Isolera™

User Manual

Biotage®

Safety and Document Conventions

Isolera™ system should only be operated and maintained by trained individuals. Please read this manual carefully before working with the system. To guarantee safe and effective operation it is absolutely necessary to follow all of the instructions in this user manual. All information is believed to be complete and accurate at the time of publication, but is subject to change.

Pay close attention to text with a NOTE or WARNING heading:

NOTE

Note text appears in the manual to provide additional or crucial information about the current topic. It may indicate a situation in which the system may not perform as expected unless specific guidelines are followed.

WARNING

The Warning note indicates a hazard that could result in serious injury to the user and/or damage to the equipment unless good laboratory practices and/or manufacturer's guidelines are followed.

Warranty and Liability

See the "Biotage Terms & Conditions of Sale" document at www.biotage.com.

Download Isolera™ User Documentation

The following Isolera user documentation can be downloaded from the system:

- Isolera™ Installation and Safety, P/N 415796
- Isolera™ User Manual (this publication), P/N 415797
- Isolera™ Quick Guide, P/N 411830
- Isolera™ Safety Translations, P/N 415798
- Isolera™ Assist Quick Guide, P/N 414767
- Isolera™ Dalton 2000 User Manual, P/N 415730

To download the user documentation from the system, connect a USB memory device to the USB port located underneath the touch screen, press **Help**, and then press **Export User Documentation**.

You can download the latest versions of the documents at www.biotage.com. If you have problems downloading, please contact your local Biotage representative.

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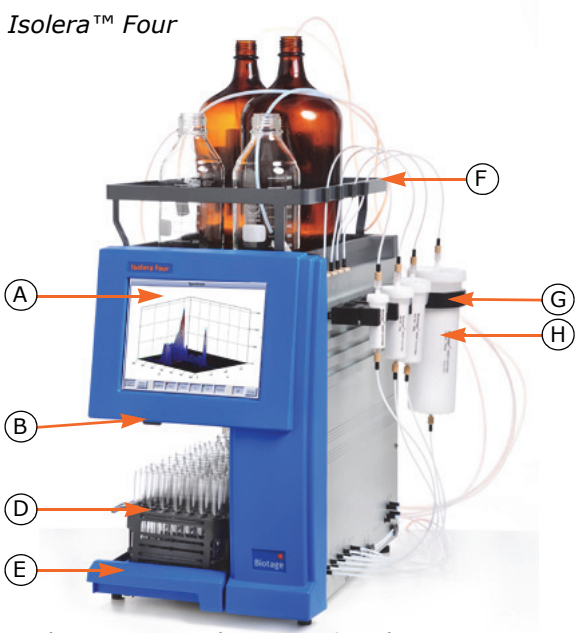
Description and Specifications

1.1 General System Description

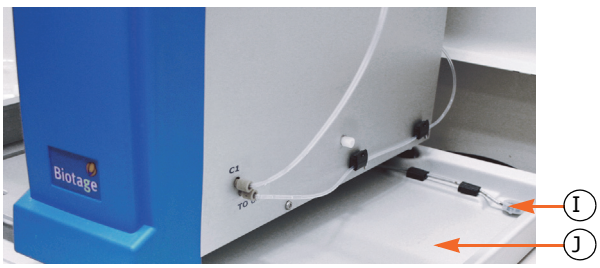
Isolera™ Four



Isolera™ LS

Isolera™ Dalton 2000

Isolera™ One with Biotage® Solvent Detector



A	Touch Screen	H	Biotage® SNAP Cartridge
B	Power Switch and USB Port	I	Solvent Detector (standard on Isolera LS and optional on all other Isolera platforms)
C	Collection Arm	J	Instrument Tray (standard on Isolera LS and optional on all other Isolera platforms)
D	Collection Rack	K	Sample Loading Pump (only on Isolera LS)
E	Collection Tray	L	Isolera™ Dalton Nanolink (optional)
F	Solvent Tray	M	Biotage® Dalton 2000 (optional)
G	Cartridge Holder		

Figure 1-1. Isolera Systems

1.1.1 Basic System Components

The Isolera™ system is a high-performance flash chromatography system, consisting of the following integrated components:

- A touch screen display, which is a solvent-resistant, color LCD screen with a resolution of 800 x 600 pixels. It serves both as a display and as the system's input device via on-screen touch controls.
- A fraction collector, which collects fractions into a wide variety of collection racks and vessels.
- A pump module, which directs liquid flow through the system. A default flow rate is specified for each cartridge but, if desired, the flow rate can be changed. If the flow rate is increased, the system will start the run at the default flow rate and then regulate towards the flow rate defined in the method. Note that the system regulates on both flow rate and pressure. If 90% of the maximum allowed pressure is reached before the defined flow rate, the flow rate at 90% pressure will be used.
- An internal detector, which provides the system with information on the light absorbance of the solvents and samples passing through the detector flow cell. (It is also possible to connect an external detector, e.g. Biotage® ELSD-A120.)

1.1.2 System Configurations

There are four system platforms available: Isolera™ Prime, Isolera™ One, Isolera™ Four, and Isolera™ LS (Large Scale). The differences are listed in the table below.

Table 1: System Differences

	Isolera Prime	Isolera One	Isolera Four	Isolera LS
Solvent Detector:	Optional	Optional	Optional	Standard
Sample Loading Pump:	No	No	No	Yes
Collection Trays:	1	1 or 2*	1 or 2*	2
Solvent Inlets:	2	4	4	4
Cartridge Positions:	1	1	4	1
Waste Channels:	1	1	4	1
Pump Type:	Single-piston	Dual-piston	Dual-piston	Dual-piston
Flow Rate:	1 to 100 ml/min	1 to 200 ml/min	1 to 200 ml/min	50 to 500 ml/min
Internal Detector:	UV†	UV or UV-VIS†	UV or UV-VIS†	UV or UV-VIS†
Mass Detector:	No	Optional	Optional	No

* One collection tray is standard, but by expanding the fraction collector, it is possible to use two collection trays (see Figure 1-1) at the same time. The maximum collection volume (with no rack change) is then 9600 ml instead of 4800 ml (with the 480 ml bottle rack). These systems are called EXP systems (Isolera One EXP and Isolera Four EXP).

† The internal detector is available in two models: 1) 200-400 nm (UV) and 2) 200-800 nm (UV-VIS). Note that the UV-VIS detector is not available with Isolera Prime.

An Isolera instrument tray with solvent detector is available for safe, unattended operation. The sample loading pump, on Isolera LS, can be used for injection of liquid samples into cartridges. This is useful when using large cartridges and sample volumes.

