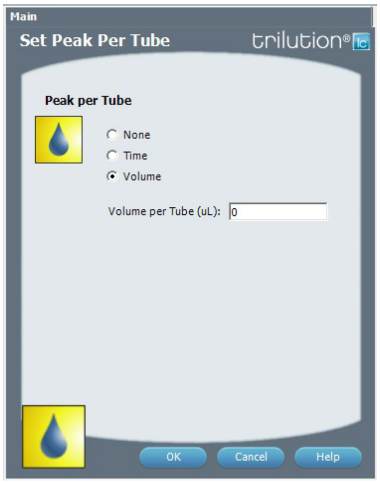


Figure 26
Set Peak Per Tube Task

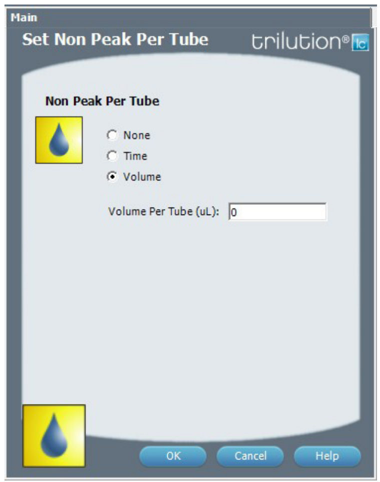


Figure 27
Set Non Peak Per Tube Task

PROTECTING YOUR SAMPLE

TRILUTION LC has safety functions that are specifically designed for fraction collection to help ensure that no compound is lost to protect samples.

Fraction Collection Simulator

TRILUTION LC has the ability to browse data from a previous run and overlay it into a method. Start and stop lines are drawn to simulate run time fraction collection. This provides a useful tool for fine tuning collection settings such as slope values without using valuable sample.

Use the Application Run Results button on the Method screen to browse for chromatograms to be overlaid into the Method Builder. Click the Update Chromatogram button to simulate the fraction collection of that chromatogram (Figure 28). Any time fraction collection settings are changed, the Update Chromatogram button must be clicked to refresh the simulation.

