

AutoPurification

System Guide

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We seriously consider every customer comment we receive. You can reach us at tech_comm@waters.com.

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Safety considerations

Some reagents and samples used with Waters instruments can pose chemical, biological, or radiological hazards. Be sure you are aware of the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's safety representative for guidance.

When you develop methods, follow the "Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory," *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol addresses good operating procedures and the techniques necessary to validate system and method performance.

Instrument-specific safety considerations

Refer to MassLynx and FractionLynx online help and operator's guides for the individual modules of the Waters AutoPurification™ System.

Safety advisories

Consult [Appendix A](#) for a comprehensive list of warning and caution advisories.

Operating this device

When operating this device, follow standard quality control procedures and the guidelines presented in this section.

Intended use

Waters® designed the Waters AutoPurification System to purify compounds by UV/Vis detection and mass spectrometry.



Warning: Although some of the modules in the AutoPurification System might carry IVDD labels, the system as a whole is not intended for clinical use.

Calibration

To calibrate HPLC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards should cover the entire range of quality-control samples, typical specimens, and atypical ones.

To calibrate mass spectrometers, consult the calibration section of the operator's guide of the instrument you are calibrating.

Quality control

Routinely run three quality-control samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality control samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

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1 System Overview

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Waters[®] AutoPurification[™] Systems are composed of chromatography instruments designed to purify your samples. FractionLynx[™] software controls these purification systems, which use various types of compound detection, fraction triggering, and fraction collection capabilities. Compound detection is achieved by combinations of ultraviolet (UV) absorbance detectors, photodiode array (PDA) detectors, evaporative light-scattering detectors (ELSD), and mass spectrometers (MS).

Capabilities and configurations

Waters AutoPurification Systems purify samples using various detectors: absorbance, mass spectrometry, and evaporative light-scattering. The most common systems are UV-directed or MS-directed.

In a UV-based purification system, the sample is separated by the HPLC column, and fractions are collected according to specific wavelengths selected by the user. After collection, the AutoPurification System can inject the fractions and further analyze the results.

In a mass-based AutoPurification System, compounds are separated by the preparative HPLC columns. They then flow into a mass detector for advanced confirmation, compound characterization, and appropriate triggering of fractions. Fractions are then collected into microplates or tubes according to molecular weight, UV absorbance, timed event, or a combination of factors.

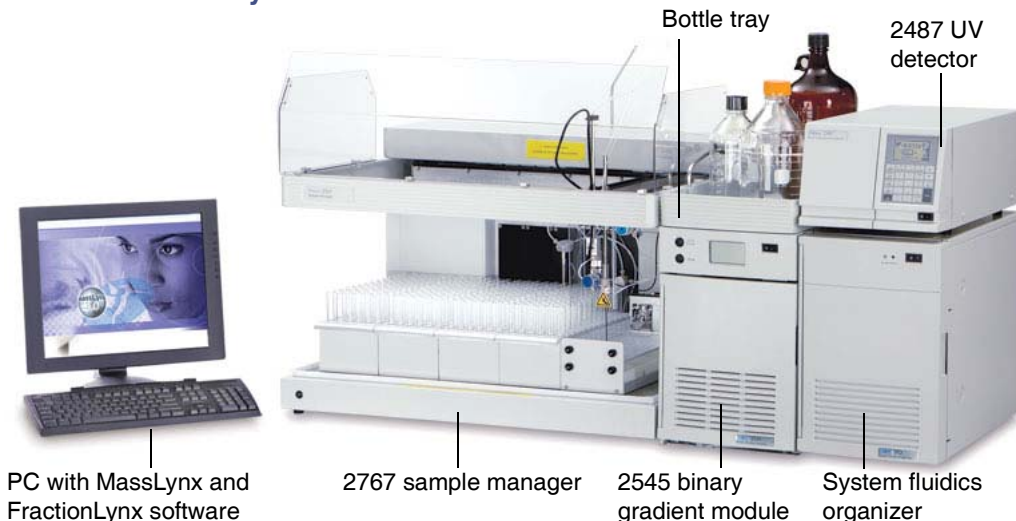
To minimize user intervention and maximize unattended operation, the system monitors mobile phases, makeup solvents, and waste levels.

UV-directed purification

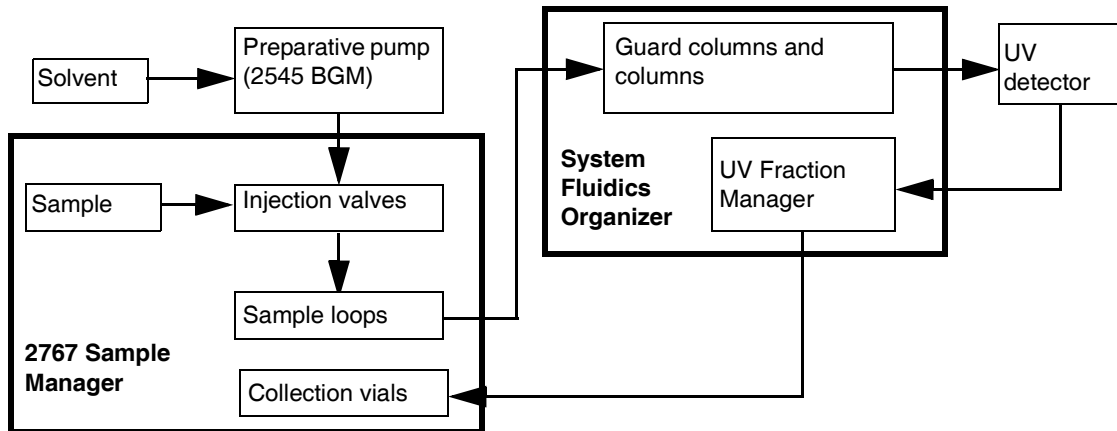
The Waters UV-directed system uses the UV detector as its primary detector.

Solvent flows from the 2545 binary gradient module to the 2767 sample manager analytical or preparative injection valves, and then to the corresponding columns in the system fluidics organizer. Analytical flow is directed to a 3-column selection valve, and preparative flow through a 10-port selection valve and prep column(s). The backpressure regulator and UV fraction manager are connected between the UV detector and the fraction collection valve of the 2767 sample manager or optional Waters Fraction Collector III.

UV-directed system



Sample and solvent flow through the UV-directed system:



UV fraction manager

The UV fraction manager provides the delay time between the detector flow cell and the fraction collector diverter or collection valve. One of four fixed channels provides for the proper delay time based on column and flow rate.

Dual-inlet flow cell

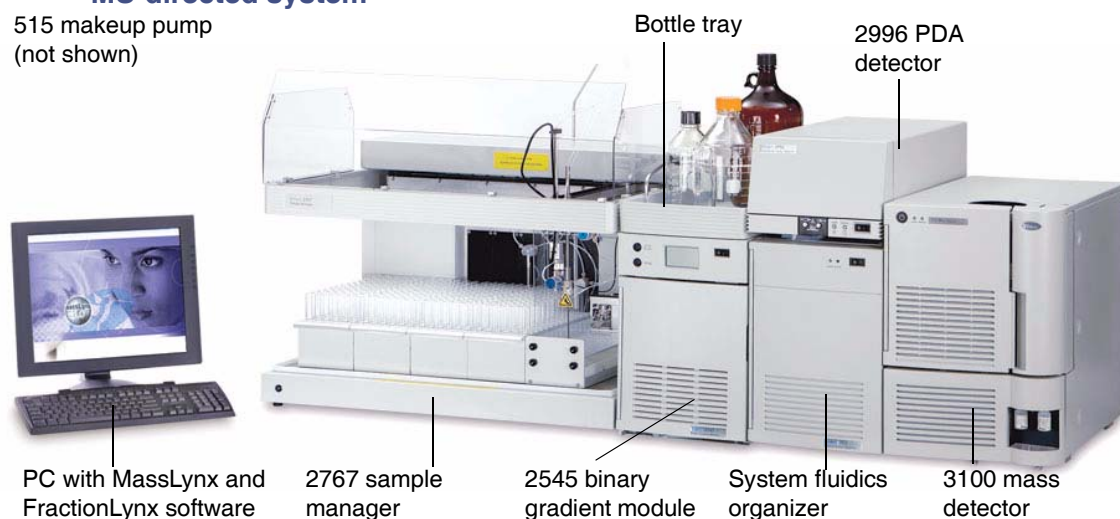
For UV-directed systems, a single-channel, dual-inlet flow cell is available for both the Waters 2487 UV and 2996 PDA Detectors. These flow cells are compatible with analytical or preparative flow rates ranging from 0.5 to 150 mL/min.

MS-directed purification

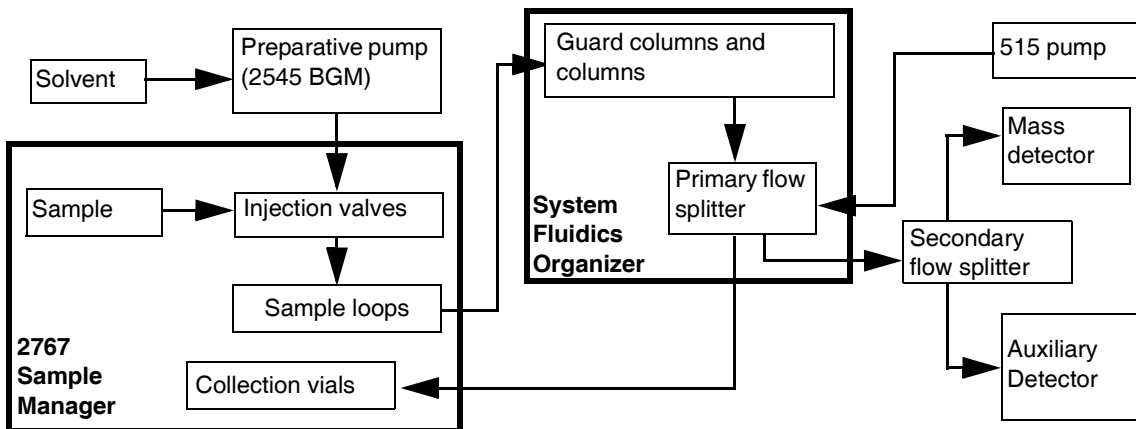
The Waters MS-directed autopurification system uses the Waters 3100 Mass Detector as its primary detector.

MS-directed system

515 makeup pump
(not shown)



Sample and solvent flow through the MS-directed system:



MS-directed systems require a flow splitter to direct a small amount of the total sample purification flow to the detectors. The splitter fits into the front of the system fluidics organizer and generates a split ratio of incoming solvent flow. You must select the appropriate flow splitter for the flow rate and application.

The primary flow splitter divides the prep flow so that 99.9% goes to the fraction collector and 0.1% is carried by the 515 makeup pump to the detector for analysis.

A secondary flow splitter (or tee) typically sends 20% of the flow to the mass detector and 80% of the flow to the UV detector.

The pump control module in the system fluidics organizer can control up to three 515 pumps. The pump control module communicates over an IEEE-488 cable connection and interfaces with the workstation using the GPIB communication protocol.



Warning: Stacking hazard. Stacking configurations for system instruments must meet all relevant safety requirements and performance standards. Each base module of the Waters AutoPurification System, whether it be the system fluidics organizer, 2545 binary gradient module, or 3100 mass detector, can physically support *no more than two* accessory modules stacked on top of it. Stacked accessory modules can include the Waters 515 pump, photodiode array detector, evaporative light-scattering detector, or dual-wavelength absorbance detectors.

Waters neither recommends nor supports stacking configurations that differ from this model. Such nonconforming configurations would invalidate warranties and shift responsibility for operator safety and system performance to the user.

System components

Solvent delivery

The software coordinates all solvents that comprise the gradient flow stream. The gradient table and flow rate automatically adjust to compensate for at-column-dilution and mobile phase modifiers (optional features).

Waters 2545 Binary Gradient Module

The Waters 2545 Binary Gradient Module (BGM) is a high-pressure binary gradient pump, the primary solvent delivery device for the system. It can deliver solvent at flow rates from 0.5 to 100 mL/min at a maximum of 6000 psi, or flow rates from 100 to 150 mL/min at a maximum of 5000 psi.

The 2545 BGM has four solvent inlet lines (A1, A2, B1, and B2). You can select two lines at one time for chromatographic runs.

- A1 and B1
- A2 and B2
- A1 and B2
- A2 and B1

See also: *Waters 2545 Binary Gradient Module Operator's Guide*.

Waters 515 HPLC Pump

The 515 HPLC pump, in conjunction with a pump control module, is required as a “makeup” pump. The pump dilutes the sample from the primary splitter and carries the flow to the detectors. Additional 515 pumps can be used for at-column dilution or mobile phase modifier addition.

See also: *Waters 515 HPLC Pump Operator's Guide* and “At-Column Dilution Application Notes” (part number 71500078010), which is available at www.waters.com.

Sample handling

Waters 2767 Sample Manager

The 2767 sample manager, injector, and collector is a high-capacity sample processing system for managing sample injections.

The 2767 sample manager operates under MassLynx software control through an RS-232 serial port.

During operation, the sample flows through the sample manager to columns and other instruments and devices in the system. Then it flows back to the sample manager for fraction collection.



Warning: To prevent injury, use the 2767 sample manager with its safety shield in place.

See also: *Waters 2767 Sample Manager Operator's Guide.*

System organizer

Waters System Fluidics Organizer

The system fluidics organizer (SFO or FO) houses the columns, the pump control module, and either the primary fraction flow splitter for MS-directed systems or a UV fraction manager for UV-directed systems.

An Ethernet switch in the system fluidics organizer serves as a connection point for the networked modules. Standard Ethernet cables provide connectivity between compatible modules and the Ethernet switch.

The system fluidics organizer communicates with the system through a rear-panel Ethernet connection. Its three electrically activated rotary valves work as follows.

- The 6-port analytical-to-prep valve provides switching between analytical and preparative analyses.
- An 8-port valve diverts flow to one of three analytical columns.
- The 10-port column selection valve provides the option to switch between two preparative columns and/or plumb an additional pump into the system to regenerate columns.

Sample detection

The AutoPurification System can control multiple detectors for fraction triggering, sample confirmation, and waste detection.

Waters Photodiode Array Detector

The photodiode array detector (PDA) is an ultraviolet/visible light (UV/Vis) spectrophotometer with 12 diodes and an optical resolution of 1.2 nm per diode. Its operating wavelength ranges from 190 to 800 nm.

Mass-directed systems use the standard analytical flow cell. UV-directed systems typically use a 2996 AutoPurification flow cell, whose shorter path length helps keep preparative peaks on scale.

See also: *Waters 2996 PDA Detector Operator's Guide.*

Waters Tunable UV Detector

The 2487 UV detector is for analytical and preparative runs. It operates from 190 to 700 nm and monitors absorbance at one or two discrete wavelengths.

Mass-directed systems use the standard analytical flow cell. UV-directed systems typically use a 2487 AutoPurification flow cell, whose shorter path length helps keep preparative peaks on scale.

See also: *Waters 2487 Dual Wavelength Absorbance Detector Operator's Guide.*

Waters Evaporative Light Scattering Detector

The evaporative light scattering detector (ELSD) measures the light scattered by analyte particles after the mobile phase evaporates from the sample. This generic method of detection is compatible with virtually all modes of chromatography. It is particularly useful for molecules without UV/Vis chromophores and those that do not ionize.