Biotage® Dalton 2000 and Isolera™ Dalton Nanolink

Biotage® Dalton 2000 and Isolera™ Dalton Nanolink are designed for use together with Isolera One or Isolera Four with a Dalton 2000 software license when purifying compounds with little or no UV absorption such as carbohydrates, steroids, lipids, and terpenes, or when high specificity is desired. The Dalton 2000 software license includes all the functionality of the Isolera Spektra software upgrade package (see page 1-6) as well as support for Biotage Dalton 2000. With this system, called Isolera™ Dalton 2000, you can detect, fractionate, and analyze chemical mixtures using flash chromatography and mass spectrometry or light absorbance.

Biotage Dalton 2000 is a compact single quadrupole mass detector with positive and negative polarity switching. Biotage Dalton 2000 uses an atmospheric pressure chemical ionization (APCI) or electrospray ionization source (ESI) to ionize substances, which can then be identified by their mass-to-charge ratios (m/z). Biotage Dalton 2000 allows you to analyze samples and automatically use the m/z ratio(s) of the identified substance(s) for collection and fractionation.

For more information, see the "Isolera™ Dalton 2000 User Manual" (P/N 415730).

Figure 1-2. Isolera Dalton 2000

1.1.3 Programmed Functions

The Isolera system can be programmed to perform the following functions:

- Generate user-defined elution gradients
- Store and implement a large number of independent chromatographic methods
- Control and collect fractions based on light absorbance or mass (if using Biotage Dalton 2000), or on a signal from an external detector
- Collect fractions into a wide variety of collection racks and vessels
- Produce detailed, printable purification reports

1.1.4 User Documentation

The following Isolera user documentation can be downloaded from the system:

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- Isolera™ Dalton 2000 User Manual, P/N 415730

To download the user documentation from the system, connect a USB memory device to the USB port located underneath the touch screen, press **Help**, and then press **Export User Documentation**.

You can download the latest versions of the documents at www.biotage.com. If you have problems downloading, please contact your local Biotage representative.

Online Help

To view context-sensitive help, press the **Help** button found in the right-hand panel. The following buttons are available in the help window:

⚠**:** View the safety requirements for the Isolera system. You must observe all these safety requirements when installing and operating the Isolera system. Failure to install or use the system in a manner specified by Biotage may result in personal injury and/or equipment damage.

: View instructions for tasks that can be performed in the present software mode (the Chemistry, Data Administration, or System mode).

: View information on the tabs and buttons in the right-hand panel.

Export User Documentation: Download the Isolera user documentation on a connected USB memory device.

▼/▲**:** Scroll down or up through the text (when a help window has more than one screenful of information).

Back: Return to the last help topic that you viewed.

Close: Exit the help.

1.1.5 Accessories

Cartridges

The Isolera system is designed to operate with Biotage® SNAP, Biotage® SNAP Ultra, Biotage ZIP® and Biotage ZIP® Sphere cartridges, which have easy-to-use Luer-lok™ connections. FLASH+® and ISOLUTE® cartridges can also be used but may require tube adapters and a separate ring stand.

All SNAP and SNAP Ultra disposable cartridge components are made from inert polypropylene, which reduces leachables that can contaminate purified compounds.

SNAP cartridges are available packed with silica (Biotage® KP-Sil), C18 (Biotage® KP-C18-HS), or NH (Biotage® KP-NH) media. SNAP 750g and 1500g are available packed with silica (KP-Sil), C18 (KP-C18-HS), or NH (Biotage® KP-NH).

ZIP cartridges are all packed with silica (KP-Sil). ZIP Sphere cartridges are packed with Biotage® KP-Sphere™, a proprietary spherical silica which is manufactured under a new process that delivers 40% more surface area.

SNAP Ultra cartridges deliver double the purification capacity of other flash cartridges by utilizing Biotage® HP-Sphere™ or Biotage® HP-Sphere™ C18, small particle, 25 µm spherical silica with a 40% increase in surface area. The higher surface area provides twice the loading capacity of lower surface area silicas. This improved load capacity means that a smaller SNAP Ultra cartridge can be used to replace a more expensive and larger competitive cartridge.

Note that SNAP and SNAP Ultra 10g to 30g and ZIP and ZIP Sphere 5g to 45g cannot be used on Isolera LS, and SNAP 1500g is not recommended for use on Isolera Prime, Isolera One, Isolera Four, or Isolera Dalton 2000. SNAP 750g, Flash 75M, and Flash 75L are not recommended for use on Isolera Prime, or Isolera Dalton 2000 when using the mass detector (the flow rate is then limited to 100 ml/min).

It is important that you do not exceed the recommended flow rates for flash cartridges (this information can be found in the flash cartridge documentation) nor allow flow at higher rates than specified through any Samplet®, dry load vessels, or pre-cartridges that you may be using. This can increase the risk of static build-up. Please read and follow the safety precautions against static electricity in the "Isolera™ Installation and Safety" document (P/N 415796) that is delivered with the system.

Figure 1-3. SNAP Ultra 50g Cartridge and Samplet (Left) and SNAP Ultra C18 Cartridges (Right)

Sample Loading Methods for Biotage® SNAP and Biotage® SNAP Ultra Cartridges

Biotage SNAP and SNAP Ultra cartridges offer several types of loading techniques including three internal dry loading options.

Liquid Loading

Liquid samples can be:

- Injected into the SNAP or SNAP Ultra cartridge inlet Luer fitting using a syringe or, when using an Isolera LS system, the sample loading pump.
- Loaded onto the frit using a pipette (not possible when using SNAP 750g or 1500g). Not recommended when using the optional mass detector (Biotage Dalton 2000).

Samples should be dissolved at the highest concentration possible in the weakest solvent. Use of a strong, polar solvent will compromise purification efficiency.

Internal Dry Loading

If internal dry loading is to be used (not possible with SNAP 750g and larger cartridges), the cartridge insert must be removed. Internal loading can be achieved using:

- Prepacked Samplet cartridges, which are inserted into the SNAP or SNAP Ultra cartridge. Prior to insertion, the solvent should be evaporated in open air or in vacuum. Prepacked Samplet cartridges are available in the same media types that are available for the cartridges.
- Empty Samplet cartridges.
  If media other than those available in prepacked Samplet cartridges is required (e.g. scavenger or catch-and-release resin, or other media), it can be easily packed into empty Samplet cartridges.
- Bulk loading.
  Dried, preadsorbed sample on media is added directly to the top of the cartridge. Specific media amounts are required; see the documentation supplied with the cartridge.