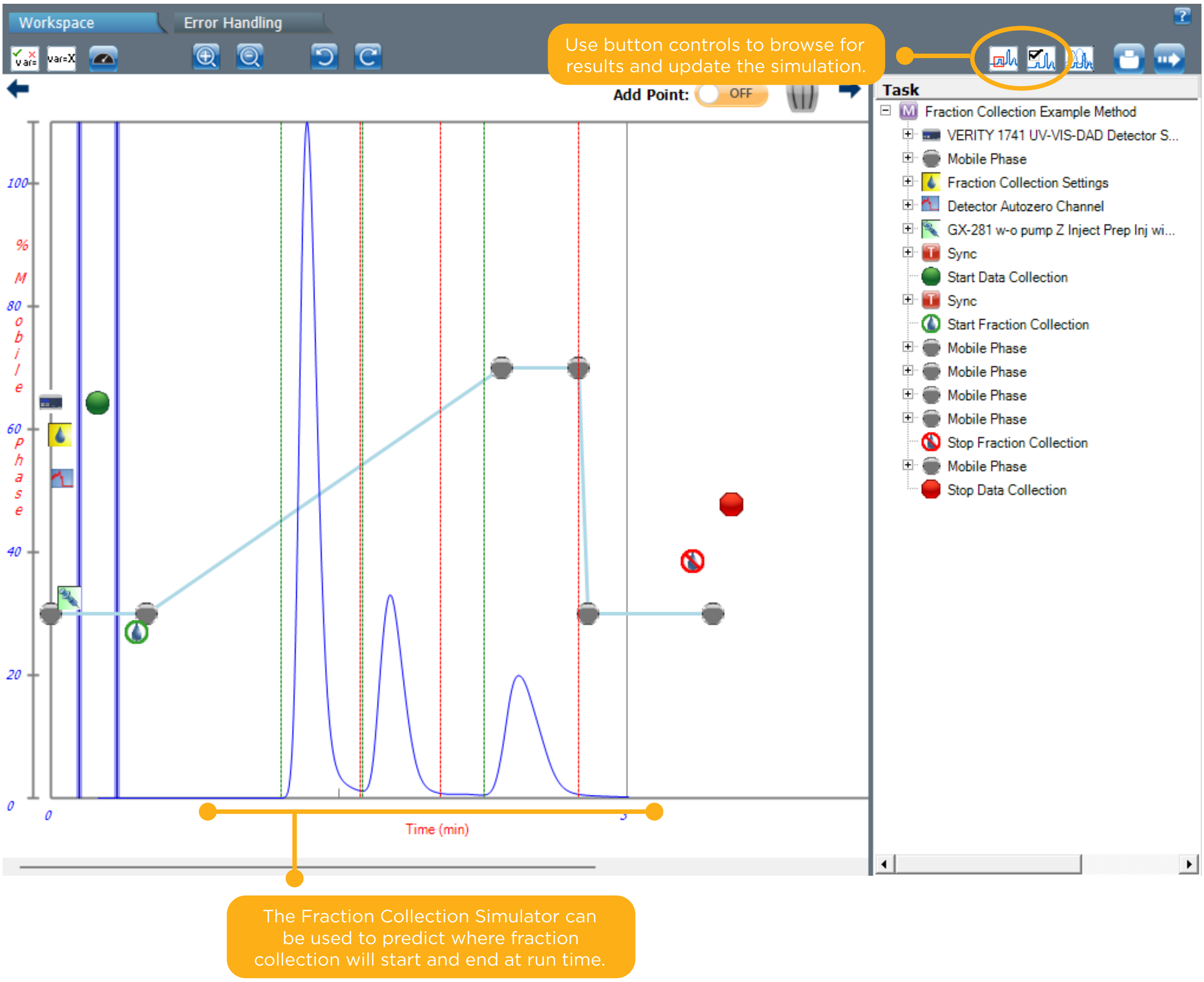


Figure 28
Fraction Collection Simulation in Method Builder

Data from multiple data channels can be used to simulate Conditional Fraction Collection. Use the Application Run Results button on the Method screen to browse for the desired chromatogram to associate with each data channel. Click the Update Chromatogram button to simulate fraction collection of the combined data (Figure 29).

NOTE	Fraction Collection Simulation does not simulate Peak or Non-peak subdivision.

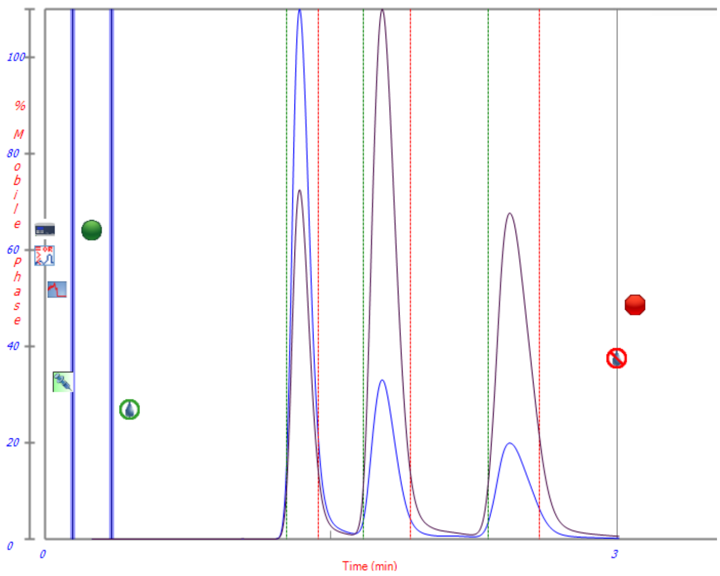


Figure 29
Fraction Collection Simulation in Method Builder with multiple data channels

Fraction Collection Error Handling and Safety Functions

When an error situation is encountered, TRILUTION LC can respond to the problem in several ways, up to and including shutting down the system entirely.

On the Application Run screen, the Minimum Fraction Sites feature can be used to ensure that a compound is not lost as a result of the fraction collector running out of collection tubes (Figure 30). The Minimum Fraction Site feature actively looks to ensure that there are a user-specified number of tubes available prior to performing the injection. If the tube requirement is not met, injection will not occur. By default, this value is set to 1. A typical or average number of fractions that is expected per injection (10 or 15, for example) should be entered.