See also: *Waters 2420/2424 Evaporative Light Scattering Operator's Guide.*

Waters 3100 Mass Detector

The 3100 mass detector, a mass spectrometer used for analytical and preparative runs, measures the mass-to-charge ratio (m/z) of individual ionized molecules. The instrument operates from 22 to 2000 Da.

See also: *Waters 3100 Mass Detector Operator's Guide.*

Software control

MassLynx software and the FractionLynx Application Manager

The MassLynx software and FractionLynx Application Manager software control the AutoPurification System.

MassLynx software provides the fundamental data platform for acquiring and managing mass spectrometry information. It includes many features to analyze and process acquired data.

FractionLynx software uses defined sample list parameters to control system modules for automatic peak tracking, fraction-collection triggering, and processing.

See also: MassLynx online Help and the FractionLynx online Help.

AutoPurify method

The AutoPurify™ method automates the stages of the purification process. Parameters set in the AutoPurify method are saved as FractionLynx processing files (*.flp). A FractionLynx processing file is required to process data or invoke automatic analytical-to-preparative processing and/or preparative-to-analytical processing.

To use the AutoPurify method, first define the method, which is stored in the MassLynx directory as a file with an *.flp extension. Then specify the AutoPurify method in the Parameter File column of the sample list, and select AutoPurify from the list of executable functions in the Process column of the sample list.

At the end of each processing stage, and if required by the FractionLynx method, AutoPurify generates a batch file for the next processing stage and a FractionLynx report file (*.fpt). The report summarizes the results of the processing stage. You can therefore review the software's choices before allowing samples to advance to the next stage of the purification process, if the system is running in manual mode. In automatic mode, the software runs all processing stages without user intervention. To automate the stages (analytical to preparative and/or preparative to analytical), the AutoLynx™ connectivity tool must be running.

See also: "Creating methods in FractionLynx" in the FractionLynx online Help.

Fraction simulation

After you run a preparative sample and collect chromatographic data, you can adjust collection parameters in the FractionLynx method using the fraction simulation function. When you change collection parameters in the chromatogram window, this feature shows how the collection would be implemented in subsequent preparative runs. Simulated fractions appear on the chromatogram, labeled “Simulated,” and depict the peak collection graphically. Once collection parameters are optimized, you can export the changes to the FractionLynx method.

Recommendation: Use fraction simulation to optimize a FractionLynx method for preparative peaks and multiple injections.

AutoMIT

FractionLynx software uses several criteria to determine when to collect fractions. One criterion is the minimum intensity threshold (MIT). The software measures the intensity level of the background response and triggers collection when the level exceeds a minimum background threshold.

You can determine the MIT manually or by using AutoMIT. Nevertheless, AutoMIT is the more efficient method. Background levels can vary by wavelength or mass within a sample, and manually reviewing each trigger to determine optimum levels consumes time and limits the effectiveness of high-throughput sample processing. AutoMIT assigns a specific MIT value for each sample based on the background response of a blank injection.

Total flow and gradient calculator

The software can calculate the gradient table of a 2545 binary gradient module (BGM) and program the instrument to coordinate the flows of up to three additional 515 HPLC pumps. In this way, each 515 pump can contribute to the system flow when necessary.

See also: “At-Column Dilution Application Notes” (part number 71500078010), which is available at www.waters.com.

Early run termination

You can set the software to end the run after the target compound is collected so that the system switches to the next sample. Early run termination saves time, collection vials, solvent consumption, and waste generation.

To terminate a run early, you must enter a value in the “Number of fractions to collect” field in the Volume tab of the FractionLynx method.

Examples: If you enter 1 in the “Number of fractions to collect” field, one fraction is collected before the run is terminated. If you enter 3 in this field, three fractions are collected before the run is terminated.

If the specified number of fractions is not reached, the run ends as specified in the acquisition methods. When using off-line column regeneration, you must specify a minimum run time setting in the Volume tab of the FractionLynx method. If you are using a single column, you must use a post-run method, which allows the column to be cleaned with high organic solvent and is run after every injection.

The post-run method requires an additional column in the sample list. A pre-run method can be used to reequilibrate the column at initial conditions before each injection. The pre-run method also requires an additional column in the sample list.

Recommendation: Off-line column regeneration might terminate the run before the off-line column is ready. To avoid this situation, enter a minimum run time in the FractionLynx method.

AutoDelay time determination

The AutoDelay time feature automatically calculates the delay time between detection and collection.

See also: [Performing a delay timing test.](#)

Open Access software

Open Access (OA) Login lets you run samples even if your experience and training in LC/MS is limited. The interface presents a series of prompts that guide you through the sample submission process. OA Login requires only that you enter your name, provide the sample ID, select a predefined FractionLynx processing method (*.flp), and then place the samples in the designated position in the autosampler. This simplified process is called “walk-up.”

System administrators must configure OA Login and create the methods for the users.

The Open Access software has solvent and waste monitoring features. In Open Access mode, you can edit the 2545 binary gradient module's gradient but not its flow rate.

Solvent and waste monitoring

You can monitor the solvent usage by the 2545 binary gradient module and auxiliary pumps. You can input the reservoir volume for each pump, and the software measures solvent usage based on the gradient conditions employed.

As the solvent reservoir is depleted, the volume in the waste drum increases. The waste volume calculation is based on the quantity of solvent pumped.

You can set two limits for each reservoir and waste container, warning and acute, each with different responses. For solvents, specify the lower limits and fill the reservoirs when empty. For waste, specify the upper limits and empty the container when full. You can configure the system to shut down when either warning level is reached.

The software informs you when a limit is reached or is below warning levels with an on-screen computer message and/or via e-mail.

Exceptions:

- The software does not monitor the usage of 2767 Sample Manager wash solvent, either as consumed from solvent reservoirs or as added to a waste container.
- The volumes of collected fractions are not subtracted from the waste calculation.

System shutdown

You can specify that the system automatically shut down at the end of the run or in the event of an adverse condition. The system response is tiered, allowing you to specify system shutdown

- immediately.
- after the current injection is completed.
- after the batch is finished.

See also: "Stopping or pausing a run" in the FractionLynx online Help.

Options

The AutoPurification system has several configurable options.

Leak detection

System-level leak sensing offers six points of detection routed to a central module. The optional leak sensor module consists of liquid sensors, tubing, and a control module in the system fluidics organizer.

The Waters field service engineer installs the sensors and sensor brackets in drip trays where leaks are most likely to occur. If a leak occurs, the drip tray of each component collects solvent and directs it through tubing to a sensor. The liquid sensor sends a signal to the control module, which signals the pump to stop the solvent flow.

Additional fraction collectors

Additional fraction collectors can be added for increased capacity or secondary collection. The collector/selector valve is recommended when using multiple fraction collectors.

Waste collection

The waste collection option enables you to add multiple collectors to the system for waste collection. All solvent that is not collected by the primary collector is collected in a single waste vessel for each injection.

Columns

The system fluidics organizer can accommodate three analytical and two preparative columns.

Analytical columns

You can use analytical columns that range in length from 50 mm to 150 mm.

Examples: Atlantis[®], SunFire[™], and XBridge[™].

Preparative columns

You can use preparative columns that range up to 150 mm in length and 30 mm in diameter.

Examples: XTerra[®] Prep, Atlantis, SunFire, and XBridge.

Guard columns

You can add a guard column before each column to protect it from precipitates, impurities, and contaminants in the samples and from potentially damaging solvents.

2 Preparing the System

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Preparing for operation



Warning: To avoid electrical shock, ensure the flow splitters and columns are properly grounded.

Perform the following tasks regularly to keep the system working optimally.

Routine tasks to prepare for operation

- Restock solvents.
- Empty waste containers.
- Check the drip trays for leaks and correct placement.
- Open MassLynx and FractionLynx.
- In FractionLynx, check the system status and ensure that there is communication with all components.
- Restock tubes in the fraction collector, reset the beds in FractionLynx, and check the status of the collection system modules.

Tip: In the FractionLynx Collection Control window, click System Overview > Control > Reset beds.

- Prime pumps.
- Remove sample plates from the sample manager.
- Check the syringe and plunger. Wash and prime the sample manager.
- Check columns and guard columns, and check the backpressure to ensure that it is stable. Replace columns if needed.
- Check the flow rate and ensure that it is suitable.

If you changed the tubing or flow rate, you must pressure balance the system. Flow rate changes and plumbing changes after the splitter affect pressure and timing.

See also: [Balancing pressures and flows on an MS-directed system on page 2-14.](#)

- Ensure the makeup pump is operating and that the pressure is stable.
- Run a QC (quality control) sample to verify system performance (for example, run the dye test).

Running samples

A sample list defines the samples available for separating by chromatography and analyzing by a mass spectrometer, UV detector, or another detector. When you create and/or run sample lists in MassLynx, you must customize them for fraction collection. The sample list contains information about the instrument configuration (inlet method), processing method, fraction trigger, and wavelength of interest. It also identifies the monoisotopic mass of interest and the FractionLynx method.

Rule: Power-on all instruments before opening MassLynx.

Before you start a run, you must perform the following tasks:

1. Check the status of the instruments. In the sample list toolbar, click Status. All the indicator lights must be green.
2. Verify that additional application managers are running:
 - If you are collecting fractions, ensure that FractionLynx collection control is activated. When you activate FractionLynx collection control, the software downloads the beds and establishes communications between MassLynx and the fraction collectors.
 - If you are using AutoPurify and making injections from the sample list, verify that AutoLynx software is running.
 - If users are submitting samples via OA Login, ensure that OA Manager and OA Login are running. However, if users are using AutoPurify with OA Login, ensure that AutoLynx is closed.

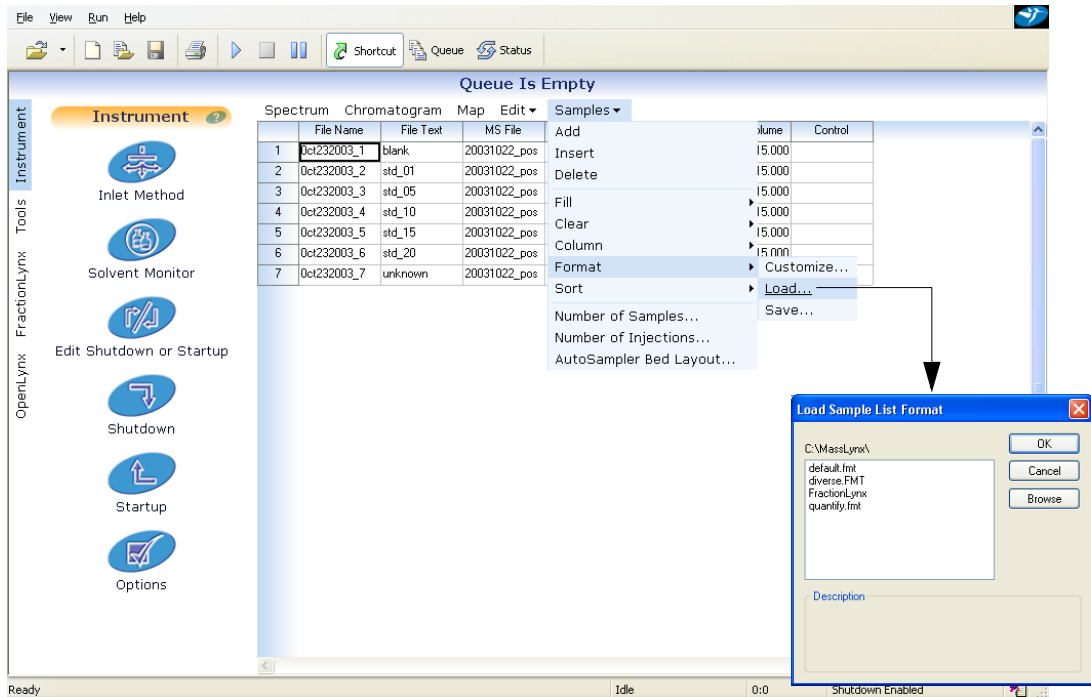
See also: “Running samples from a sample list” in the FractionLynx online Help.

To create a sample list:

1. In MassLynx, the last project you worked on automatically opens. If that project is not the one you want, find and open the correct project by clicking File > Open Project.
2. Load samples in the sample manager.

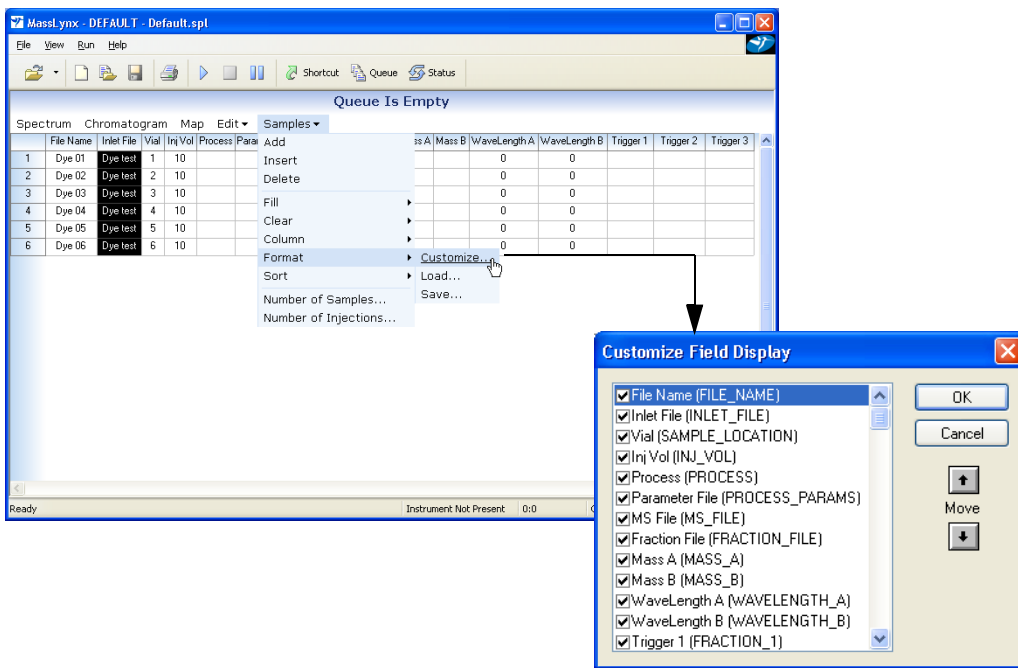
See also: *Waters 2767 Sample Manager Installation and Maintenance Guide*.
3. Define the sample list in MassLynx. From the MassLynx Main window, click Samples > Format > Load.

Load sample list format:



4. In the Load Sample List Format dialog box, click FractionLynx > OK.
5. To customize the sample list, click Samples > Format > Customize.

Customize field display dialog box:



6. In the Customize Field Display dialog box, select the check box for the required column to add or remove the column.

Tip: Before you remove a column, ensure its cells are empty. MassLynx software executes the commands in the column even if they are hidden from view.

Using the arrow keys, arrange the order of the columns, and then click OK.

See also: “Creating sample lists to collect fractions” in FractionLynx Help.

7. Customize the format to meet your needs, and then save it with a new name.




Caution: Using the same file name in more than one sample list can cause an error. Append the file name with a suffix to make it unique in multiple sample lists.

Examples: TestDyes02, TestDyes03, TestDyes04.