External Dry Loading

Dried, preadsorbed sample on media can be loaded externally using a DLV-030, DLV-070, DLV-500 (for SNAP 750g and larger cartridges). They all attach directly to the SNAP or SNAP Ultra cartridge inlet Luer fitting; DLV-500 uses a tube connection. For further information, see the user documentation supplied with DLV-30 and DLV-70 (P/N 413107), and DLV-500 (P/N 412625).

Sample Loading Methods for Biotage ZIP® and Biotage ZIP® Sphere Cartridges

Liquid samples can be injected into the ZIP and ZIP Sphere cartridge inlet Luer fitting using a syringe or, when using an Isolera LS system, the sample loading pump.

Dried, preadsorbed sample on media can be loaded externally using a dry load vessel (DLV-030 or DLV-070).

Collection Racks and Vessels

The Isolera software comes with a preconfigured list of Biotage collection racks. The vessel size range is from 9 ml test tubes to 480 ml bottles. You may add other racks to this list (see "Add a Rack Type" on page 2-6).

Rack Type	Vessel Diameter x Length	Vessel Volume	No of Vessels	No of Racks per Tray
13 x 100 mm*	13 mm x 100 mm	9 ml	48	4
16 x 100 mm*	16 mm x 100 mm	14 ml	35	4
18 x 130 mm	17.5 mm x 130 mm	18 ml	28	4
16 x 150 mm*	16 mm x 150 mm	22 ml	35	4
18 x 150 mm	18 mm x 150 mm	27 ml	28	4
25 x 150 mm	25 mm x 150 mm	45 or 53 ml	15	4
120 ml	120 ml bottles	120 ml	6	4
240 ml	240 ml bottles	240 ml	18	1
480 ml	480 ml bottles	480 ml	10	1
Funnel rack†	Any vessel up to 10 l	Up to 10 l	32	1

* Not recommended on Isolera LS, and therefore not included in the preconfigured list for Isolera LS.

† Only included in the preconfigured list for Isolera LS.

Figure 1-4. Typical Racks Used with Isolera

Isolera™ LS Funnel Rack Kit

The maximum collection volume for Isolera LS can be increased from 9.6 liters up to 320 liters by using a funnel rack kit from Biotage. The funnel rack kit comes with two racks (16 positions each) and a cart with wheels that holds the Isolera LS system and the collection vessels. For more information, please contact your local representative.

Isolera™ Spektra Upgrade

Isolera Spektra is a software upgrade package that features a TLC-to-Step gradient calculator, λ-all detection, baseline correction, 3D absorbance spectrum for the whole detector range, and real-time PDA scanning.

Biotage® ELSD-A120

ELSD-A120 (evaporative light-scattering detector) is a universal detector that can be used with Isolera One and Isolera Four systems when purifying compounds with little or no UV absorption such as carbohydrates, steroids, lipids, and terpenes.

ELSD-A120 provides intelligent method design which enables the chemist to independently set nebulizer and evaporator temperatures for a particular compound or compound class. Independent temperature control helps ensure that all compounds are detected.

Figure 1-5. Biotage ELSD-A120

Order Accessories

To order accessories, please contact your local representative. A condensed accessory list is available on page 5-1.

1.2 Software Description

1.2.1 Main Menu

The system's main menu provides access to the three different software modes:

- **New:** Set up and run a purification using the Assist Workflow. The user is guided through the workflow. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.
- **Chemistry:** Set up, control, monitor, and review a purification.
- **Data Administration:** Administrate cartridge types, methods, rack types, results, solvents, and user accounts.
- **System:** Change detector, network, reporting, and runtime settings, set the system clock, set the language used in the Chemistry mode, enable the λ-all detection mode and the TLC to Step Gradient editor (requires an Isolera Spektra or Dalton 2000 software license), enable the Assist Workflow, calibrate the fraction collector, internal detector, and mass detector (optional), release stuck check valves, clean the collect valve, back up and restore the database, export system logs, view and reset usage statistics, etc.

The main menu also provides software version information through its **About** button, access to context-sensitive help through its **Help** button, and contains the **Shut Down** button, which is used in the system shutdown process.

NOTE

Only users with system owner privilege can log into the Data Administration and System modes.

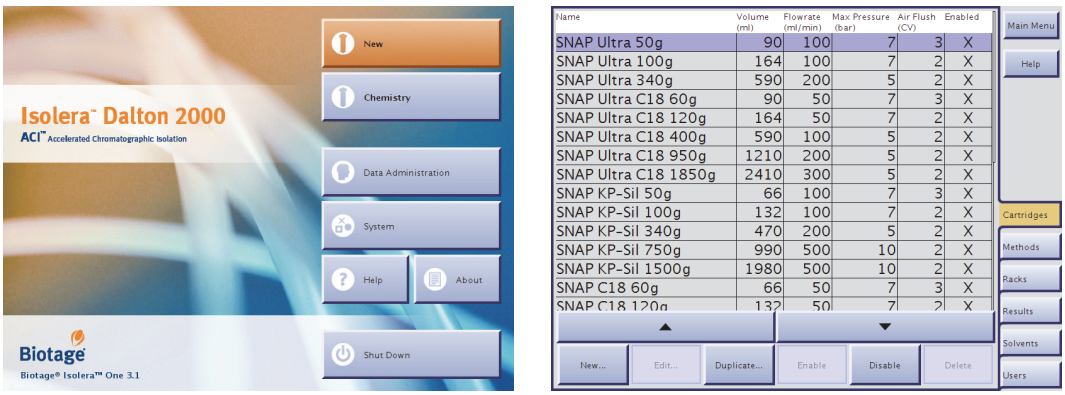


Figure 1-6. Main Menu and Data Administration Mode (Isolera LS Shown)

1.2.2 Data Administration Mode

In the Data Administration mode, the software is divided into six (6) tabs that can be accessed in the right-hand panel (see Figure 1-6). Here is a short description of what you can do at each tab:

- **Cartridges:** Add, edit, and delete user defined cartridge types. It is also possible to select the cartridge types that will appear in the cartridge selection list when setting up a method in the Chemistry mode.
- **Methods:** Export, import, and delete methods. It is also possible to set default methods, i.e. a list of methods that the users can choose from when creating a new method at the Method tab in the Chemistry mode.
- **Racks:** Add, edit, and delete user defined rack types. It is also possible to select the rack types that will appear in the rack selection list when setting up a method in the Chemistry mode.
- **Results:** Export and delete result records, and clean up the system's database. The records can be exported as CSV files (comma-delimited text files), PDF files, and XML files (including all raw data), or DATX files (including the mass detector data).
- **Solvents:** Add, edit, and delete user defined solvents. It is also possible to select the solvents that will appear in the solvent selection list when setting up a method in the Chemistry mode.
- **Users:** Add, edit, and delete user accounts.