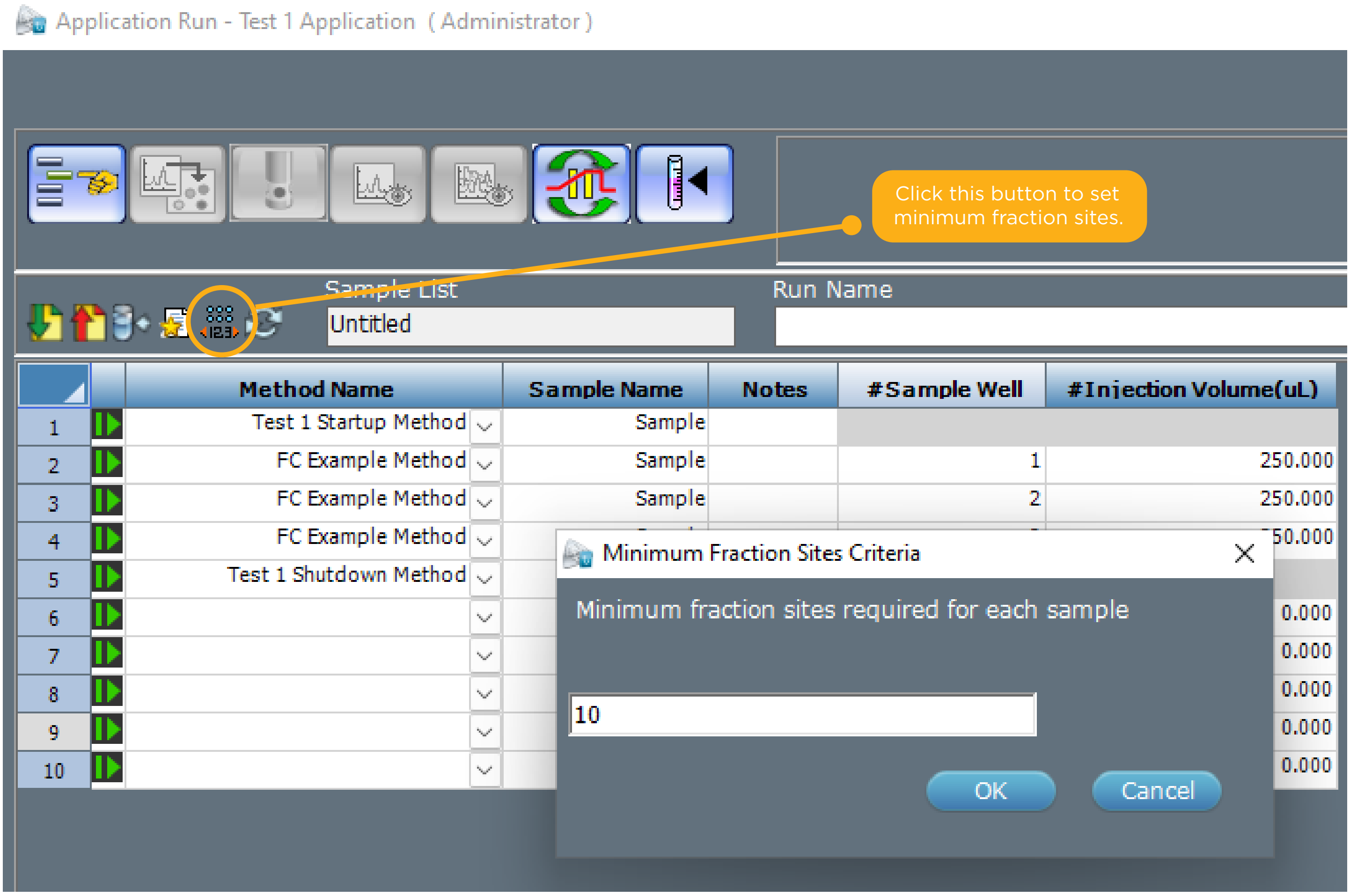


Figure 30
Minimum Fraction Sites in Application Run screen

Two error handling options are available in the method related to fraction collection (Figure 31).

- **No Fraction Sites**
  This error results when no fraction wells are available or when not enough wells are available based on the Minimum Fraction Sites criteria set in the Sample List. This error handling condition is best used in conjunction with the Minimum Fraction Sites setting to prevent the sample from being injected. If Minimum Fraction Sites criteria is not set and all available fraction sites are used, this error will stop the current method from running, preventing further sample loss.
- **No Fractions Collected**
  This condition results when no fractions are collected for a sample. This only applies if the Method contains tasks to establish fraction collection parameters.

The method can be set up to stop the run if one of these errors occur and to optionally run a stop method to shut down the system.