8. Enter sample information into the sample list.
9. Equilibrate the system: run it at initial conditions for three to five column volumes. Ensure that the pump pressure meets your expectations and that no excessive fluctuations occur.

To run samples from a sample list:


1. From the MassLynx main window, click  (Start Run) on the toolbar.
2. In the Start Sample List Run dialog box, select one or more of the following options:
 - To acquire data for all the highlighted rows in the sample list, select the Acquire Sample Data check box. Do not select this option if you are post-processing previously acquired data.
 - To process previously acquired data using a previously created AutoPurify method (*.flp file), clear the Acquire Sample Data check box, and select the Auto Process Samples check box. The AutoPurify method you specified in the sample list processes the data and creates a browser report with the results.
See also: “Creating and modifying an AutoPurify method” in FractionLynx online Help.
 - To acquire and process data automatically, select the Acquire Sample Data and Auto Process Samples check boxes.
Tip: Do not select the Pre-Run, Auto Quantify Samples, and Post-Run check boxes. They do not apply to FractionLynx.
3. In the From and To sample fields, specify the samples you want to run by performing one of these tasks:
 - Highlight the samples in the sample list that you want to run.
 - In the From Sample and To sample fields, specify a range of samples.
 - You need not do anything further to run all samples in the sample list.
Default: Samples are run in sequential order.
4. To assign priority to a sample list so that it runs ahead of non-priority lists in the queue, select the Priority check box.
5. If you want to run a sample list overnight, select the Night Time Process check box.

6. To add the sample list to the MassLynx queue, click OK.
See also: “Running samples from a sample list” in FractionLynx Help.
Result: The sample queue appears at the side of the window. Within it, you can delete, prioritize, and pause sample lists.
7. Process and review results in the FractionLynx browser.
See also: “Viewing fractions on a chromatogram” and “View results in the FractionLynx browser” in FractionLynx online Help.
Tips:
 - To monitor the status of the current fraction collection, including peak detection and collection system versus waste system volume, click FractionLynx > Collection Control > System > FractionLynx - Collection Control window.
 - To monitor the status of a run in MassLynx, click Instrument > Inlet Method. The Inlet Method window appears with the LC and detector status. Click the 2545 Additional Status tab in the Inlet Method window to monitor the status of the 2545 binary gradient module and the 515 pumps. Check the solvent levels tab to view solvent and waste status.
 - To monitor the MS status, LC status, and System status, click Status at the top of the MassLynx sample list screen.



To pause a run:

1. Click  (Pause) on the MassLynx toolbar. When you pause a run, an initiated task finishes, but the next task does not begin. If the sample manager is in the middle of injections, it finishes loading the injector loops, and then pauses the run.

For example, if the sample manager is rinsing injector ports, the run is paused after the rinse cycle.

2. To resume the run, click  (Start Run).

To stop a run:

1. Click  (Stop) on the Inlet Method toolbar or select LC > Stop Method, and then click  (Stop) on the MassLynx toolbar.
2. To verify that the run stopped, check the Status Overview page in the Inlet Method window.

Running samples using OA Login

OA Login simplifies the process of running samples on the autosampler. An OA Login administrator defines the sample information that you must provide to run samples. A wizard guides you through the sample submission process. OA Login requires only that you enter your name, provide the sample ID, select a predefined method, and then place the samples in the designated position in an autosampler, a process called walk-up. You can print results in a report, or e-mail them for review.

To run samples using OA Login:

1. From the FractionLynx main window, click OA Manager.
2. In the OA Login Manager window, click Administration > Set Bed Layout.
3. In the Select Bed Layout dialog box, select the “Use current MassLynx autosampler bed layout” check box, and then click OK.
4. Click Start > Programs > OALogin > OALogin.

Tip: If Secure mode is enabled, the term “Security” appears in the menu bar. To disable Secure mode, click Security > Swap Mode, and then enter your name and password.

5. In OA Login window, click Admin > Login Options.
6. In the OA Login Options dialog box, click AutoPurify.

Tip: If you selected the “Allow single sample file login” check box and you are submitting barcoded plates, select the check box “User Batch ID as Sample List File Name.” Doing so enables OA Login to read a tab-delimited file with the same name as the batch ID from the defined path. If such a file does not exist, or it includes more parameters than these defined in the walk-up method selected, an error message appears.

OALogin Options dialog box with AutoPurify selected:

The screenshot shows the OALogin Options dialog box with the following settings:

- Mode of operation:** OpenLynx, AutoPurify, Prevent Multiple Instances
- Default Job Identifier:** Base Job name: [], Next Job number: 1, Allow user to change job identifier
- Default Data File Name:** Use Sample ID for file name, Use Incremental File Naming, Base File Name: [], Increment Number: 0
- E-Mail:** Enable e-mailing of user report
- QuanLynx Results Location:** Enable directory specification
- Plate Layout Display:** Display Plate Layout
- Login Screen Appearance:** Title Bar Text: OALogin, Login Button Text: Login Samples...
- Batch Identifier Text:** []
- User Name:** Allow user to create own user name
- Single Sample File Login:** Allow single sample file login
- Printing of OALogin Report File:** Enable User Print Control
- Barcode Support:** Use Batch ID as Sample List File Name, Path: []
- Sort Methods By:** No Sort

Buttons: OK, Cancel

7. Log in samples using either the wizard or the single-page log-in.

Shutting down the system

You can create a shutdown method in MassLynx software to specify when and how the software performs the shutdown procedure. You can specify shutdown procedures when one or more of these conditions occurs:

- At the end of the run or when the sample list (queue) is paused.
- In the event of an adverse (error) condition.
- In the event of a warning or acute level message relating to solvent or waste monitoring.

As part of a shutdown method, you can specify how the software performs shutdown tasks as follows:

- Defining shutdown tasks such as turning the LC pump off, putting the mass spectrometer in standby mode, and turning the UV lamp off.
- Specifying a delay time before the software initiates shutdown procedures. Delay time is the time period that the system waits before the software begins performing shutdown tasks.
- Optimizing shutdown procedures by directing the software to omit unnecessary tasks. When you select the Optimize check box, the software compares the current status of the instrument against each configured task. If the instrument is already in this state (for example, LC pump is turned off), the task is not performed.
- E-mailing the system administrator. When you select the “E-mail recipient” check box and enter an e-mail address, the software notifies the recipient of the shutdown.

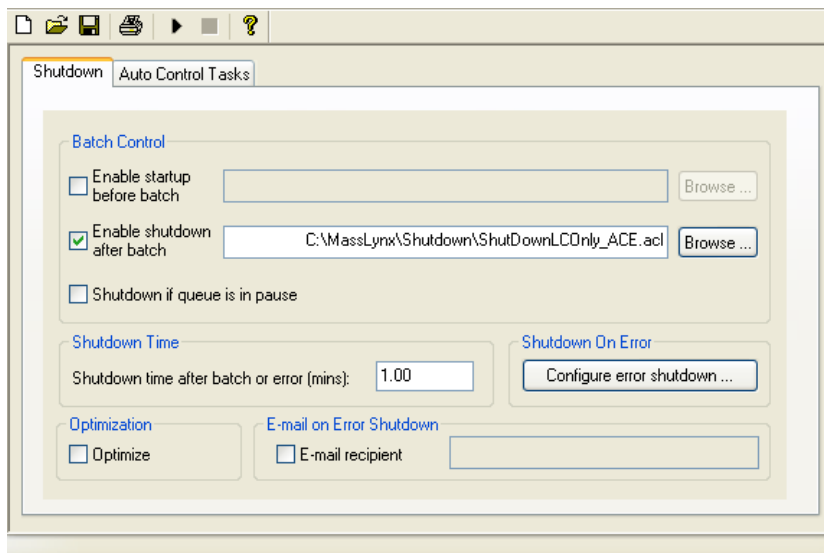
See also: “Shutdown tab” in the MassLynx help.

To create a shutdown method in MassLynx:

1. Click Instrument > Edit Shutdown or Startup.
2. In the Shutdown dialog box, select shutdown option check boxes in the Shutdown tab, and select shutdown tasks in the Auto Control Tasks tab.
3. Click File > Save As to save the shutdown method.

After you define these tasks, you must specify the shutdown method you created in the Shutdown dialog box.

Specifying shutdown conditions:



See also: Startup and shutdown topics in MassLynx Help.

Specifying emergency shutdown conditions

In case of an emergency, you can specify that the software run the shutdown method immediately or after a specified delay time. You must specify the error conditions that constitute an emergency, such as an error with the mass spectrometer, an external device, or with an inlet device, such as an LC or GC. In addition, you can specify that the software run the shutdown method when the solvent monitor detects one or more vessels at the warning or acute level. You can also specify that a person receive e-mail notification of the shutdown.

To specify emergency shutdown conditions:

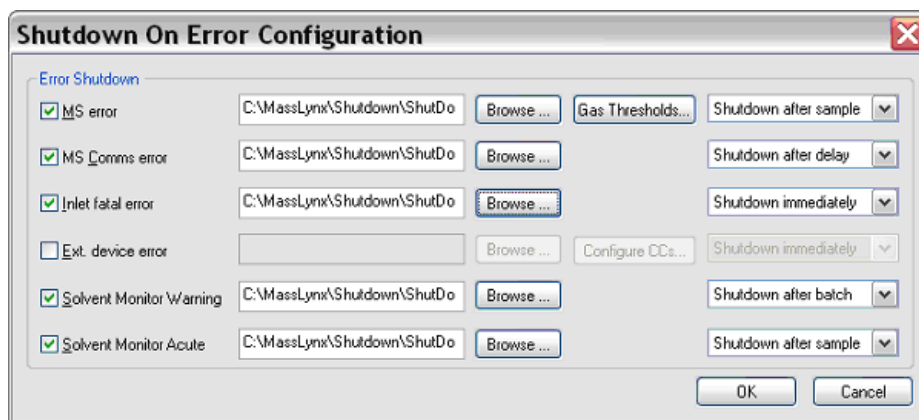
1. In the Shutdown dialog box, click the Configure error shutdown button.
2. In the Shutdown on Error Configuration dialog box, select the following parameters:
 - The error conditions (mass spectrometer error, solvent monitor at the acute level).
 - A previously created shutdown method.
 - When to run the shutdown method: immediately or after a specified delay time.

Tip: The solvent monitoring capability has additional shutdown responses: Shutdown after the current sample or Shutdown after the current batch.

3. Click OK > Save As to save the shutdown method.

Recommendation: If you use OpenAccess to run samples, select the Optimize check box and specify a shutdown time delay.

Specifying shutdown error conditions:



At the bottom of the MassLynx window, the status bar indicates the current shutdown status of the instrument, such as Shutdown Disabled, Shutdown Enabled, or Only Error Shutdown Enabled.

Testing system performance

After installing FractionLynx, you must test the system to confirm that the instruments are functioning correctly, and that the system produces the expected chromatographic results.

Testing involves using the Sample Dye Kit (part number 716000765) to inject dyes and subsequently collect them. A passing result for this system-level test means that the system instruments function properly. If the test results differ from those in the examples provided, ensure that collection parameters are set appropriately.

See also: [Chapter 3, Maintenance and Troubleshooting](#), and “Testing system performance” in FractionLynx online Help.

The conditions defined in each of the following tests are designed to help you rapidly determine whether all the components function as a system. Actual system performance (column load per run) depends greatly on conditions such as the HPLC solvents or modifiers, and the dimension of the columns.

For the MS-directed system test and UV-directed system test, many FractionLynx method parameters are identical, including the gradient and UV detector parameters. The chromatogram examples represent results that you should expect, although they can differ somewhat from system to system. As part of the system test, you also perform these procedures:

- [Balancing pressures and flows on an MS-directed system on page 2-14.](#)
- [Performing a delay timing test on page 2-18.](#)
- [Performing a chromatography test on page 2-34.](#)

Before running system tests

Confirm that these system components are set up correctly to avoid possible damage to the injectors and to prevent solvent leaks:

- Before running the pumps, ensure that the bed layout and reference position values are correct in the sample manager and fraction collector.
- Ensure that the syringe size, injection loops, and holding loops are correctly set, matching the actual installed instruments on the system and as defined in the Inlet method.

See also: The *Waters 2767 Sample Manager, Injector and Collector Maintenance Guide* and the *Waters Fraction Collector II or III Operator's Guide*.

Balancing pressures and flows on an MS-directed system

Before performing the timing test, balance system pressures.

Guideline: To function correctly, the splitter requires approximately 100 psi or more backpressure on the preparative side of the fraction collector system. Any additional restrictions downstream can affect the split ratio and require rebalancing the system pressures. Changing the flow rate or the plumbing requires rebalancing the pressures and flow to the mass spectrometer.

Recommendation: Use 50% organic mobile phase, as 50% is the midway point for most gradients. The pressure must be constant to obtain accurate readings.



Warning: To prevent injury, wear solvent resistant gloves and eye protection when performing this test.

To measure system pressure:

1. In the Inlet Editor, specify flow rates for both the preparative and makeup pumps.
 - For the preparative pump, create a method to run 50:50 acetonitrile/water.
 - For the makeup pump, set the flow rate at 1 mL/min. with 100% methanol.
2. Run the system at the desired flow rate, and wait for the pressure to stabilize.
3. Record the system pressures from the front panels of the pumps on the System Pressure Form.
4. To measure the pressure on the makeup side of the pump, disconnect the fitting on the splitter, wait for the pressure to stabilize, and record the makeup pump pressure on the System Pressures Form.
5. Reconnect the 1/1000 or 1/10,000 fitting and wait for the pressure to stabilize.
6. To measure pressure on the preparative side of the splitter, disconnect the collection fitting on the preparative side of the splitter, wait for the pressure to stabilize, and record the pressure on the System Pressures Form.

7. Reconnect the fitting and wait for the pressure to stabilize. Due to the high flow rate, you might need to stop the pump to reconnect the fitting.
8. Calculate the difference in pressure between the preparative and makeup sides, and enter the values on the form.

Rule: The pressure must be greater by approximately 100 psi on the preparative side than on the makeup flow side of the splitter.

Tip: You can adjust the pressure difference by adding or removing any restriction from the flow path. To increase the backpressure, you can add a short piece of small-diameter tubing, usually 0.010 inch on the prep side of the flow path.

9. After adding or removing tubing, repeat the system pressure test.

System pressure form:

System Pressure Readings		
Prep side before disconnection:	<input type="text"/> _psi	
		Delta: <input type="text"/> _psi
Prep side after disconnection:	<input type="text"/> _psi	
		Difference between prep side delta and make-up side delta: <input type="text"/> _psi
Make-up flow side before disconnection:	<input type="text"/> _psi	
		Delta: <input type="text"/> _psi
Make-up flow side after disconnection:	<input type="text"/> _psi	

Pressure test performed by: _____
Title/Dept.: _____ Date: _____
Comments and notes: _____ _____ _____
Mobile phases: _____ _____ _____
Flows: _____ _____ _____

Recommendation: Keep a copy of this page as a template for future pressure tests.

To measure the flow to the mass spectrometer:

1. Set the makeup pump to deliver methanol at 1 mL/min.
2. Disconnect the fitting going to the tee or the splitter before the probe.
3. Collect the solvent for one minute into an Eppendorf tube. Measure the solvent collected using a syringe. It should be the same as the programmed flow (1 mL).
4. The mass spectrometer should be receiving 20% of the 1 mL/min flow from the makeup pump. You can confirm this percentage as follows:
 - Place an Eppendorf tube at the outlet of the waste line from the UV detector. Wait for a steady waste stream to exit the waste line from the UV detector.
 - Measure the flow output for 1 minute.
 - Use a syringe to measure the solvent collected, which should not be less than 800 μL .
5. If the volume of collected solvent is less than 800 μL , then too much solvent is going to the mass spectrometer, and you need to adjust the split. You can adjust the split by changing the tubing lengths coming from the tee.