Buttons in the Right-hand Panel

The following buttons are available in the right-hand panel:

- **Main Menu:** Go to the main menu to enter the System mode, enter the Chemistry mode (either by pressing Chemistry or New to go through the Assist Workflow), view software version information, or shut down the system.
- **Help:** View context-sensitive help and download user documentation.

1.2.3 System Mode

In the System mode, the software is divided into six (6) tabs that can be accessed in the right-hand panel (see Figure 1-7). Here is a short description of what you can do at each tab:

- **Detector:** Enable or disable the internal detector, an external detector (e.g. Biotage ELSD-A120), mass detector (optional), and a solvent detector (standard on Isolera LS and optional on all other Isolera system platforms). The mass detector and external detector cannot be connected at the same time.
- **General:** Set the system clock, the pressure unit to be used by the system (bar or psi), and the language used in the software's Chemistry mode, enable the λ-all detection mode and the TLC to Step Gradient editor (requires an Isolera Spektra or Dalton 2000 software license), enable the Assist Workflow, and enable or disable the mouse pointer.
- **Maintenance:** Calibrate the fraction collector and internal detector, release stuck check valves, clean the collect valve, back up and restore the database, export the system configuration and logs, restore the system configuration, and view and reset usage statistics. If using an Isolera Dalton 2000 system, you can also calibrate and pump down or vent the mass detector, open a wizard to replace the microfilter, and reset the counter for the active splitter switches.
- **Network:** Configure a network connection and set up file sharing so that reports, methods, backups, and the system configuration and logs can be saved in a share folder on your network.
- **Reporting:** Connect a network printer with postscript support or a USB printer to the system. It is also possible to enable or disable automatic printing, saving (in the share folder), and e-mailing (to the e-mail address specified in the user's account) of purification reports.
- **Runtime:** Specify how flushes are performed, change collection and fractionation parameters, and enable or disable automatic rack allocation, Peak Mode (in the status view), auto extend of the gradient, audible alarm (e.g. when rack change is needed), solvent and waste monitoring, and vessel, solvent, and waste estimation.

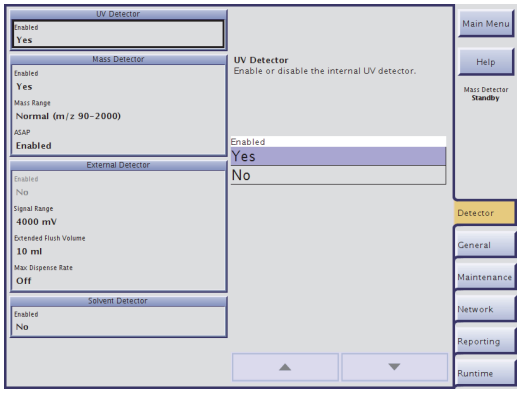


Figure 1-7. System Mode (Isolera Dalton 2000 Shown)

Buttons in the Right-hand Panel

The following buttons are available in the right-hand panel:

- **Main Menu:** Go to the main menu to enter the Data Administration mode, enter the Chemistry mode (either by pressing Chemistry or New to go through the Assist Workflow), view software version information, or shut down the system.
- **Help:** View context-sensitive help and download user documentation.

1.2.4 Chemistry Mode

Enter the Chemistry mode either by pressing **New** (to go through the Assist Workflow) or **Chemistry** in the main menu. In the Chemistry mode, the software is divided into four (4) tabs that can be accessed in the right-hand panel (see Figure 1-8). Here is a short description of what you can do at each tab:

- **Method:** Create, open, and edit a method, and assign it to a cartridge mounted on the system, select the rack position(s) to be used, and start or queue up* the purification.
- **Status:** View information on a cartridge mounted on the system, monitor and edit the purification in progress, enable injection of a liquid sample, and start or queue up* a purification after a finished equilibration step. It is also possible to enable or disable the Collect All mode, manually instruct the system to switch to a new collection vessel, start and stop collecting through the waste channel, start and end an isocratic segment, change the processing order of queued purifications*, and manually end (or abort) and remove a purification.
- **Results:** Search and view the results of all purifications stored in the system's database, on a USB memory device (if connected), or in a share folder on your network (if connected). Result reports can be printed to a network printer with postscript support or a local USB printer and saved as PDF files on a USB memory device or in a share folder on your network. It is also possible to create a new method with the same purification parameters as the ones used in a previous purification. If desired, the system can help you to optimize the gradient to isolate one of the peaks and reduce the amount of solvent used.
- **Setup:** Assign solvents to the solvent inlets, set the solvent and waste reservoirs' volumes (if monitoring of solvent and waste are enabled), prime the solvent inlets and, when using an Isolera LS system, control the sample loading pump. It is also possible to flush a cartridge with air to empty it of remaining solvents† and purge a cartridge to release any remaining pressure before unloading it. If using an Isolera Dalton 2000 system, you can also prime the makeup solvent inlet, flush the mass detector and Isolera Dalton Nanolink tubing, perform a mass detector function test, and view the mass detector spectrum.

* Purifications can be queued up when using a system with four cartridge positions.

† The Air Flush feature is not available when using an Isolera Prime system.

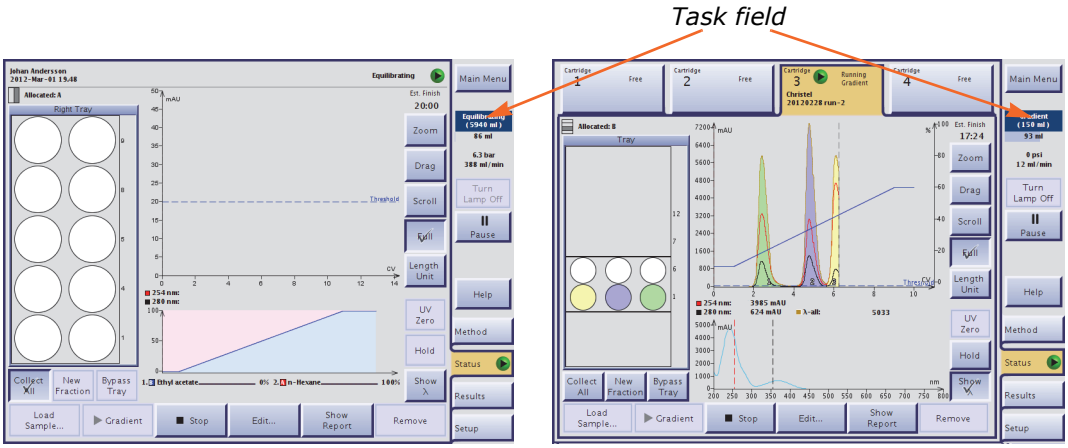


Figure 1-8. Chemistry Mode (Isolera LS and Isolera Four EXP with Isolera Spektra License Shown)

NOTE

When using the Assist Workflow, some of the features are not available for a user with the basic workflow editor enabled. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.