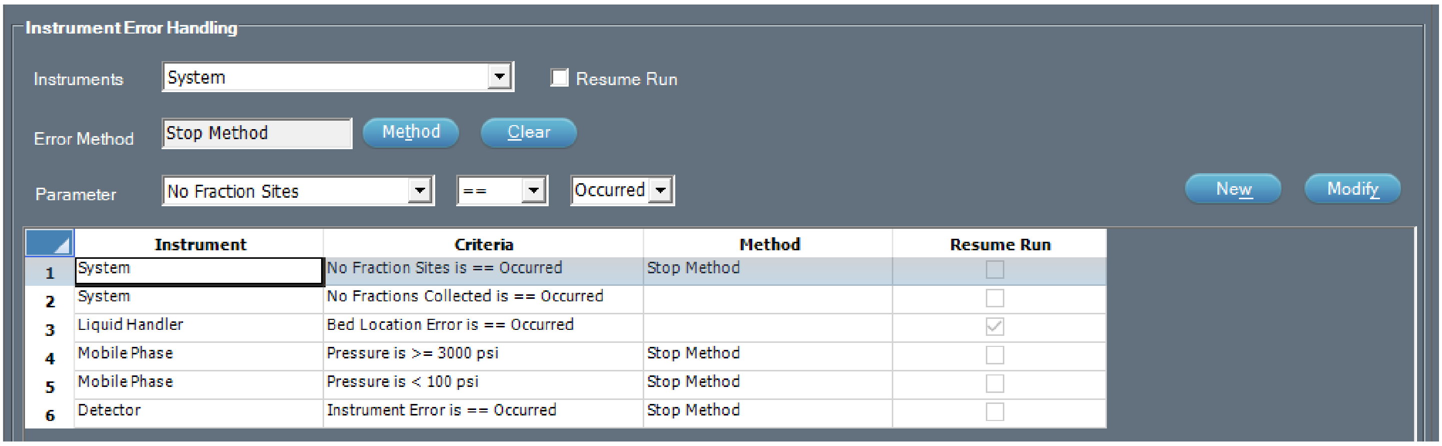


Figure 31
Method Builder error handling options

Manual Collection

The Application Run screen has two main fraction collection functions, Manual Advance and Manual Divert (Figure 32). These allow a user to interrupt the method-controlled fraction collection that is occurring and collect peaks manually. The Manual Advance and Manual Divert buttons are only active during a Fraction Collection window (time between the start and stop fraction collection tasks). The Manual Advance function switches to the next fraction tube position and begins collection. The software then continues to collect fractions based on the method-defined parameters. The Manual Divert function switches the valve to divert and then moves to the next fraction tube position. Collection will begin again when the method parameters have been met, such as when a slope of another peak is detected.

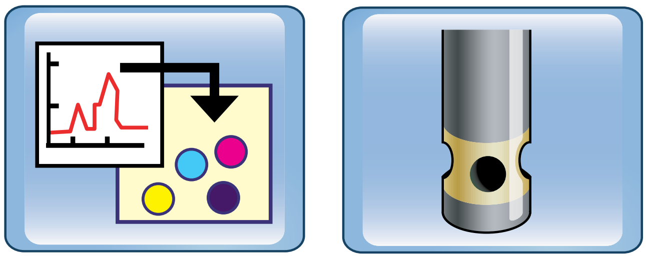


Figure 32
Manual Advance and Manual Divert

Fraction Collection Delay Volume

To accurately collect fractions, it is necessary to know the fraction delay time, the time it takes a peak to travel from the detector to the fraction collector. Gilson's Fraction Collection Delay Volume Calculator, a companion software to TRILUTION LC, allows inputting system configuration information such as tubing length, detector flow cell, and fraction collection valve, and provides the corresponding delay to input into TRILUTION LC (Figure 33).

Simply input the delay volume calculated by the Fraction Collection Delay Volume Calculator in the TRILUTION LC Method configuration (Figure 34), and the software will automatically convert to delay time based on the method flow rate, allowing the method flow rate to be variable.

Gilson recommends verifying the system is fully optimized by performing a recovery study.