Example: When 1000 $\mu\text{L}/\text{min}$ is flowing to the split, which is upstream of the mass spectrometer and UV detector, and 800 $\mu\text{L}/\text{min}$ is flowing out of the detector, then 200 μL of solvent must be flowing to the mass spectrometer.

Rule: The optimum value for an electrospray mass spectrometer source is 200 $\mu\text{L}/\text{min}$.

Tip: When creating a split using a tee and tubing of different diameters, you can adjust it by adding or removing tubing. Replacing a piece of tubing with a longer piece of the same diameter tubing creates more pressure on that part of the split, reducing the volume of solvent passing through. If insufficient solvent is flowing to the mass spectrometer, add more tubing to the UV detector side of the split, or remove some tubing from the mass spectrometer side.

Performing a delay timing test

The FractionLynx AutoDelay feature automatically determines the delay time between the detectors that trigger fraction collection and the fraction collector. You can determine the delay time manually or automatically. To calculate the delay time manually, follow the procedure outlined on [page 2-32](#). In the automatic method, the delay time is determined using a series of injections from a caffeine standard. You can enter the delay time into the FractionLynx method manually, or the software can export the delay time into the FractionLynx method.

Determining the delay time automatically

You can run the AutoDelay wizard in one of two modes: simple or advanced. The detectors configured in the AutoPurification system influence which mode you run.

Simple mode automatically considers the first analog channel to be the waste detector data channel. If there are no analog traces, then the software uses the last detector configured in the inlet method as the waste detector channel. In simple mode, the injections required for delay time determination run consecutively without user input.

For example, if the AutoPurification System has a mass spectrometer or PDA detector as the fraction trigger detectors and either a 2487 detector under IEEE control or an analog device as the waste channel detector, use simple mode.

In Advanced mode, the user defines which detector is the waste detector and which detector trace is the trigger.

Requirement: If one of the detectors is configured as a Waters ELSD, you must use advanced mode. The ELSD is considered an IEEE-controlled device with analog data, which complicates the automatic identification of the waste detector channel.

After determining the delay time, the software reports its findings, and you can copy or export the results to the FractionLynx method. FractionLynx software can create an AutoDelay report that you can print and archive. The AutoDelay report contains the chromatography performed as a part of this test.

Tip: Good chromatography and good integration of the peaks are essential for success. It is important to maintain good peak shape and have only a single peak.

Requirements:

- Caffeine Test Kit (included in the Waters Sample kit), part number 700003233.
- A detector in the waste stream plumbed to the fraction collector using appropriately-sized tubing. Ensure that the tubing does not increase peak width and does not add more than 100 psi backpressure.
- Mobile phases:
 - A % Water/TFA or Water/formic acid, 0.1% (v:v).
 - B % Acetonitrile/TFA or acetonitrile/formic acid, 0.1% (v:v).
- Waters 19-mm × 50-mm column.

Exception: If you are using a column of a different size and type, you must adjust the flow and injection volumes accordingly. You must pressure balance the purification system at the purification flow rate.

Prerequisites:

You must perform the following tasks before you can automatically determine the delay time:

- Perform the system test and balance system pressures, and if required, test the flow to the MS probe.
- Determine the length and internal diameter of the tubing that connects the fraction collector to the waste detector.
 - The standard tubing that is pre-installed in the instrument is 3048 mm (120 inches) in length and 1.575 mm (0.062 inches) in diameter. If the tubing has been replaced or cut since installation, measure the length to within the nearest 15 cm (6 in).
 - Determine the ID of the tube installed. Different diameter lengths of tubing ship with the instrument and depend on the operational flow rate of the AutoPurification system:
 - 1.016 mm (0.40 in)
 - 0.762 mm (0.30 in)
 - 0.508 mm (0.02 in)

Tip: Once you run the AutoDelay wizard and enter the tubing dimensions, the software retains these dimensions. The next time you run the wizard, you need not enter the tubing dimensions, as they were automatically retained from the previous run. The

AutoDelay report contains the tubing dimensions, which you can reference the next time you perform the timing test.

- In MassLynx software, create inlet and MS methods.
- Configure and plumb the waste detector in the waste stream after the fraction collector.
- Ensure that the fraction collector is loaded with sufficient fraction tubes. The fraction must fit into a single tube.

Creating methods for the AutoDelay test

Before you run the AutoDelay wizard, you must create inlet and MS methods in MassLynx software. Once created, you can reference these methods any time you run the AutoDelay wizard. The total run time for the inlet method is 3 minutes.

Perform the AutoDelay test at the flow rate that you use for purification. You might need to scale the inlet method to account for your column diameter, length, and flow rate.

See also: [Total flow and gradient calculator on page 1-11.](#)

To create an inlet method for the AutoDelay test:

1. Enter the flow rate used to check system pressures.
2. Enter the inlet method in the table below for the 2545 binary gradient module.

Waters 2545 Binary Gradient Module parameters for the inlet method

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	30.00	95.0	5.0	Initial
2	0.25	30.00	95.0	5.0	6
3	1.50	30.00	30.00	70.0	6
4	1.60	30.00	95.0	5.0	6
5	3.00	30.00	95.0	5.0	6

3. Enter a total run time of 3.00 minutes.

4. In the Inlet Editor, configure the primary and waste detectors as follows:
 - If the detector is IEEE 2487, enter a wavelength of 254 nm.
 - If the detector is a PDA, enter a scan range of 240 to 290 nm.
 - For the waste detector, enter a collection rate of at least 5 Hz.
 - Enter a run time of 3 minutes.
5. Save the method.

Example: 3min_caff_gradient

To create an MS method:

1. Set the scan range from 150 to 250 Da.
2. Use the default settings for scan speed and interscan delay.
3. Enter a total run time of 3 minutes.
4. Save the method.

Example: 3min_caff

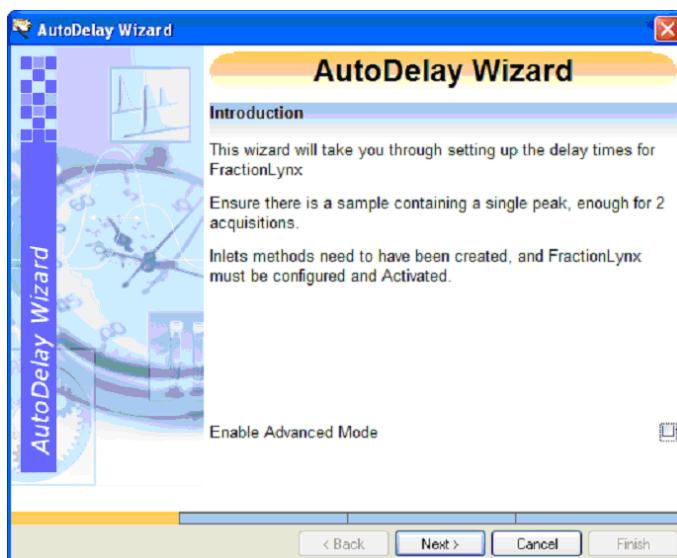
Using AutoDelay in simple mode

After you create an inlet and MS method, you can run the AutoDelay wizard.

To determine the delay time automatically in simple mode:

1. In FractionLynx, click Collection Control > AutoDelay Wizard.
2. In the AutoDelay wizard's Introduction page, do not select the Advanced Mode check box, and click Next.

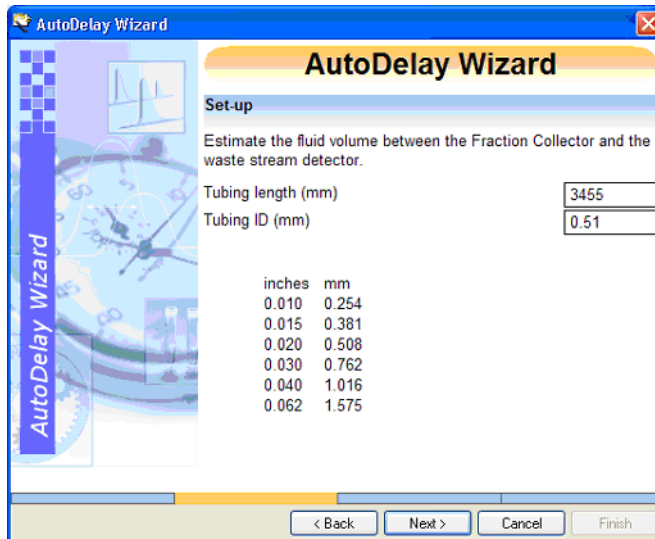
AutoDelay Wizard Introduction page:



Do not select this check box.

3. In the AutoDelay wizard's Set-up page, enter the tubing length and ID, and then click Next.
 - In the Tubing length field, enter the length of the tubing that connects the waste detector to the fraction collector.
 - In the Tubing ID field, enter the tubing's internal diameter.

AutoDelay Wizard Set-up page:



Example.
Your tubing might vary.

4. In the AutoDelay wizard's Make Injections page, enter the following parameters, and then click Run Injections:
- In the Inlet and MS Method fields, select the Inlet and MS methods that you created earlier.
 - In the Bottle Location field, enter the caffeine standard vial location.
- Tip:** Use the same naming convention as in the sample list.
- In the Injection Volume field, enter the injection volume.
- Recommendation:** 100 μ L of the 1.00 mg/mL test solution.
- In the Mass field, enter the M + H value (195 Da) as the mass of the caffeine sample to be monitored.

AutoDelay Wizard Make Injections page:

The screenshot shows the 'AutoDelay Wizard' window with the 'Make Injections' tab selected. The 'Methods' section contains the following fields and values:

Field	Value
Inlet Method	3min_caff_gradient
MS Method	3min_caff.exp
Bottle Location	4,1 32
Injection Volume	100
Mass	195

Navigation buttons at the bottom: < Back, Next >, Cancel, Finish.

Result: Two injections are automatically performed, the first one without fraction collection, the second with fraction collection.

Exception: If you installed SCN 562, a third injection is performed. This injection confirms that the software determined the correct delay time.

Once the injections finish successfully, the Results page appears ([page 2-30](#)).

Using AutoDelay in advanced mode

In advanced mode, the two injections required for delay time determination do not run automatically. After the first injection, you must specify which detector triggers fraction collection and which detector monitors the waste stream. You must also start the second injection.

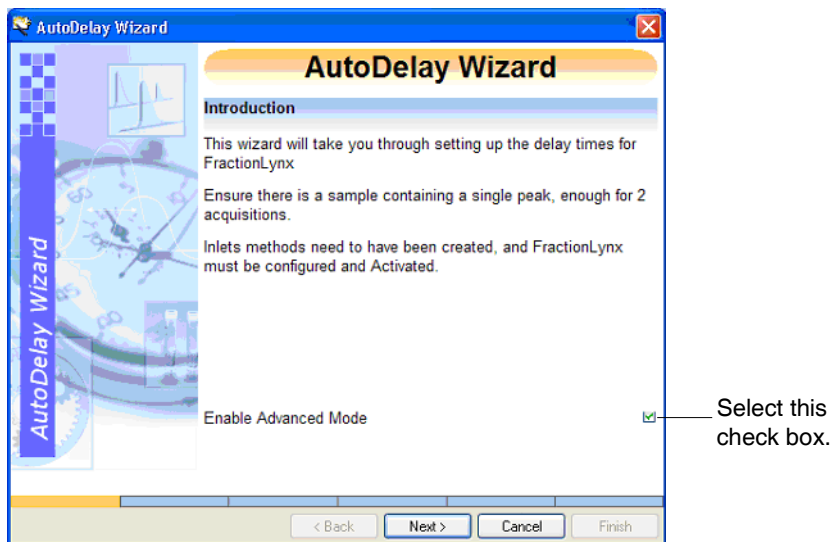
Rule: For those systems that contain a Waters evaporative light-scattering detector, use AutoDelay in advanced mode.

To regenerate a delay time report, you can reprocess data files collected earlier.

To determine the delay time automatically in advanced mode:

1. In FractionLynx software, click Collection Control > AutoDelay Wizard.
2. In the AutoDelay wizard's Introduction page, select the Enable Advanced Mode check box, and then click Next.

AutoDelay Wizard Introduction page:



3. In the AutoDelay wizard's Set-up page, enter the tubing length and ID, and then click Next.
 - In the Tubing length field, enter the length of the tubing that connects the waste detector to the fraction collector.
 - In the Tubing ID field, enter the tubing's internal diameter.

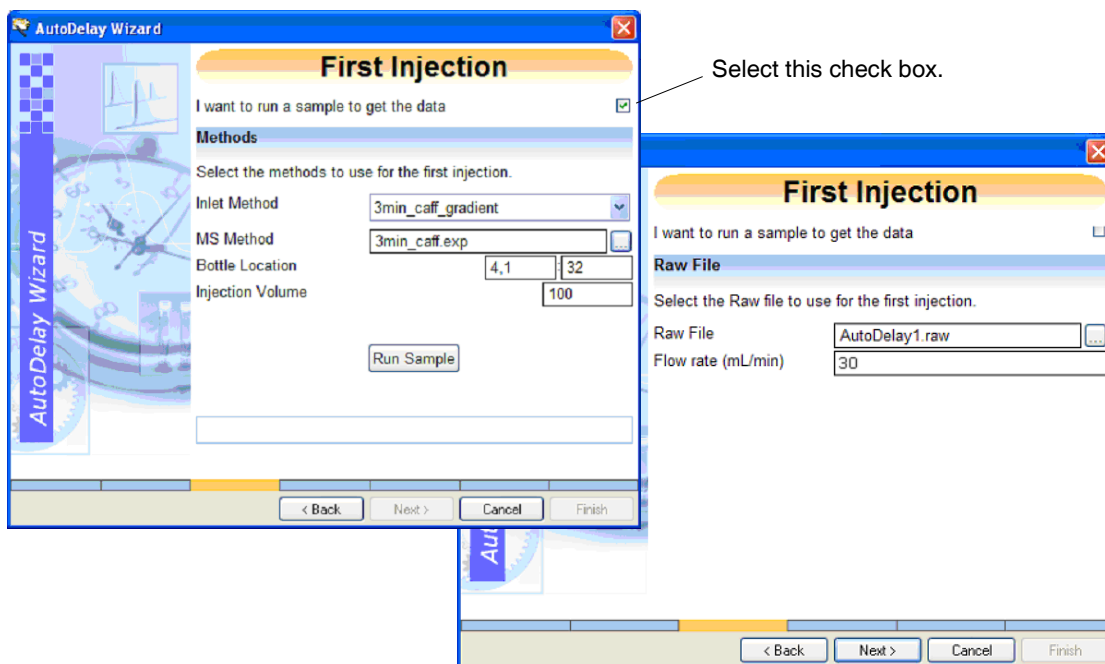
4. In the AutoDelay wizard's First Injection page, take one of these actions:
- If this is the first time you are running this wizard, select the “I want to run a sample to get the data” check box, and enter these parameters:
 - In the Inlet and MS Method fields, select the Inlet and MS methods that you created earlier.
 - In the Bottle Location field, enter the caffeine standard vial location.
 - In the Injection Volume field, enter the injection volume, and then click Run Sample.