Status and Task Field

The system can be idle, paused (), or processing (). When the system is processing, the following tasks can be in progress:

- **UV ON:** The system turns on the detector lamp(s). If using an Isolera Dalton 2000 system, the mass detector and makeup flow are activated during this phase.
- **Eq. Flush:** Before an equilibration is started, the system runs an equilibration flush to empty the solvent inlets of solvents used in the previous purification and fills them with new solvents.
- **Equilibrating:** The system is running an equilibration.
- **UV Warm-up:** The UV lamp is being warmed up. This takes approximately 7.5 minutes.
- **UV Zero:** The system is setting the UV zero level.
- **Baseline Det.:** The system is measuring the solvent absorbance of the gradient so that the baseline can be subtracted from the signal during the gradient run. For more information on UV baseline correction, see "Specify Collection Parameters at the Collection Tab" on page 4-9. (Only when using systems with an Isolera Spektra or Dalton 2000 software license installed.) If using an Isolera Dalton 2000 system, the mass detector baseline is also calculated.
- **Gradient Flush:** After a baseline detection, the system flushes the system with the solvent mix used in the start of the gradient.
- **Sample Pump:** The sample pump is in use. (Only when using Isolera LS.)
- **Gradient:** The system is running a purification. If the system is not collecting or is collecting on the tray, the background color is blue. If fractions are collected through the waste channel (i.e. the Bypass Tray option is enabled), the background color is magenta.
- **Line Flush, Air Flush, Purge, and Detector Flush:** After the gradient purification stage is completed, the system performs enabled flushes (in the System mode) and a system decompression process (Purge). It is also possible to manually instruct the system to perform an Air Flush and a Purge. Note that the Air Flush feature is not available with Isolera Prime. The background color is green when a flush is collected on the tray and yellow when it goes to waste.
- **MD Finishing:** The mass detector data is saved in the system's database. (Only when using Isolera Dalton 2000.)
- **Prime:** The system is priming the solvent inlet(s).

When using an Isolera Dalton 2000 system, the mass detector status is shown above the **Help** button:

- **Initializing:** The mass detector is initializing.
- **Pumping:** The mass detector is pumping down the vacuum.
- **Standby:** The vacuum system is on, but all other parts of the mass detector are powered off. The mass detector automatically goes into standby mode after two hours of inactivity.
- **Operate:** Mass detector voltages and temperatures are at operating level, but no acquisition is in progress.
- **Acquiring:** The mass detector is collecting data.
- **Paused:** Acquisition has been paused, but the mass detector is in acquiring mode.
- **Fault:** The mass detector reports a fault condition.

Buttons in the Right-hand Panel

The following buttons are available in the right-hand panel:

- **Main Menu:** Go to the main menu to enter the Data Administration mode, enter the System mode, view software version information, or shut down the system.
- **Turn Lamp On/Skip Warm Up/Turn Lamp Off:** Manually turning the detector lamp(s) on and off. The UV lamp should be turned on approximately 7.5 minutes before operation so it can warm up and stabilize. When the lamp(s) has/have been manually turned on, the Turn Lamp On button toggles to a Skip Warm Up button, which can be used when you want to skip the UV lamp warm-up period and begin the run. However, if the UV lamp has not reached operating temperature, purification results may not be optimal. To prolong lamp life, the lamp(s) is/are turned off automatically if the system is idle (no purification is started and no user interaction is detected) for approximately two hours. Note that the Skip Warm Up button is disabled for the first 45 seconds of the UV lamp warm-up period.
- **Pause/Resume:** To pause a purification, press the Pause button. The collection arm returns to its home position (the inner right corner), the system is paused, and the Pause button is toggled to a Resume button. To resume the operation, press the Resume button.

WARNING

Keep your hands out of range of the collection arm until it has stopped moving (with the collection arm in the inner right corner).

- **Help:** View context-sensitive help and download user documentation.

1.2.5 Method Tab (Chemistry Mode)

At the **Method** tab, in the software's Chemistry mode, you can create, open, and edit a method, and assign it to a cartridge mounted on the system, select the rack position or positions to be used, and start or queue up* the purification.

There are different ways of creating a new method:

- **Create a new method.** If using the method wizard, it will guide you step-by-step through the setup of a run. You can also use the TLC to Linear Gradient editor or the TLC to Step Gradient editor (requires an Isolera Spektra or Dalton 2000 software license) to calculate a purification gradient and get cartridge and sample load recommendations. After setting up the method, you can save it by entering a unique method name.
- **Base the new method on an existing method.** You can either base the method on one of the preconfigured Biotage methods or a method created and saved in the system's database, on a USB memory device, or in a share folder on the network. After editing, you can save the method under a new name by entering a unique method name. The original method remains unaltered under its original name.
- **Base the new method on a previous purification (at the Results tab).** After editing the purification parameters, you can save it by entering a unique method name.

* Purifications can be queued up when using a system with four cartridge positions.

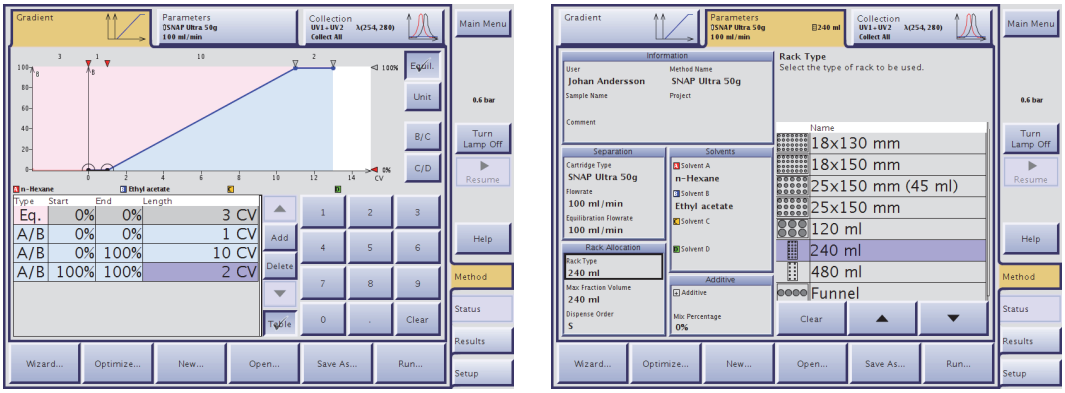


Figure 1-9. Method Tab (Isolera LS Shown)

NOTE

You do not have to save your method to be able to run it.