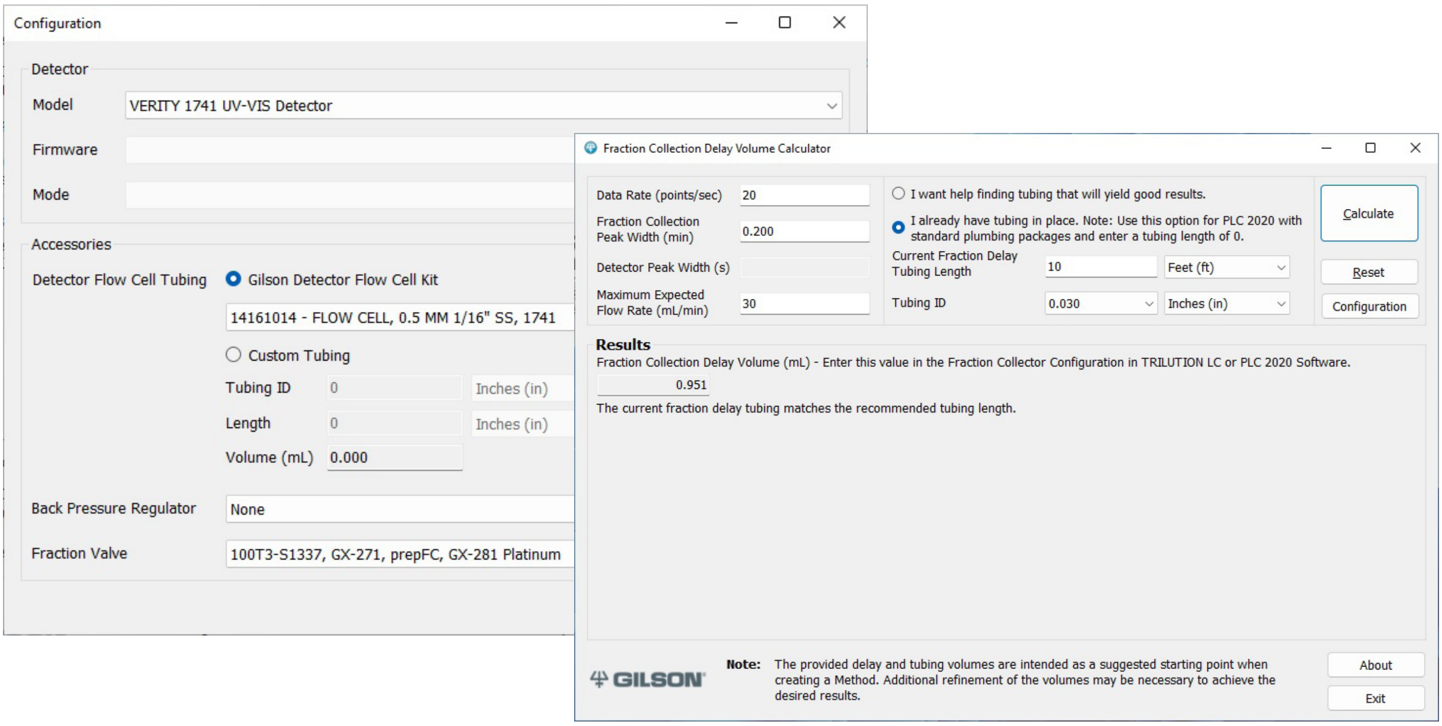


Figure 33
Fraction Collection Delay Volume Calculator

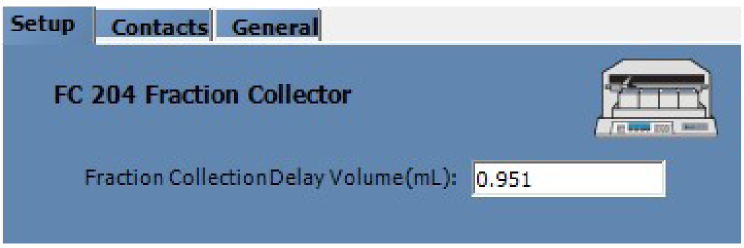


Figure 34
Fraction Collection Delay Volume in Method Configuration

Collection of Waste

To ensure no sample is lost during a run, the waste from each injection can be collected. By connecting a VALVEMATE® II to the system after the fraction collection valve, the waste can be collected into separate bottles for each sample injected. This setup allows recovery of any compounds that were not collected during the run.

COMPLEX COLLECTIONS

For more complex collections, TRILUTION LC allows changing fraction collection parameters during a method, allowing collection modes to vary over the course of a purification run. In addition, TRILUTION LC can extend capacity by controlling multiple fraction collectors or by bulk collecting fractions into larger vessels.

Collection Windows

TRILUTION LC has the ability to define specific and multiple collection windows. A collection window is a time frame in which fraction collection is enabled in the method. Each window can be configured to collect with different parameters. For new fraction collection setting parameters to be used, the prior window must end with a Stop Fraction Collection task. Below is an example method with three distinct collection windows (Figure 35). When setting collection time windows, it is important to consider that start/stop times set in the method are based on method time, not data collection time. For precise windows, it may be necessary to adjust the collection window times accordingly.

Figure 35
Method with three collection windows

Multiple Fraction Collectors

Multiple fractions collectors can be connected on a system to increase fraction capacity. TRILUTION LC is able to use a Fraction Collection System in conjunction with a VALVEMATE® II Valve Actuator or by simply daisy chaining multiple fraction collectors together. The Fraction Collection System offers an efficient method for incorporating additional fraction collectors into a system for increased collection capacity.

Daisy Chaining Fraction Collectors

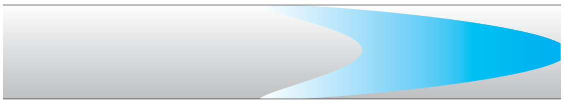


Figure 36
Example of laminar flow through HPLC tubing

Not using a VALVEMATE II in a Fraction Collection System requires that multiple fraction collectors are daisy chained together. This means that each additional fraction collector will have a larger delay volume (Figure 37). This can result in peak spreading and fractions that are more diluted, which in turndecreases fraction recovery. Depending on viscosities, the laminar flow effect can cause a sample spread of up to 2 inches for every one inch of tubing (Figure 36). The amount of spreading will vary depending on flow rates and internal tubing diameter. If multiple fraction collectors have increasing delay volumes, the compounds are spreading out as they travel through the tubing making them less concentrated than when they came off the column.