Recommendation: 100 μ L of the 1.00 mg/mL test solution.

Result: The software performs the first injection.

- If you do not want to make an injection but want to regenerate data, do not select the “I want to run a sample to get the data” check box. Select a data file that was collected previously in the Raw File field, and enter the flow rate.

AutoDelay Wizard First Injection page:



5. In the AutoDelay wizard's Select Chromatograms page, enter the following parameters, and then click Next:
- For the detector that triggers fraction collection, select the scan function and mass or wavelength trigger.
Example: For the mass spectrometer, the mass that triggers collection is 195 Da for caffeine. In a UV-directed system, set the scan function to 254 nm.
 - For the waste detector, select the scan function and either TIC or 254 nm.
Example: In this example, the IEEE 2487 is the waste detector and it is configured as Function 3: Diode Array.

AutoDelay Wizard Select Chromatograms page:

AutoDelay Wizard

Select Chromatograms

Select the chromatograms from the Trigger and Waste detectors

Trigger detector

Function: Function 1 : ES+

Mass: TIC 195.1

Waste detector

Function: Function 3 : Diode Array

Wavelength: TIC 254

< Back Next > Cancel Finish

6. If you selected “I want to run a sample to get the data” in the First Injections page, enter the following parameters in the Second Injections page, and then click Run Sample:

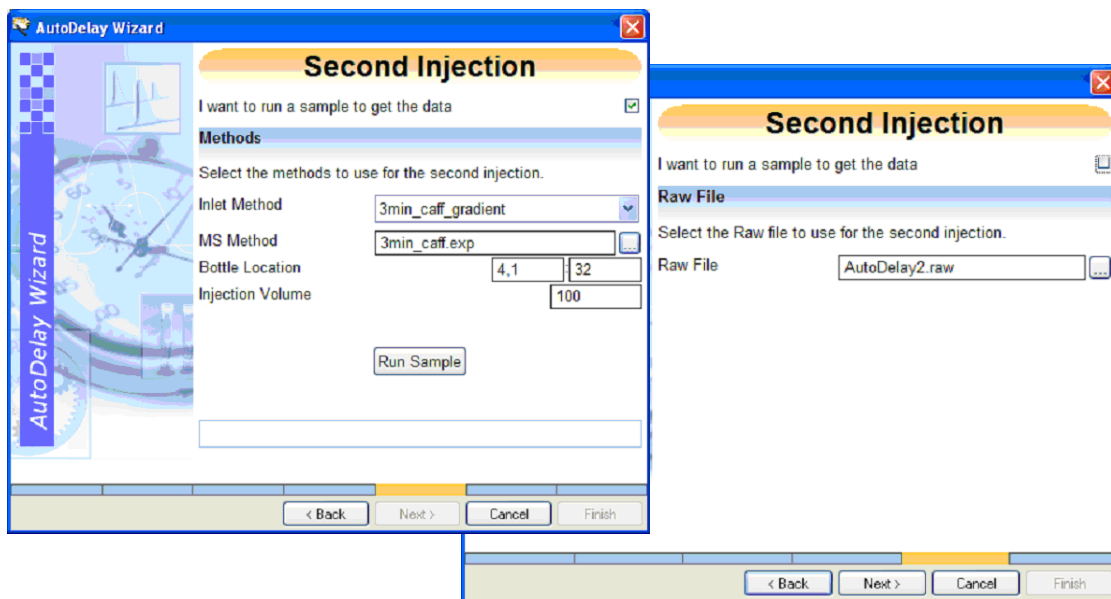
- In the Inlet and MS Method fields, select the Inlet and MS methods that you created earlier.
 - In the Bottle Location field, enter the caffeine standard vial location.
 - In the Injection Volume field, enter the injection volume.

Recommendation: 100 μ L of the 1.00 mg/mL test solution.

Result: The system performs the second injection.

- If you do not want to make an injection and want to regenerate data, do not select the “I want to run a sample to get the data” check box. Select a data file that was collected previously in the Raw File field, and enter the flow rate.

AutoDelay Wizard Second Injection page:



Result: FractionLynx performs the second injection or regenerates the data, and then the Results page appears.

7. If you installed SCN (Software Change Note) 562, the Confirmation Injection page appears. If you installed SCN 568, go to step 8. The Confirmation Injection page confirms that the software correctly determined the delay time in the previous two injections.

Tip: To determine the SCN that is installed on your system, click Help > About MassLynx from the MassLynx main window.

In the Confirmation Injection page, do one of the following:

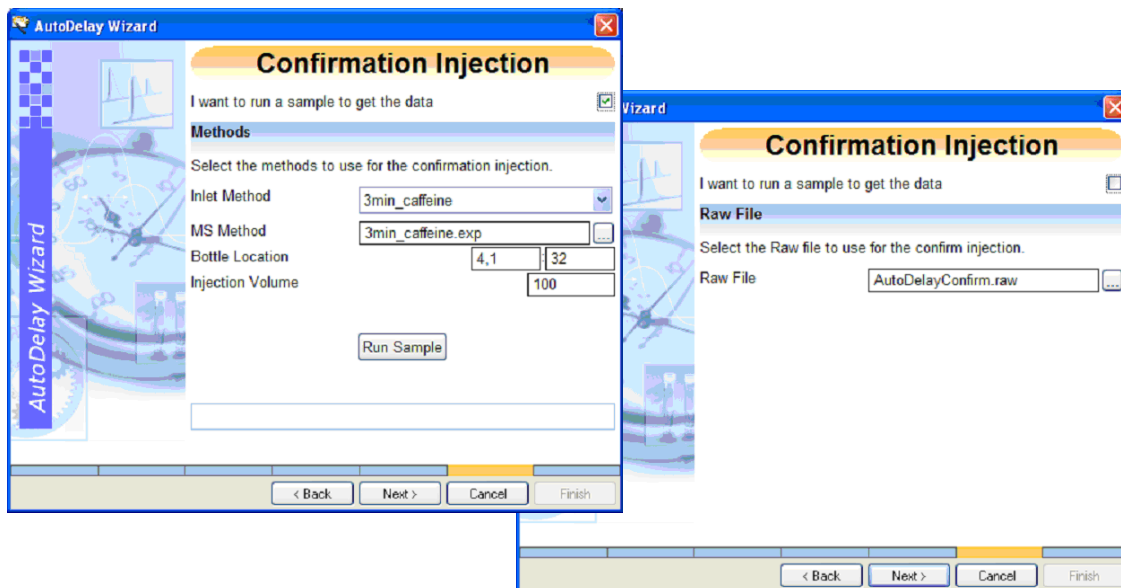
- In the Inlet and MS Method fields, select the Inlet and MS methods that you created earlier.
 - In the Bottle Location field, enter the caffeine standard vial location.
 - In the Injection Volume field, enter the injection volume.

Recommendation: 100 μ L of the 1.00 mg/mL test solution.

Result: The system performs the confirmation injection.

- If you do not want to make an injection and want to regenerate data, do not select the “I want to run a sample to get the data” check box. Select a data file that was collected previously in the Raw File field, and enter the flow rate.

AutoDelay Confirmation Injection page:



8. In the AutoDelay Wizard's Results page, perform one of the following tasks:

- To view the AutoDelay report, click View Report.

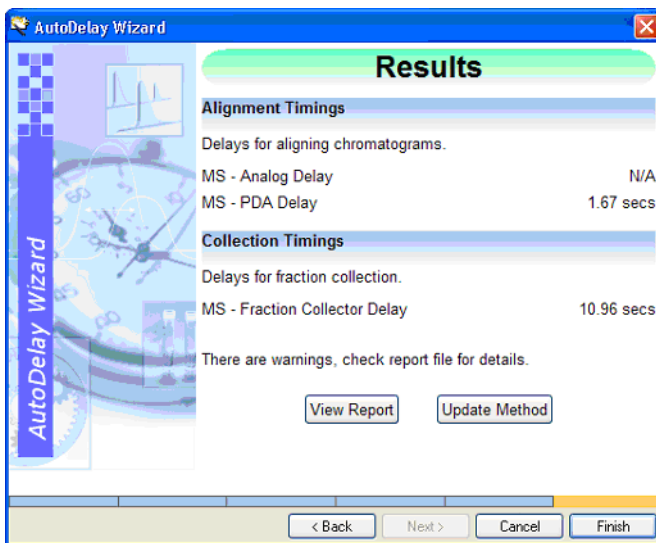
Tip: The AutoDelay report is an .html file that is automatically saved. You can click File > Save As to save it with a new name.

- To update a FractionLynx method with the delay time parameters, click Update Method.

Recommendation: For UV-directed systems, record the delay time, and then manually enter it into the FractionLynx method.

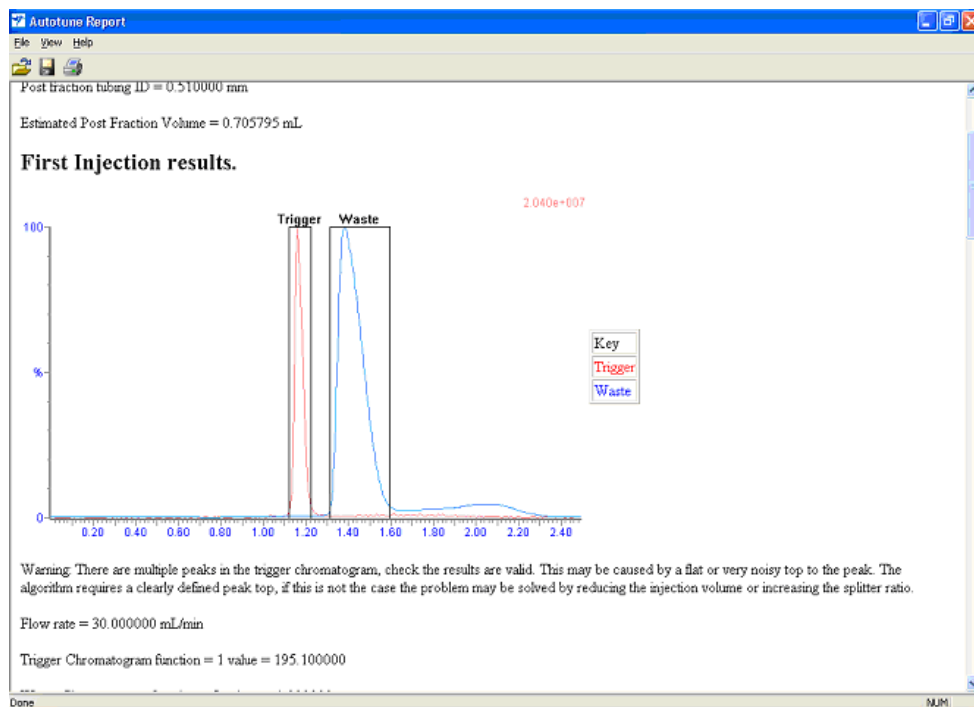
- If there are warnings in the report, the Results page instructs you to check the report for details.
- To repeat the test, click Finish, and start the wizard again.
- To exit, click Finish.

AutoDelay Wizard Results page:



In this example, the delay time between the MS and the waste detector is 10.96 seconds, and the delay time between the MS and the PDA detector is 1.67 seconds.

AutoDelay Report:



The AutoDelay report displays the chromatograms from the injections. In this example, the report depicts an overlay of the two chromatograms, and the boxes show the integration points, beginning, and end for each peak.

If the integration start of the waste peak occurs before the integration start of the trigger peak, a timing error is generated. If this situation occurs, it could be caused by a noisy baseline. To correct this problem, inject a larger amount of sample.

Requirement: Use the single compound standard. A compound with multiple peaks interferes with the software's ability to determine which peak should be used to determine the delay time.

To save the AutoDelay report:

1. Click File > Save As.
2. In the File Save As dialog box, enter the name of the AutoDelay report, and then select the file location to save the report.

Result: The AutoDelay report is saved in the Data folder of the current project. The next time you run the AutoDelay wizard, these files will be overridden unless you save them to a new location.

Performing the delay timing test manually

You can determine the delay time manually by injecting a dye sample from the sample dye kit. The dye is a mixture of three compounds. The following test is conducted using a Waters 19-mm × 50-mm XTerra column.

Rule: When using a different size and type of column than the one prescribed here, you must adjust flow and injection volumes accordingly. Pressure balancing and flow to the mass spectrometer must be performed at the purification flow rate. When using the column isocratically, the retention time is about 90 to 120 seconds, based on the column size and flow rate.

Required materials:

- Stopwatch
- Mobile phases:
 - A Water/TFA, 0.1% (v:v)
 - B Acetonitrile/TFA, 0.1% (v:v)
- Sample dye kit, part number 716000765 (in System Startup Kit)

Test sample dye compounds

Item	Thionin acetate	Thioflavin T	Crystal violet
m/z	227.1	282.1	371.2
UV _{max}	590 nm	418 nm	590 nm
Formula	C ₁₂ H ₉ N ₃ SC ₂ H ₄ O ₂	C ₁₇ H ₁₉ CIN ₂ S	C ₂₅ H ₃₀ CIN ₃
Sigma part number	T3337	T3516	C3886

To set up the delay timing test:

1. In the FractionLynx method, set the split collector delay to 30 seconds.
2. In MassLynx, create an inlet method with the following settings:
 - Run at the required flow rate for 3 minutes with 10% A and 90% B.

- For UV-directed systems, set the UV detector to the following parameters, and ensure that the connections on the UV Fraction Manager correspond to the flow rate used for this test:
 - Waters 2996 PDA Detector to scan from 200 to 600 nm.
 - Waters 2487 UV Detector to monitor 418 nm and 590 nm.
- 3. For MS-directed systems, create an MS method for a 3-minute scan acquisition with electrospray + ve, and specify these parameters:
 - Centroid = 150 to 550 amu scan for 0.5 seconds.
 - Inter-scan delay = 0.1 seconds.
 - Cone Voltage = 25 V.

To run the delay timing test:

1. In the MassLynx sample list, specify these values:
 - Specify the Injection Volume as 100 μ L.
 - Enter these values depending upon the detectors configured in the system:
 - For an MS-directed system, enter 227.1 in the Mass A column, 282.1 in the Mass B column, and 371.2 in the Mass C column. In the Fraction Trigger 1 column, enter Mass A. In the Fraction 2 column, enter Mass B. In the Fraction Trigger 3 column, enter Mass C.
 - For a UV-directed system, enter 418 in the Wavelength A column and 590 in the Wavelength B Column. In the Fraction Trigger 1 column, enter Wavelength A for a PDA detector or UV1 for an UV detector, such as a Waters 2487. In the Fraction Trigger 2 column, enter Wavelength B for a PDA detector or UV2 for an UV detector.

Tip: UV1 and UV2 correspond to Channel A and Channel B wavelengths in the Inlet method. The setting in the Wavelength column is for reporting purposes only; it does not control the 2487 detector. The software uses the value set in the Fraction Trigger column, UV 1, UV 2, against the value set in the Inlet Editor.

2. When the injection occurs, monitor the waste line from the fraction collector valve. As soon as the dye appears in the waste line of the fraction collector valve, start the stopwatch.