1.2.6 Status Tab (Chemistry Mode)

At the **Status** tab, in the software's Chemistry mode, you can:

- View information on a cartridge mounted on the system. Available information is: user, sample name, status, allocated rack(s), and the estimated time when the system will have completed the purification.
- Enable injection of a liquid sample using a syringe or the sample loading pump (the Load Sample button) and start or queue up* a purification after a finished equilibration step. (Only Isolera LS is equipped with the sample loading pump.)
- Change the processing order of queued purifications.*
- Start and end an isocratic segment (the Hold button).
- Enable or disable the Collect All mode and manually instruct the system to switch to a new collection vessel.
- Monitor and edit a purification in progress. While a purification is running, the programmed gradient and a dynamic chromatogram are displayed. The chromatogram can be magnified, dragged to the desired position, etc. If an Isolera Spektra or Dalton 2000 software license has been installed on your system, the absorbance spectrum for the whole detector range can be viewed in the gradient view by pressing the Show λ button. The gradient is then displayed in the chromatogram.
- Start and stop collecting through the waste channel (the Bypass Tray button).
- Stop – end (the enabled flushes and a purge will be performed) or abort – a purification.
- Clear a cartridge position in the software (the Remove button).

* Purifications can be queued up when using a system with four cartridge positions.

WARNING

When pressing the ■ Stop button, keep your hands out of range of the collection arm until it has stopped moving (with the collection arm in the inner right corner).

NOTE

If you want to end or abort a purification in progress, press the ■ Stop button.

It is not possible to edit a purification during the flushes and purge.

When using a system with four cartridge positions, queued equilibrations take priority over queued gradient runs.

Cartridge Status

The status of a cartridge can be one of the following:

- **Free:** No purification is assigned to the cartridge.
- **Preparing UV Detector:** The UV lamp is being warmed up in order for the signal to be accurate and stable.
- **Queued Equilibration/Gradient Run*:** The equilibration or gradient run is queued and will be performed when the system has completed the task(s) in progress.
- **Equilibrating:** The system is running the isocratic or gradient equilibration. If gradient equilibration is enabled for the used cartridge, gradient equilibration is used when the percentage of the strongest solvent is above 10% in the initial solvent mix. When the percentage is less or equal to 10%, isocratic equilibration is used. Note that gradient equilibration is not applicable when using an Isolera Prime system.
- **Equilibration Finished:** The equilibration is completed. Press the Load Sample button to load the liquid sample and then the ▶Gradient button to start or queue up* the gradient run.
- **Running Gradient:** The system is either preparing for the run (e.g. performs a UV Zero) or running the gradient. For more information on the task being performed, see the task field in the right-hand panel.
- **Finished:** The purification is completed.
- **Manually Extended:** The purification was manually extended after it was completed. Press the ▶Gradient button to start or queue up* the extended run.

* Purifications can be queued up when using a system with four cartridge positions.

NOTE

When using a system with four cartridge positions, queued equilibrations take priority over queued gradient runs.

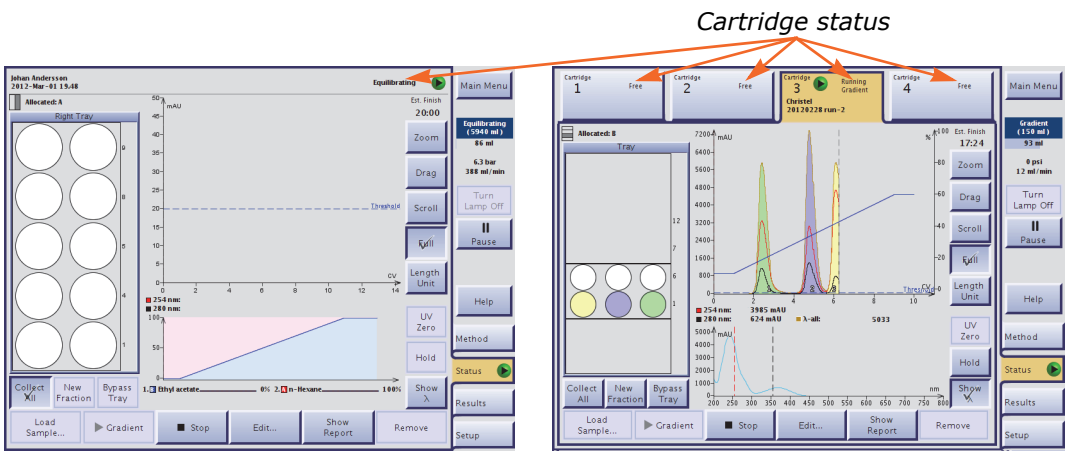


Figure 1-10. Status Tab (Isolera LS and Isolera Four EXP with Isolera Spektra License Shown)

Color Legends

Fractions

Fractions that are already collected can be located by matching their colors on the chromatogram with the vessel colors in the tray overview:

- ○ = The vessel is allocated but has not been used.
- ●, ●, ●, and ● = The vessel contains a fraction.
- ● = The vessel contains flush liquid.

If fractions are collected through the waste channel (i.e. the Bypass Tray mode is enabled), the fractions are colored in magenta in the chromatogram and numbered W1, W2, and so on.

Light Absorbance

Light absorbance and the Start Threshold (if defined) are displayed in the chromatogram using the following colors:

- = Absorption measured by the internal detector, channel 1.
- = Absorption measured by the internal detector, channel 2.
- = Absorption measured by the internal detector for the whole detector range. (Only available with an Isolera Spektra or Dalton 2000 software license.)
- = Absorption measured by the external detector (when connected).
- = The defined threshold in mAU.

The defined wavelengths and the measured absorption in mAU are displayed underneath the chromatogram. If the signal is used for collection, the frame around the color icon is white. If it is used for monitoring, the frame is black.

If an Isolera Spektra or Dalton 2000 software license has been installed on your system, the absorbance spectrum for the whole detector range ( ) can be viewed in the gradient view by pressing the **Show λ** button.

Mass

When using an Isolera Dalton 2000 system, the XICs are displayed in the chromatogram using the following colors:

- = Ion 1.
- = Ion 2.
- = Ion 3.
- = Ion 4.

The specified m/z values are displayed underneath the chromatogram. If the signal is used for collection, the frame around the color icon is white. If it is used for monitoring, the frame is black.