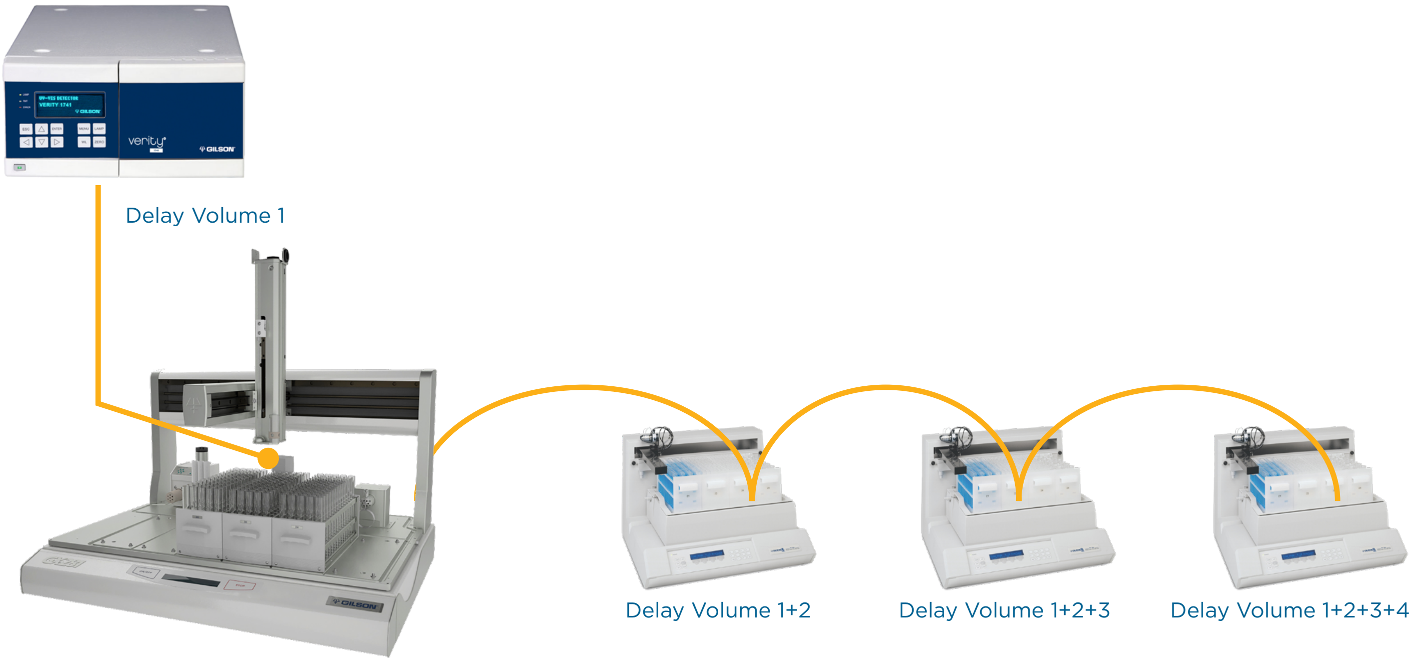


Figure 37
Fraction Collection System without VALVEMATE II

Fraction Collection System with a VALVEMATE® II Valve Actuator

The Fraction Collection System in conjunction with a VALVEMATE II allows for plumbing multiple fraction collectors together in a way that all delay volumes for each fraction collector can be the same (Figure 38). With this setup, tubing lengths can be minimized to reduce peak spreading.