3. When you hear the fraction collector valve click to the dispense position, stop the stopwatch. The dye should have eluted before the collection valve opened.
4. Calculate the actual Splitter/Collector delay as follows:
The actual delay is equal to original Splitter/Collector delay specified in the FractionLynx method (e.g., 30 seconds) minus the time recorded on the stopwatch.
Example: If the dye is seen in the waste line 15 seconds before the valve clicks to the collection position, the delay time is calculated as follows:
Actual splitter collector delay = $30 - 15 = 15$ seconds.
5. In the Timing tab of the FractionLynx method, change the Splitter/Collector delay to the value just calculated, and rerun the experiment to ensure that the delay time is correct.
6. Repeat the procedure for all flow rates, such as 10, 15, 20, or 25 mL/min.
7. For the MS-based system, complete the procedure for APCI and ESI if you are using both probes.
8. Perform a chromatography test of the entire system, as described below.

Performing a chromatography test

Requirements: Sample dye kit, which contains three compounds for testing the ability of the AutoPurification System to perform high-flow-rate targeting and fractionation of specified masses and wavelengths.

See also: The table titled [Test sample dye compounds on page 2-32](#).

The compounds elute in this order:

- Thionin acetate loses the acetate salt ($C_2H_4O_2$) to give this [M+H] ion:
Thionin ([M+H] = 228.1), lambda max. = 590 nm
- Thioflavin T loses a Cl to give this [M+H] ion:
Thioflavin T ([M+H] = 283.1), lambda max. = 418 nm
- Crystal violet loses HCl to give this [M+H] ion:
([M+H] = 372.2), lambda max. = 590 nm

Tip: Report masses to one decimal place.

Requirement: To perform the chromatography test, create an inlet method using the parameters provided in this procedure. Use the XTerra 19-mm × 50-mm column to perform the tests. Scale the method appropriately for different flow rates and/or differently-sized columns. Use the flow rate that will be used for purification.

To create an inlet method for the system test:

1. Set up the Inlet Editor to run a 10-minute method labeled “Dye.”
2. Set up the Waters 2545 binary gradient module to use these parameters:
 - Mobile Phase A: Water with 0.1% TFA or 0.2% formic acid
 - Mobile Phase B: Acetonitrile with 0.1% TFA or 0.2% formic acid
 - Flow Rate: specify the same rate that you used in the delay timing test.
 - Run Time: 10 minutes
 - Gradient conditions as specified in the following table:

Gradient conditions for the system test

Time	A%	B%	Curve Number
T = 0 minutes	95	5	6
T = 1 minutes	95	5	6
T = 7 minutes	30	70	6
T = 7.5 minutes	5	95	6
T = 8.5 minutes	5	95	6
T = 9 minutes	95	5	6
T = 10 minutes	95	5	6

3. In the Inlet Editor, set the wavelength range you want to acquire, based on your detector type:
 - For a Waters 2996 PDA Detector, in the Inlet Editor, set the wavelength range to 210 nm to 650 nm.
 - For a Waters 2487 dual wavelength absorbance detector, in the Inlet Editor, set the single channel wavelength to 418. Set the dual channel wavelength to 590.

4. For an MS-directed system, create a mass spectrometer method with these parameters, and then set it to run for 10 minutes:
 - Set up a Scan Function: 150 to 500 amu
 - ES+
 - Centroid Data
 - 0.5-second scan time with a 0.1-second Inter Scan Delay
 - Cone Voltage = 35 V
5. In the MassLynx sample list, perform these tasks:
 - Specify an injection volume of 100 μ L.
 - For an MS-directed system, enter 227.1 in the Mass A column, 282.1 in the Mass B column, and 371.2 in the Mass C column. In the Fraction Trigger 1 column, enter Mass A. In the Fraction Trigger 2 column, enter Mass B. In the Fraction Trigger 3 column, enter Mass C.
 - For a UV-directed system, enter 418 in the Wavelength A column and 590 in the Wavelength B column. In the Fraction Trigger 1 column, enter Wavelength A for a PDA detector or UV1 for a UV detector, such as the Waters 2487 dual wavelength absorbance detector. In the Fraction Trigger 2 column, enter Wavelength B for a PDA detector, or UV2 for an UV detector.

Tip: The Mass and Wavelength columns are for information only, unless the corresponding fraction information is entered in the Fraction Trigger column.
6. For systems that include a ZMD mass detector, set the instrument's multiplier voltage to 550 V. For systems that include a ZQ mass detector, set the multiplier voltage to 500 V. For systems that include a 3100 mass detector, retain the default gain setting of 1.
7. From the shortcut bar, click FractionLynx > Collection Control, and verify that your system is activated.

To set the FractionLynx method parameters for a system test:

1. In the FractionLynx main window, click Edit FractionLynx method.
2. In the FractionLynx method, click each tab to set the parameters you want to define for this fraction collection method (see the [Table titled "FractionLynx method settings:"](#) on page 2-37.)

Exception: The FractionLynx method parameters for the UV-based system test apply to Waters UV detectors. When using the detectors of other manufacturers, the threshold values can differ slightly. They might also differ from system to system. Use the values specified in the table below as guidelines.

FractionLynx method settings:

Parameter tab	Settings you can specify
General	<ul style="list-style-type: none"> • Set Fraction Collection to On and Peak Type to Preparative. • Select the Max Fractions per injection check box, and specify a value of 10. • Select the Max Tubes per injection check box, and specify a value of 10. • Rinse time = 0. • Span = 0.5 amu.
Timing	<ul style="list-style-type: none"> • Solvent Front Delay = 0 seconds. • Split/Collector Delay = x seconds, where x = the split collector delay determined earlier.
Volume	<ul style="list-style-type: none"> • Minimum Fraction Width = 3 seconds. • Maximum Fraction Width = 120 seconds. • Maximum Tube Fill = 90 percent.

3. Based on your detector, enter the values specified in the table below.

Tip: Where values are not specified in the table, retain FractionLynx method's defaults.

FractionLynx method settings based on detector type:

Detector type	FractionLynx method tab	Settings you can specify
2487	UV	<ul style="list-style-type: none"> • MIT = 1000. • Peak Start = Leading Edge Gradient % with a value of 30. • Terminate Peak = Below Gradient % with a value of 60.
PDA	PDA	<ul style="list-style-type: none"> • Span = 3 nm. • MIT = 5,000. • Peak Start = Leading Edge Gradient % with a value of 30. • Terminate Peak = Below Gradient % with a value of 60.
Mass Spec	ES+	<ul style="list-style-type: none"> • ES+ Ion Adducts = 1. • MIT = 5,000,000. • Peak Start = Use MIT only. • Terminate Peak = Use MIT only.
Analog	Analog	<ul style="list-style-type: none"> • MIT = 5,000. • Peak Start = Leading Edge Gradient % with a value of 30. • Terminate Peak = Below Gradient % with a value of 60.

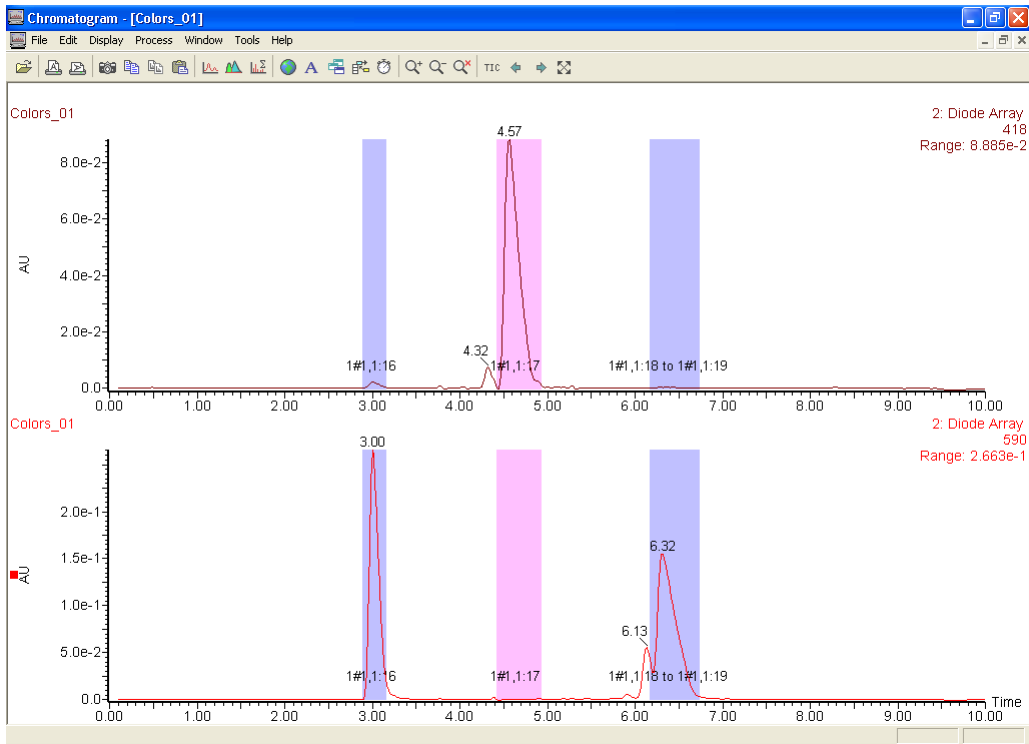
To collect fractions for the system test:

1. From the shortcut bar, click FractionLynx > Collection Control, and verify that your system is activated.
2. From the MassLynx toolbar, click Start.

UV Chromatography Dye Test Results

These chromatograms represent typical results of the dye test. The chromatograms show three separate color samples of blue, yellow, and violet collected into separate vials labeled 1:16, 1:17, and 1:18 to 1:19.

UV dye test chromatogram:

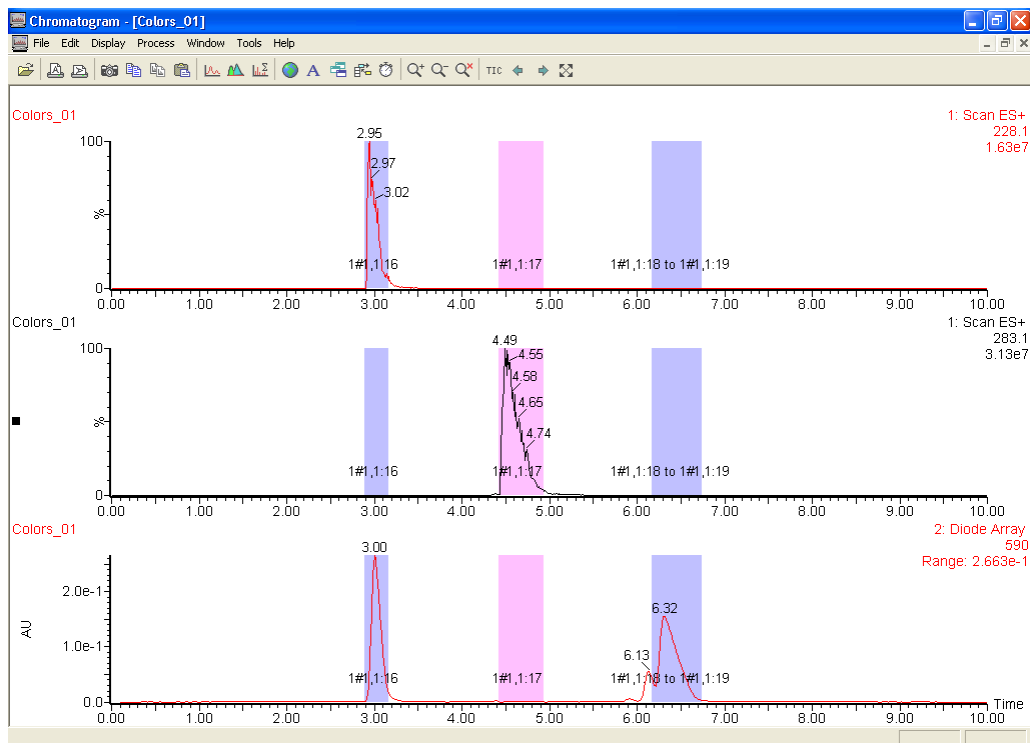


This sample was collected using the Waters 996 PDA Detector. The wavelength values were put in the sample list, and the fraction triggers were set to Wavelength A and B.

- The extracted wavelength shows the correct picture of what was actually collected.
- Click Display > Wavelength to input the wavelengths of choice, and then click OK to get the extracted wavelengths.
- To extract the spectrum, double-click the peak in the Total Absorbance Chromatogram (TAC).

Actual results can vary if different column sizes, flow rates, and injection volumes are used.

MS chromatography dye test results:



The chromatograms above represent typical results of the dye test. The three components of the dye mix have been separated and collected into separate vials labeled 1:16, 1:17, and 1:18 to 1:19.

The extracted ion shows the correct picture of what was actually collected into each vial. Double-click the peak in the Total Ion Chromatogram (TIC) to extract the spectrum, and double-click the spectrum of the mass of interest to extract the mass chromatograms. Always consider the extracted ion or wavelength when troubleshooting as it will give the correct representation of what was actually collected.

Viewing reports

Within the FractionLynx browser, you can view, print, or export results to other applications (such as a LIMS system or an Microsoft Excel file) using the clipboard or tab-delimited text files. From the MassLynx shortcut bar, click FractionLynx > Browser.

FractionLynx creates a report file (*.fpt) when you perform these tasks:

- Use a sample list and select AutoPurify in the Process column and an *.flp method in the Parameter column. When starting a sample list, you must also select Acquire Sample Data and Auto Process Samples.
- Submit a job using OA Login.

Requirement: For compound targeting, when using OA Login or a sample list, you must specify the mass value that you want to search for.

Printing reports

Report schemes (*.frs files) specify the information included in printed reports. From the FractionLynx Browser, click File > Report Scheme Settings to define the information you want in a printed report.

Using the waste detector

By including an additional detector downstream of the fraction collector in an AutoPurification system, you can monitor the solvent stream for samples or portions of samples not collected by the fraction collector. Waste detectors are commonly used to evaluate the efficiency of fraction collection. A waste detector trace that shows no evidence of the sample indicates successful fraction collection. However, the presence of the sample in the waste stream does not necessarily indicate a failed collection.

The waste detector trace differs from the trigger detector trace. This section explains how to use the waste detector trace to monitor the quality of fraction collection.

Prerequisite: To monitor the uncollected portions of the peak, you must connect a waste detector to the outlet of the fraction collector. Ensure that the detector flow cell is designed for the correct flow rate.

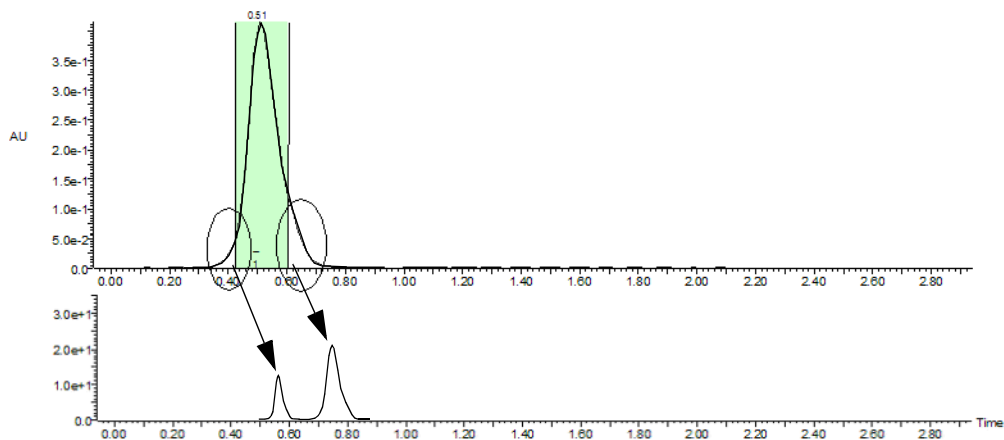
Tip: The illustrations in this section depict a single compound eluting from the column. If multiple peaks elute and are collected by the waste detector, the resulting chromatograms may differ from the illustrations provided in this section.