Positive and negative TIC signals are displayed in the chromatogram as blue and red dotted lines (····· and ·····).

Gradient Graph and Table

The colors used in the gradient graph and table are:

- = Solvent A and the equilibration line (only in the graph).
- = Solvent B and the gradient line in segments with Solvent A and B (only in the graph).
- = Solvent C and the gradient line in segments with Solvent B and C (only in the graph).*
- = Solvent D and the gradient line in segments with Solvent C and D (only in the graph).*

The gradient graph shows the defined gradient (excluding the additive). The actual percentage of pumped solvents (including the additive*, if used) are displayed underneath the graph.

* Not available when using an Isolera Prime system.

1.2.7 Results Tab (Chemistry Mode)

Purifications that are processed on the system are stored as individual records in the system's database. The records can, if desired, be saved by the user as XML files (including all raw data) and SPECTRUM files (with the raw 3D UV spectrum) on a USB memory device or in a share folder on the network. If the run was performed using the mass detector (optional), you will also get DATX files with the mass detector data.

At the **Results** tab, in the Chemistry mode, it is possible to search and view the results of all purifications stored in the system's database, on a USB memory device (if connected), or in a share folder on your network (if connected). Two result reports are available for each purification: 1) an archive report with the spectra from the mass analysis (if performed, only on Isolera Dalton 2000), purification details, a chromatogram, a 3D absorbance spectrum for the whole detector range (requires an Isolera Spektra or Dalton 2000 software license), TLC data (if entered in the TLC to Step Gradient editor) and 2) a fraction report. These reports can be printed to a network printer with postscript support or a local USB printer and saved as PDF files on a USB memory device or in a share folder on your network.

At the **Results** tab, it is also possible to create a new method with the same purification parameters as the ones used in a previous purification. If desired, the system can help you to optimize the gradient to isolate one of the peaks and reduce the amount of solvent used.

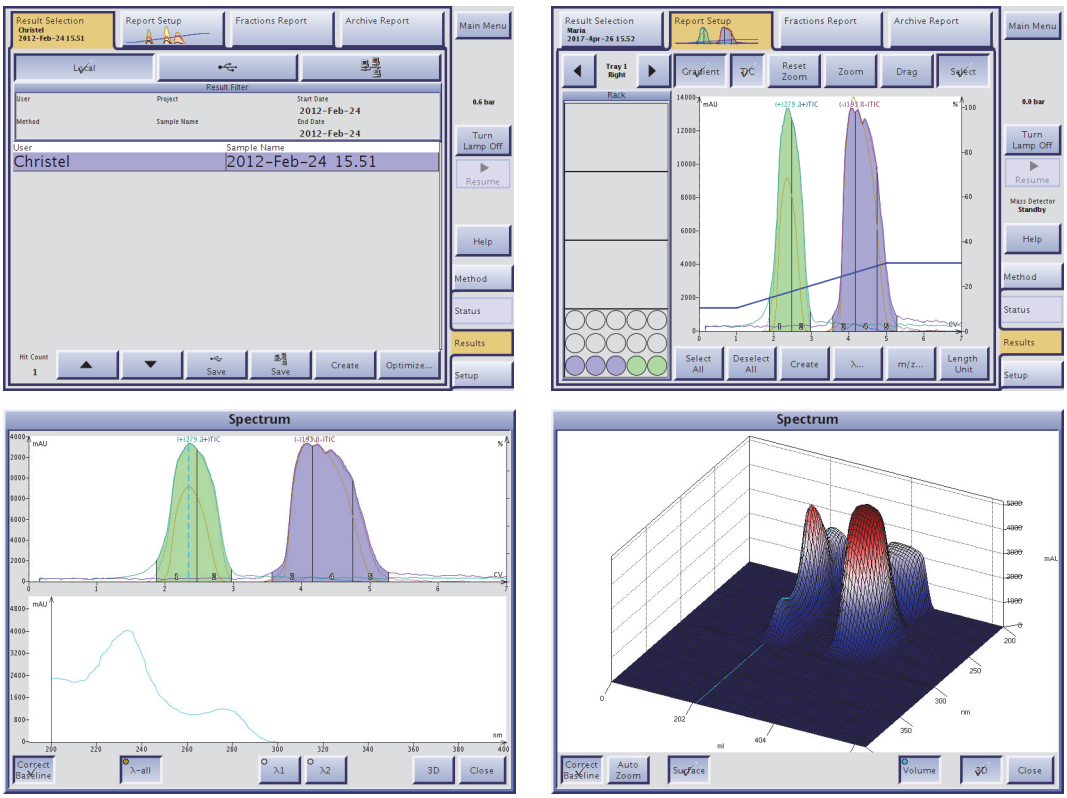


Figure 1-11. Results Tab with Isolera Spektra License

Result and Fraction Selection

All records that match the specified filter settings are listed at the **Result Selection** tab. Select the record you want to view; both a fraction and an archive report are displayed. If a record that you want to view is not listed, change the filter settings by pressing the **Result Filters** field. Possible search criteria are 1) user name, 2) project name, 3) method name, 4) sample name, and 5) date when the purification was run. Note that the result filter is case sensitive.

At the **Report Setup** tab, you can select the fractions to be displayed in the reports. The colors used in the tray overview are:

- = The vessel has not been used for the viewed purification.
- = The vessel is deselected.
- = The vessel is selected and contains flush liquid.
- , , , and =The vessel is selected and contains a fraction. (The vessel color corresponds with the fraction color in the chromatogram.)

If an Isolera Spektra or Dalton 2000 software license has been installed on your system, you can view 2D and 3D absorbance spectra for the whole detector range by pressing the **λ...** button. Here you can also modify how the 3D spectrum is presented in the archive report. If the run was performed using a mass detector (Biotage Dalton 2000), you can view the chromatogram and the negative and positive mass spectra by pressing the **m/z...** button.

To create a method with the same purification parameters as the ones used in the selected record, press the **Create** button at the **Result Selection** or **Report Setup** tab, or if you want help optimizing the gradient, press the **Optimize** button.