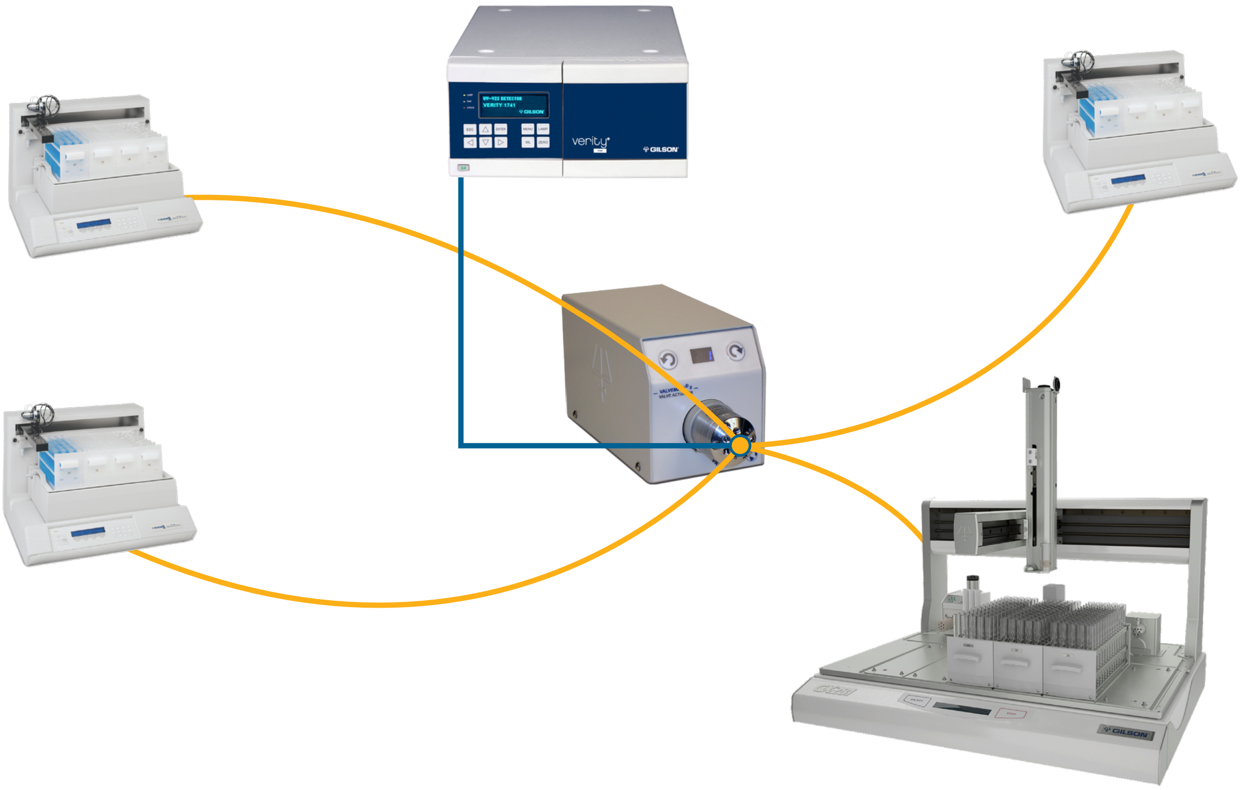


Figure 38
Fraction Collection System with VALVEMATE II

To configure a Fraction Collection System, do the following:

- Add each fraction collector that will be used to the Method Configuration workspace.
- Add a VALVEMATE II into the Configuration. The position of this switching valve will direct the flow of mobile phase to the appropriate fraction collector.
- Next add the Fraction Collection System. In the configuration property page select which port each fraction collector is connected to on the VALVEMATE II (Figure 39).
- Enter a delay volume for each fraction collector on its own configuration property page.

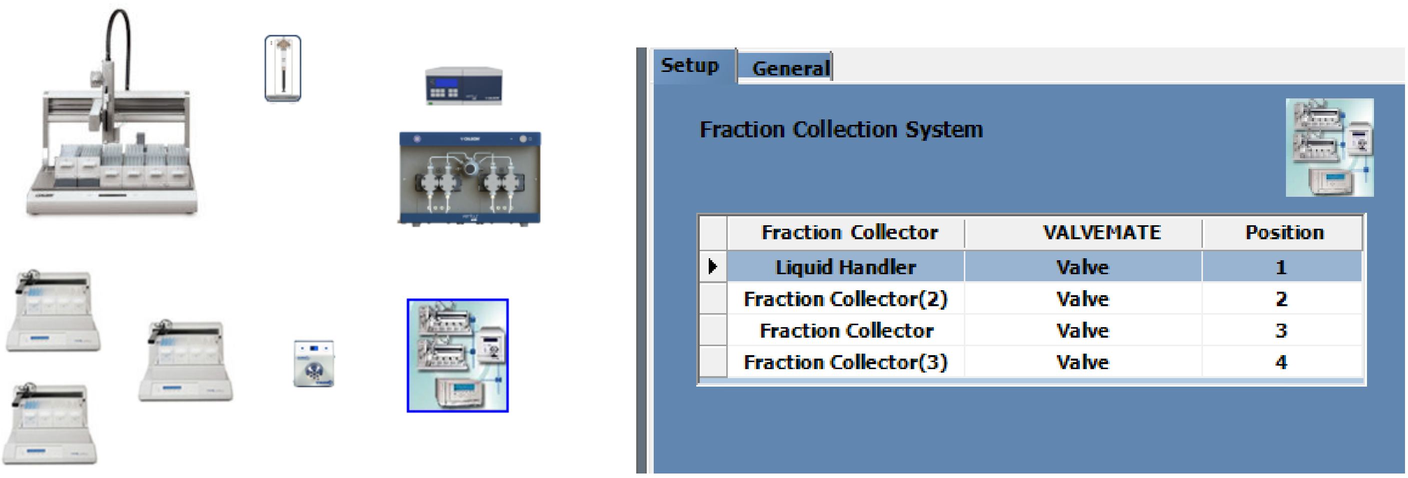


Figure 39
Fraction Collection System configuration

Advanced Fraction Collection

An add-on is available for TRILUTION LC to enable bulk collection. Large volume fractions can be collected into bottles via a VALVEMATE II. In this setup, a special VALVEMATE II Collect instrument is included in the configuration as a fraction collector (Figure 40). One port on the valve is connected to waste and the other ports are connected with tubing to collection bottles. The ports are numbed in the method to allow selection of the appropriate port for collection (Figure 41).

Another add-on allows collection of a peak into different vessels. As shown in the chromatogram (Figure 42), it is possible to collect the front and rear of a peak into tubes while collecting the purest part of the peak into a bottle. In this setup, two VALVEMATE II modules are used in conjunction with a Fraction Collection System. The Fraction Collection System is configured similar to that described on the previous page, using a special VALVEMATE II Collect instrument. Refer to Technical Note 228 for additional details.