Expected versus actual collection

The illustration below contains two chromatograms. The upper chromatogram displays the trace from the trigger detector, where some of the front and the tail of the peak were intentionally not collected. To ensure some of the sample was not collected and to more clearly describe the functionality, the collection threshold in the FractionLynx method was set artificially high.

The illustration below is a drawing that depicts the expected chromatogram from the waste detector, when the front and tail of the peak pass through the trigger detector.

Expected collection when the threshold is set too high:

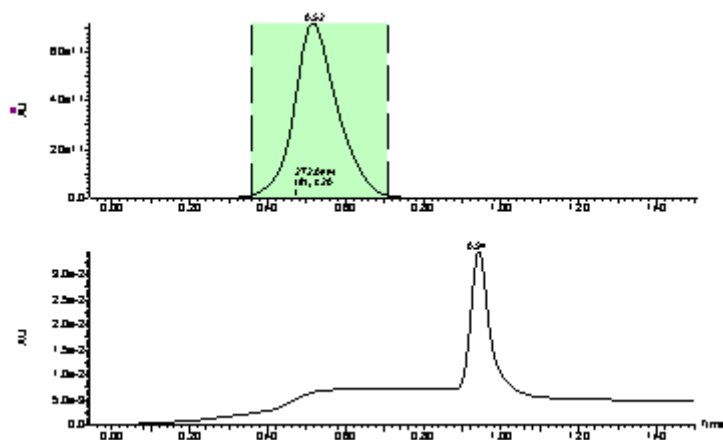


Although the illustration appears to depict two distinct peaks passing through the waste detector, it is actually a single peak. When collection starts, the collection valve opens and the solvent flow to the waste detector stops. The portion of the sample that was not collected remains in the tubing beyond the collection valve. Whether or not the sample reaches the waste detector depends on three factors:

- Volume of the tubing between the collector valve and the waste detector
- System flow rate
- Collection threshold

When collection finishes, the tail of the peak that was not collected moves through the collection valve and combines with the front of the peak, which remained in the tubing during fraction collection. The two portions of the sample elute as a single peak in the waste detector.

Actual collection with correct threshold settings:



The illustration above shows two chromatograms. The upper chromatogram depicts the trigger detector trace and the portion of the peak that was collected successfully. This example is more representative of an actual collection than the example on the previous page. If you magnified the baseline of the upper chromatogram, you would notice that a small amount of sample was not collected from both the front and tail of the peak, which is not unusual.

The lower chromatogram depicts the waste detector trace, and shows a small plateau before the waste peak and then the peak itself. The plateau corresponds to the small amount of sample that entered the waste detector before collection started and the flow to the waste detector stopped. The peak corresponds to the pre- and post collections, which combine to form a single peak.

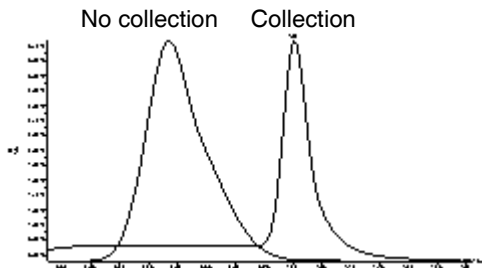
In the illustration above, the waste detector was relatively close to the fraction collector, so a small amount of the uncollected fraction reached the waste detector, resulting in the slight increase in absorbance and the plateau before the peak. When the distance, and therefore the volume, between the fraction collector and the waste detector is increased, slightly different peak shapes can result.

Monitoring the quality of the fraction collection

When comparing the waste detector trace before and after a collection, the loss of the peak's signal indicates successful collection. You can successfully collect a fraction, yet still see a peak in the waste detector.

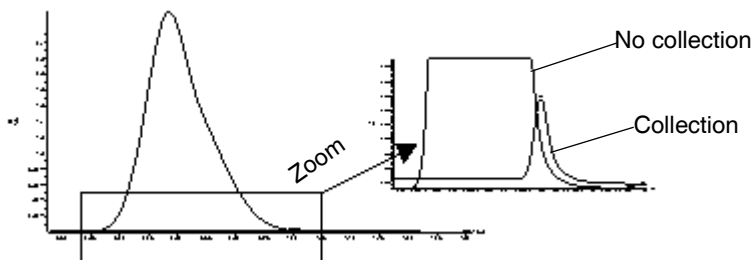
The illustration below depicts waste chromatograms with and without fraction collection. The peak on the left shows the waste detector chromatogram when no collection occurred. The peak on the right shows the waste detector chromatogram when collection did occur. The right-hand trace confirms that some sample was not collected and passed through to the waste detector. When you overlay the two chromatograms and compare them to one another, it appears that the collection was not successful.

Overlay of waste detector traces with and without collection:



However, when you link the vertical axis and overlay the two chromatograms, as shown below, it is difficult to see the waste peak detected when fraction collection occurred, compared to when collection did not occur. The inset chromatogram, which displays a magnified section of the chromatograms, shows a better representation of the relationship between the two chromatograms. The retention time delay results from the temporary flow stoppage in the tubing between the fraction collector and the waste detector.

Overlay of waste detector traces with and without collection, with the vertical axis linked:



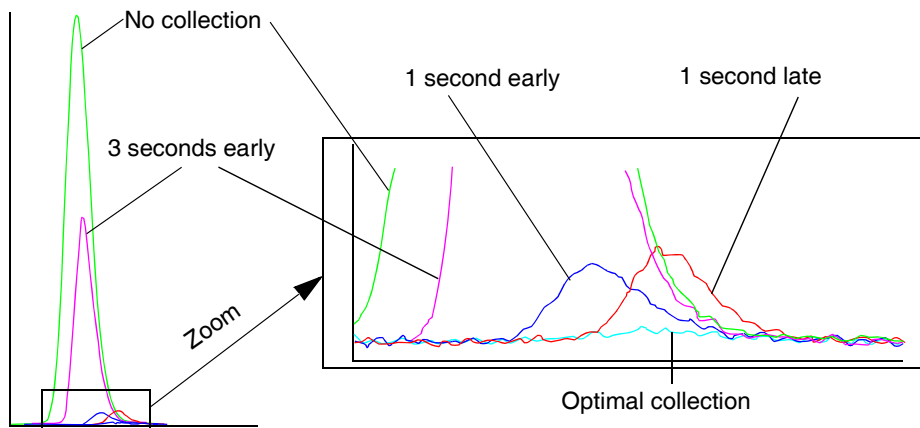
Comparing chromatograms with different delay times

Delay times affect the efficiency of fraction collection. To optimize collection, you can adjust the delay times in the FractionLynx method, and monitor the waste detector chromatograms. The illustration below depicts how delay time can affect the efficiency of the collection.

Example: The following illustration depicts five overlaid chromatograms from the waste detector. The largest peak is the waste peak with no collection at all. During collection, the other four peaks elute as follows:

- 3 seconds early
- 1 second early
- 1 second late
- At the correct delay time

Effect of delay time on chromatograms:



To see the differences in the chromatograms easily, zoom on the base of the peaks (shown at right).

When the delay time is set to the optimal value, the amount of sample that passes from the fraction collector to waste is minimal.

Determining the system dwell volume

System dwell volume is the amount of liquid that resides in the tubing from the point where the mobile phase solvents are mixed until they reach the head of the column.

Gradient methods depend on gradual change in the mobile phase concentration to separate compounds. By determining the system dwell volume, you can program the appropriate hold time at the beginning of a gradient method. This ensures consistent chromatography when scaling between systems or between columns.

Determine the dwell volume for both the analytical and preparative sides of the AutoPurification system. Do the analytical side first.

Prerequisite: The day before you perform this procedure, prepare a solution of acetonitrile with 0.05 mg/mL uracil. This ensures the uracil will be completely solubilized when you perform the system dwell volume procedure.

To determine the system dwell volume:

1. Remove the column.
2. Set up the AutoPurification system with acetonitrile as mobile phase A and acetonitrile with 0.05 mg/mL uracil as mobile phase B.
3. Program the analytical method as follows.

Tip: Use the scaled flow rates appropriate for the selected analytical and preparative columns.

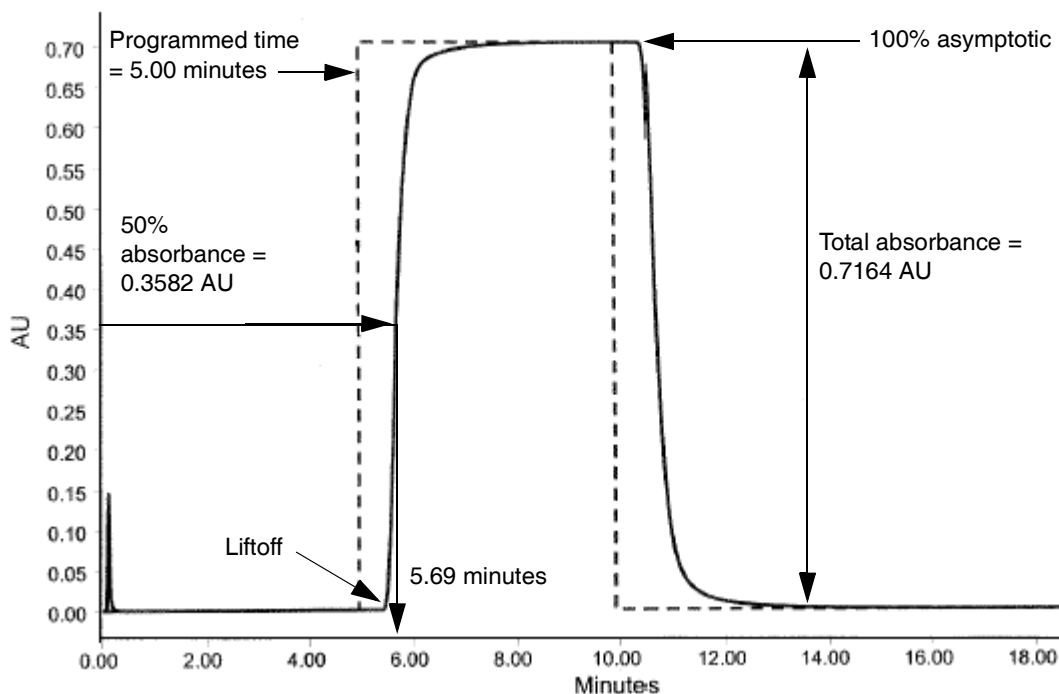
Programming the analytical method: 1_System_Volume

Time	Flow	%A	%B	Curve shape
0.00	1.46	100	0	
5.00	1.46	100	0	6
5.01	1.46	0	100	1
10.01	1.46	0	100	1
10.02	1.46	100	0	1

4. Measure the absorbance difference between 100% A and 100% B.

Example: In the figure below, the absorbance difference between 100% A and 100% B is 0.7164 AU.

Example: Measuring absorbance at mid-point:



5. Measure the time at 50% of the absorbance difference.
6. Calculate the time difference between liftoff and the 50% point, and then multiply the time difference by the flow rate.

Calculation example:

Actual time at 50% absorbance = 5.69 min

Programmed time = 5.00 min

Delay time = Actual time – programmed time = 0.69 min

System volume: 0.69 min \times 1.5 mL/min = 1.04 mL

- Repeat steps 3 through 6 for the preparative side, programming the preparative method using the values in the following table.

Programming the preparative method: 2_System_Volume

Time	Flow	%A	%B	Curve shape
0.00	25	100	0	
5.00	25	100	0	6
5.01	25	0	100	1
10.01	25	0	100	1
10.02	25	100	0	1

3

Maintenance and Troubleshooting

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System maintenance

See also: MassLynx online Help, FractionLynx online Help, and the operator's guides for the individual modules of the AutoPurification System:

- *3100 Mass Detector Operator's Guide*
- *2545 Binary Gradient Module Operator's Guide*
- *System Fluidics Organizer Operator's Guide*
- *2767 Sample Manager, Injector, and Collector Operator's Guide*
- *2996 PDA Detector Operator's Guide*
- *2420 Evaporative Light Scattering Detector Operator's Guide*
- *2487 Dual Wavelength Absorbance Detector Operator's Guide*
- *Fraction Collector III Operator's Guide*

General operating procedures

To keep your system running smoothly, follow the operating procedures and guidelines in each system instrument's documentation.

Spare parts

For spare parts and part numbers, go to <http://www.waters.com>. Parts not listed in the Waters web site are not recommended for customer replacement.

Contacting Waters Technical Service

If you are located in the USA or Canada, report malfunctions or other problems to Waters Technical Service (800 252-4752). If you are located anywhere else, phone Waters' corporate headquarters in Milford, Massachusetts (USA), or contact your local Waters subsidiary. Our Web site includes phone numbers and e-mail addresses for Waters locations worldwide. Go to www.waters.com, and click About Waters > Worldwide Offices.

However you contact Waters, be prepared to provide the serial number of your mass detector and other instruments in the system.

Troubleshooting and clearing errors

FractionLynx displays three types of errors that can occur in the AutoPurification system:

- Collection system problems
- Fraction collection problems
- Instrument problems

You can view communication errors in the FractionLynx - Collection Control window and the MassLynx Inlet Editor. To view communication errors in the FractionLynx - Collection Control window, click Overview > System Overview > View Status Log. In the Status Log dialog box, a green check mark or red X appears next to the system name indicating whether communications are working or have failed. Pause the cursor on the symbol to view the status message.

To clear errors in the FractionLynx - Collection Control window:

1. Deactivate the fraction system.
2. Close the FractionLynx - Collection Control window, and then reopen it.
Guideline: If the solutions in these troubleshooting topics do not resolve the problem, consider reviewing the fraction file (Fract.txt) that resides in the *.raw data directory of your project. The Fract.txt file contains values such as threshold settings for triggers, minimum and maximum fraction times, and adduct information. Also keep in mind that FractionLynx cannot create a fraction file if the file is missing from the sample list.
3. Click the Activate button to activate collection control.

Collection system error messages

Collection system error messages and solutions

Error message	Solution
Bed Layout Error!	Determine which of these conditions might be causing the problem, and then correct it as instructed: <ul style="list-style-type: none">• Ensure that you specified the correct bed for each fraction collector.• The bed layout might be corrupt. Re-create the bed.
Failed to load Barcode file	Determine which of these conditions might be causing the problem, and then correct it as instructed: <ul style="list-style-type: none">• Create the required bar code file using the BarCode Generator, or specify the correct bar code file.• If your access to the file is denied, check the permissions on the file, or determine whether another user is editing the file.
Collector: [CollectorNumber], Plate:[PlateNumber]- Barcode File [BarcodeFile] does not match plate format, or does not exist.	Load the bar code file into the BarCode Generator. Review the file's format to be sure that it is the same as the plate format you are using.
Need to reset beds after Emergency Collection in Injection Mapped Reserved Vessels mode.	Reset all beds for this system. Rule: You cannot resume normal collection from the injection vial after running an emergency collection from that vial in Injection Mapped Reserved Vessels mode.