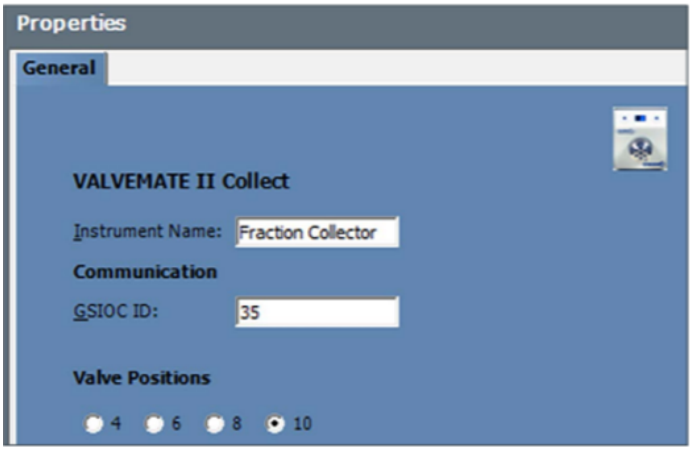


Figure 40
VALVEMATE II Collect configuration properties

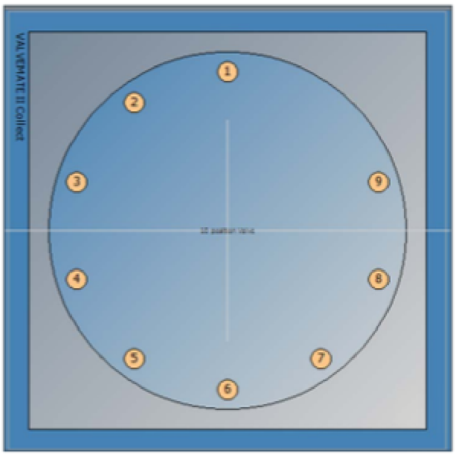


Figure 41
Numbering of ports for collection (port 10 is waste)

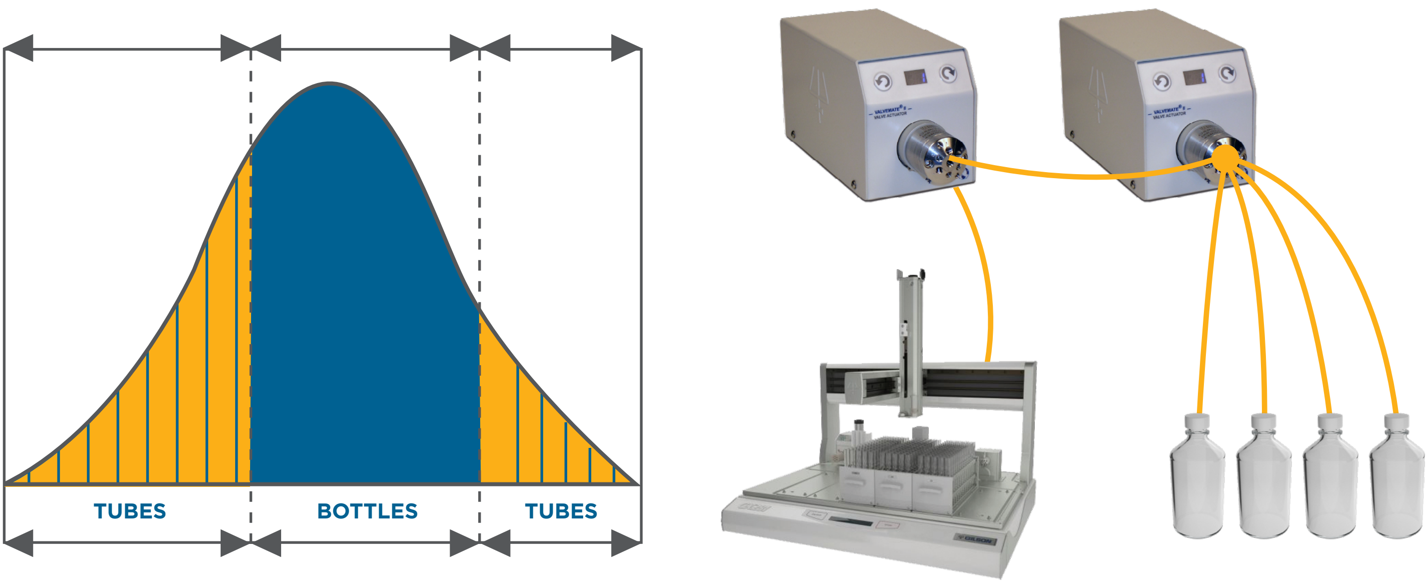


Figure 42
Collecting fractions into different vessels with a VALVEMATE II

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