Collection system error messages and solutions (Continued)

Error message	Solution
Cannot reset the plates on the current collector.	<p>For safety reasons, correct this problem in one of two ways:</p> <ul style="list-style-type: none"> • Wait until the collection moves to another collector. • Pause the sample list, wait until the sample run finishes, and then reset the plates and beds. <p>Rule: When running samples in mapped mode, such as for one-to-one collection, you cannot reset the plates. Pause the sample list, wait for the run to finish, and then reset the beds.</p>
The tube has zero volume or the max tube fill is 0%.	<p>Determine which of these conditions might be causing the problem, and then correct it as instructed:</p> <ul style="list-style-type: none"> • The collection tube volume is 0 μL. Use the Bed Layout Editor to set the volume of the tube on the plate. • In the FractionLynx method, specify a value for Max tube fill greater than 0%.
Fraction System, Comms: Failure, Check connections and settings. Device, Not Initialized, Bed not loaded.	<p>In the FractionLynx - Collection Control window, review the Status Log to determine the nature of the problem.</p>

Fraction collection problems

Fraction collection problems and solutions

Problem	Solution
Identified wrong mass.	<p>Review the sample list to ensure that the correct monoisotopic mass was specified, and ensure that the correct sample was injected at the beginning of a run. If you reuse a sample list, you may inadvertently specify the wrong sample, mass of interest, or set the wrong triggers.</p> <p>From the MassLynx Main window, click Instrument > MS Method. In the Mass Spec Method, check the scan settings.</p>
Incorrect trigger entries.	<p>If the compound of interest was not collected, in the sample list, note the Fraction Trigger column and ensure the mass of interest is selected.</p> <p>Check the wavelength settings for your detector type:</p> <ul style="list-style-type: none">• For a 996 or 2996 PDA Detector, you must specify a numerical value in the Wavelength column. In the Fraction Trigger column, select wavelength A, B, C, and so on.• For a Waters 2487 Detector, specify the wavelength in the Inlet Editor. In the Fraction Trigger column of the sample list, select UV1 for Channel A, and UV2 for Channel B.
An error message notifies you that you are outside the calibration range.	<p>The MS scan speed is too fast or too slow. Adjust your scan speed to stay within the calibration range.</p> <p>See also: <i>Waters 3100 Mass Detector Operator's Guide</i>.</p>

Fraction collection problems and solutions (Continued)

Problem	Solution
Target mass was not in the MS Scan range.	<p>If the compound of interest was not collected, ensure that the mass spectrometer method has a range that includes the target mass. For example, if a target mass is 253, and the mass scan range is 300 to 500, the fraction is not collected.</p> <p>From the MassLynx Main window, click Instrument > MS Method. In the Mass Spec Method, check the scan settings.</p>
Not collecting the target mass of interest.	<p>In the Tune window, examine the cone voltage and compound ionization parameters.</p> <p>Set the cone voltage low (30) to prevent too much fragmentation.</p>

Instrument problems

Instrument problems and solutions

Problem	Solution
Error initializing [selector] valve.	Determine which of these conditions might be causing the problem with the selector valve, and then correct it as instructed: <ul style="list-style-type: none">• Ensure the communication port is set correctly. Ensure cables are correctly and securely attached.• Change the control mode on the selector valve from local to remote.
Error moving the selector valve to [Position].	Determine which of these conditions might be causing the problem, and then correct it as instructed: <ul style="list-style-type: none">• Ensure communication port is set correctly and ensure cables are correctly and securely attached.• If the error occurred while moving the selector valve, reset it, and then reactivate FractionLynx.• Ensure you are using the correct type of valve for the configuration (analytical, preparative, or selector).
Failed to communicate with the Waters 2767: Communication Fault.	Ensure the instrument's communications settings are correct, that cables are secure in their proper terminals, and that the 2767 Sample Manager's on/off switch is set to On.
Waters 2767 Fraction Collector: [ReportedError].	See the <i>Waters 2767 Sample Manager, Collector Installation and Maintenance Guide</i> for details. From the FractionLynx Main window, deactivate, and then reactivate FractionLynx.

Instrument problems and solutions (Continued)

Problem	Solution
<p>Waters F C II/III: Error during initialization. Comms Error</p>	<p>When any of these errors occur on the Waters Fraction Collector II/III, determine whether these conditions exist:</p> <ul style="list-style-type: none"> • Cables are correctly and securely connected, and the on/off switch is set to On. • Communications settings are correctly specified. • Ensure cables are correctly and securely attached. • Ensure the on/off switch is set to On.
<p>Error occurred entering idle state.</p>	<p>You attempted to close the fraction collector valve on the Waters Fraction Collector II/III. Inspect the cables making sure they are correctly and securely connected.</p>
<p>Error moving to vessel Error moving to rinse position Error moving home</p>	<p>If these errors occur while trying to move the Waters 2767 Sample Manager to the home position or rinse position, see the Inlet Method window for status details.</p> <p>If these errors occur while trying to move the Waters 2767 Sample Manager to the Waters Fraction Collector II/III home position, check the cables and power.</p>
<p>Error during move</p>	<p>Inspect the cables for improper or poor connection. Power-on the Waters Fraction Collector II/III. Ensure that the fraction collector is not obstructed.</p>
<p>This instrument is in the SET state. This instrument has to be returned to the MENU state manually before initialization can be completed. Reset comms when instrument has been returned to MENU.</p>	<p>Use the control panel on the Waters Fraction Collector II/III to return to the Main menu, and then try again.</p>

2545 multi-module instrument problems

The error messages might involve the 2545 Binary Gradient Module or other instruments (1525, 600, SFO, or 1500).

Multi-module instrument error messages and solutions

Error message	Solution
Please reset communications	<p>Any time you reconfigure a set of controlled instruments, you must reset communications.</p> <p>To reset communications, from the Inlet Method window, click LC > Reset Communications.</p> <p>Close the Inlet Method window, and then reopen it to apply the new configuration to the additional status view.</p>
Message (m)	<p>Check the operator's guide for the particular hardware module for corrective action instructions.</p> <p>Note: (m) indicates the name of the instrument that generated this error message.</p>
Module is in failed state (m)	<p>The instrument, indicated by (m), experienced an error and is no longer functioning.</p> <p>Try resetting communications. From the Inlet Method window, click LC > Reset Communications.</p> <p>If this does not restore the green OK indicator, refer to the instrument's operator's guide.</p>
Failed to read module status (m)	<p>Determine whether these items on the autopurification device (m) are correct:</p> <ul style="list-style-type: none">• The on/off switch is set to On.• Cables are connected securely and correctly.• Configuration is correct.• Try to scan 2545 binary gradient modules in the Inlet Method window.• Try to scan from the Inlet Method > Inlet > Modify Instrument Method > Configure Waters Pump Control Modules.

Peak detection problems

Peak detection problems and solutions

Problem	Solution
One or both detectors did not detect a peak.	<p>If the mass spectrometer did not detect a peak, confirm the condition of these mass spectrometer components and settings:</p> <ul style="list-style-type: none">• Ensure that the sample ionizes in the selected ionization mode.• Ensure there is flow to the detector. Tee and/or the probe must not be blocked. Look for flooding in the mass spectrometer and ensure flow emanates from the tip of the probe.• Fittings do not leak.• The polarity parameter settings (+ and –) must be correct.• Mass spectrometer cone must be clean.• Try injecting a known compound such as setup solution or the dye standards. <p>If the UV detector did not detect a peak, confirm the condition of these UV detector components and settings:</p> <ul style="list-style-type: none">• Sample contains an UV chromophore.• Lamp must be turned on and working properly.• Tee must not be blocked.• Wavelength must be set correctly (for example, at 240 nm, not 254) in the Inlet method. <p>If both the UV detector and mass spectrometer fail to detect peaks, confirm the condition of these components and settings:</p> <ul style="list-style-type: none">• Makeup pump must be running and pumping flow.• Sample and solvent must be flowing to both detectors.• Vial must contain the correct sample for the injection.• The injector Z position must be set correctly.

Peak detection problems and solutions (Continued)

Problem	Solution
One or both detectors did not detect a peak. (continued)	If the ELSD detector did not detect a peak, confirm the condition of these components and settings: <ul style="list-style-type: none">• Lamp must be turned on and working properly.• Tee must not be blocked.• Gas must be flowing freely to the nebulizer and the nebulizer and/or drift tube must be clean.
Poor peak shape due to overloaded or degraded column.	Decrease injection volume. Decrease concentration of solubilized sample. Replace the column. Check the solvents and ensure that the mobile phase is composed of high-quality HPLC water and organic solvents.

A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

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Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.



Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)



Warning: (Risk of burn injury from contacting hot surfaces.)



Warning: (Risk of electric shock.)



Warning: (Risk of fire.)



Warning: (Risk of needle puncture.)



Warning: (Risk of injury caused by moving machinery.)



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



Warning: (Risk of exposure to a toxic substance.)



Warning: (Risk of personal exposure to laser radiation.)



Warning: (Risk of exposure to biological agents that can pose a serious health threat.)

Warnings that apply to particular instruments, instrument components, and sample types

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.



Warning: Pressurized nonmetallic, or polymer, tubing can burst. Observe these precautions when working around such tubing:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.



Warning: Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 400 kPa (4 bar, 58 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the HPLC system so that the LC solvent flow stops if the nitrogen supply fails.

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.



Warning: To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.

This warning applies to certain instruments when they are in Operate mode.



Warning: High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid non-lethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

Biohazard warning

This warning applies to Waters instruments that can be used to process material that might contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.



Warning: Waters' instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe good laboratory practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.



Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*.

Caution symbol

The caution symbol signifies that an instrument's use or misuse can damage the instrument or compromise a sample's integrity. The following symbol and its associated statement are typical of the kind that alert you to the risk of damaging the instrument or sample.



Caution: To prevent static charges and resultant circuit damage, do not remove the covers that protect integrated circuit chips.

Warnings that apply to all Waters instruments

When operating this device, follow standard quality control procedures and the the equipment guidelines in this section.



Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Important: Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

Achtung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: eventuali modifiche o alterazioni apportate a questa unità e non espressamente approvate da un ente responsabile per la conformità annulleranno l'autorità dell'utente ad operare l'apparecchiatura.

Atencion: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

注意：未經有關法規認證部門允許對本設備進行的改變或修改，可能會使使用者喪失操作該設備的權利。

注意：未經有關法規認證部門明確允許對本設備進行的改變或改裝，可能會使使用者喪失操作該設備的合法性。

주의： 기기 검교정 담당자의 승인 없이 무단으로 기기를 변경 또는 수정하는 경우에는, 그 기기 운영에 대한 허가가 취소될 수 있습니다.

注意：規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザとしての承認が無効になる可能性があります。



Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Attention: Manipulez les tubes en polymère sous pression avec précaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Éteignez toute flamme se trouvant à proximité de l'instrument.
- Évitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Évitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Vorsicht: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.



Attenzione: prestare attenzione durante l'utilizzo dei tubi di polimero pressurizzati:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Estinguere ogni fonte di ignizione circostante.
- Non utilizzare tubi soggetti che hanno subito sollecitazioni eccessive o son stati incurvati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrato.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamento nei tubi non metallici, riducendo notevolmente la resistenza alla rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點：

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。



警告: 当在有压力的情况下使用管线时, 小心注意以下几点:

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚砜会导致非金属管线膨胀, 大大降低管线的耐压能力。

경고: 폴리머재질의 튜빙을 압력하에서 사용할 때는 다음 사항에 유의하십시오.

- 압력을 받은 폴리머 튜빙 부근에서는 반드시 보호안경을 착용할 것
- 모든 화기의 접근을 금함
- 늘리거나 뒤틀린 튜빙은 사용하지 말 것
- 비금속 튜빙을 테트라히드로퓨란(THF)이나 염산 및 황산과 함께 사용하지 말 것
- 디글로로메탄(methylene chloride)와 디메틸설폭사이드(dimethyl sulfoxide)는 비금속 튜빙을 팽창시켜 쉽게 파열되므로 주의할 것

警告: ポリマーチューブに圧力をかけて取り扱う場合は、次のように注意してください。

- 加圧したポリマーチューブの付近では、常に保護めがねを着用してください。
- 付近の火はすべて消してください。
- 激しい応力やねじれを受けたチューブは使用しないでください。
- テトラヒドロフラン(THF)、濃硝酸、あるいは濃硫酸には、非金属製のチューブを使用しないでください。
- ジクロロメタンやジメチルスルホキシドは非金属製のチューブを膨張させ、チューブの破断圧力を大幅に低下させますので、注意してください。



Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Attention: L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

Vorsicht: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes unter Umständen nicht ordnungsgemäß funktionieren.

Attenzione: l'utente deve essere al corrente del fatto che, se l'apparecchiatura viene usata in un modo specificato dal produttore, la protezione fornita dall'apparecchiatura potrà essere invalidata.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告：使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。

警告：使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。

경고： 제조사가 지정한 것 이외의 방법으로 기기를 사용하는 경우에는, 사용자가 위험으로부터 보호될 수 없는 경우가 발생할 수 있음에 유념하십시오.

警告：ユーザは製造業者が指定していない方法で装置を使用した場合は装置が提供する保護が損なわれることがあるということを承知しているものとします。



Warning: To protect against fire hazard, replace fuses with those of the same type and rating.

Attention: Remplacez toujours les fusibles par d'autres du même type et de la même puissance afin d'éviter tout risque d'incendie.

Vorsicht: Zum Schutz gegen Feuergefahr die Sicherungen nur mit Sicherungen des gleichen Typs und Nennwertes ersetzen.

Attenzione: per una buona protezione contro i rischi di incendio, sostituire i fusibili con altri dello stesso tipo e amperaggio.

Advertencia: sustituya los fusibles por otros del mismo tipo y características para evitar el riesgo de incendio.

警告: 為了避免火災的危險，應更換同種類型及規格的保險絲。

警告: 為了避免火災的危險，應更換同種類型及規格的保險絲。

경고: 화재를 방지하기 위해서는 퓨즈 교체 시 같은 종류, 같은 등급의 것을 사용하십시오.

警告: 火災の危険防止のために、ヒューズの交換は同一タイプおよび定格のもので行ってください。



Warning: To avoid possible electrical shock, disconnect the power cord before servicing the instrument.

Attention: Afin d'éviter toute possibilité de commotion électrique, débranchez le cordon d'alimentation de la prise avant d'effectuer la maintenance de l'instrument.

Vorsicht: Zur Vermeidung von Stromschlägen sollte das Gerät vor der Wartung vom Netz getrennt werden.

Attenzione: per evitare il rischio di scossa elettrica, scollegare il cavo di alimentazione prima di svolgere la manutenzione dello strumento.

Precaución: para evitar descargas eléctricas, desenchufe el cable de alimentación del instrumento antes de realizar cualquier reparación.

警告：要避免觸電，請在修理或保養器材前把電源線拔出。

警告：为避免可能引起得触电危险，在修理前请切断电源连接。







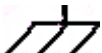
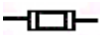

경고: 전기 충격의 가능성을 피하기 위해서는, 기기를 수리하기 이전에 전원 코드를 차단하십시오.

警告：感電の危険性を避けるために、装置の保守を行う前には装置の電源コードを引き抜いてください。

Electrical and handling symbols





Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Protective conductor terminal
	Frame, or chassis, terminal
	Fuse
	Recycle symbol: Do not dispose in municipal waste.

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!

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