1.2.8 Setup Tab (Chemistry Mode)

At the **Setup** tab, in the software's Chemistry mode, you can:

- **Assign a solvent to each of the solvent inlets** on the right side of the system. When a purification is run, the software references the solvent assignments to determine which solvent inlets that are connected to the solvents used in the method. Therefore, it is important that the solvents be assigned accurately.
- **Enter the capacities and current fluid levels for the waste and solvent reservoirs** each time you empty a waste reservoir or replenish a chromatography solvent. The system will issue a warning when it is time to replenish a chromatography solvent or empty a waste reservoir. (This feature is only available if monitoring is enabled, see page 3-11.)
- **Prime the solvent inlets** to remove air bubbles from the pump and solvent inlets or empty the solvent inlets of solvents used in the previous purification and fill them with new solvents.
- **Prime the makeup solvent inlet.** (Only Isolera Dalton 2000.)
- **Flush the mass detector and Isolera Dalton Nanolink tubing.** (Only Isolera Dalton 2000.)
- **Perform a mass detector function test.** (Only Isolera Dalton 2000.)
- **View the mass detector spectrum.** (Only Isolera Dalton 2000.)
- **Flush a cartridge with air** to empty it of remaining solvents before unloading it. This feature is not available with Isolera Prime.
- **Purge a cartridge** to release any remaining pressure before unloading it.
- **Control the sample loading pump** when cleaning the pump tubing or emptying the tubing of solvent and/or sample before replacing it. (Only Isolera LS has the sample loading pump.)

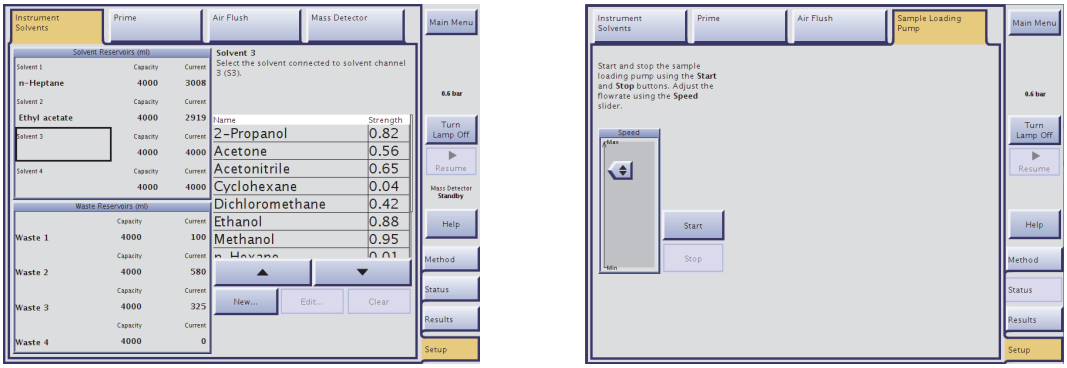


Figure 1-12. Setup Tab (Isolera Four and Isolera LS Shown)

1.2.9 Isolera Remote Viewer

With the system connected to your network, it is possible to perform the following tasks through a standard web browser (through the Isolera Remote Viewer feature):

- Create a new method. If desired, the method can be based on a previous purification found in the Results view.
- Check the status of the system and the progress of a purification.
- Instruct the system to start and stop collecting fractions by clicking the **Collect All** button (✓ = fractions are collected).*
- Instruct the system to switch to a new collection vessel by clicking the **New Fraction** button.*
- Instruct the system to start and end an isocratic segment by clicking the **Hold** button (✓ = isocratic hold is enabled).*
- Instruct the system to pause the purification in progress by clicking the **Pause** button. For safety reasons, it is only possible to pause and not resume a run from your office.
- Instruct the system to set the current UV level to 0 (zero) AU by clicking the **UV Zero** button.*
- View, print, and export purification results.

* You must be logged in as the user defined in the purification run.

To access the Isolera Remote Viewer, see page 4-32.

Figure 1-13. Isolera Remote Viewer (System with Four Solvent Inlets and Spektra License Shown)

1.3 Solvent Specifications

WARNING

Many solvents are considered to be hazardous to humans and the environment, so take appropriate safety precautions when using them. Comply with Safety Data Sheets (SDS) and any other applicable regulations for the safe use, handling, transporting, storage, and disposal of these solvents.

Solvent	CAS No.	EC No.	Strength[1,2]	Selectivity Class[3]	UV Cutoff (nm)	Vapor Pressure at 20°C (psi)	Vapor Pressure at 20°C (mbar)
Acetone	67-64-1	200-662-2	0.56	6 (VIa)	330	3.6	247.4
Acetonitrile	75-05-8	200-835-2	0.65	6 (VIb)	190	1.4	93.6
Cyclohexane	110-82-7	203-806-2	0.04	0	210	1.5	103.4
Dichloromethane (Methylene chloride)	75-09-2	200-838-9	0.42	5 (V)	235	6.9	475.3
Ethanol	64-17-5	200-578-6	0.88	2 (II)	210	1.3	90.0
Ethyl acetate	141-78-6	205-500-4	0.58	6 (VIa)	255	1.4	98.3
n-Heptane	142-82-5	205-563-8	0.01	0	210	0.7	47.4
n-Hexane	110-54-3	203-777-6	0.01	0	210	2.3	161.6
Methanol	67-56-1	200-659-6	0.95	2 (II)	210	1.9	129.7
2-Propanol	67-63-0	200-661-7	0.82	2 (II)	210	0.6	44.0
Tetrahydrofuran	109-99-9	203-726-8	0.57	3 (III)	220	2.5	172.4
Toluene	108-88-3	203-625-9	0.29	7 (VII)	286	0.4	29.1
Water	7732-18-5	231-791-2	1.00*	0	190	0.3	23.4

1 Neue U. D. *HPLC Columns Theory, Technology, and Practice*, Wiley-VCH (1997).

2 Dean J. A. *Lange's Handbook of Chemistry*, 15th edition, McGraw-Hill (1999).

3 Snyder L. R. and Kirkland J. J. *Introduction to Modern Liquid Chromatography*, Wiley (1979).

* When water is used in reversed phase chromatography, the strength value is 0.

Chapter 2

Data Administration
(Data Administration Mode)

WARNING

Before performing any procedures in this chapter, please read and observe the safety requirements in the "Isolera™ Installation and Safety" document (P/N 415796). Failure to follow those requirements may result in personal injury and/or equipment damage.

2.1 Log into the Data Administration Mode

You must log into the Data Administration mode to be able to administrate cartridge types, methods, rack types, results, solvents, and user accounts.

1. If you are not at the main menu, press **Main Menu** in the right-hand panel.
2. Press **Data Administration**. All user accounts with system owner privilege are listed in the **Select User** dialog.
3. Select your user name. If you have not been assigned a user name and/or system owner privilege, please contact your system supervisor.
4. Press **OK**. If your account is password-protected, the **Password** dialog opens.
   a. Enter your password using the keypad. For the purpose of security, password characters appear as asterisks.
   b. Press **OK**. If you have entered a valid password, the software is opened in the Data Administration mode.

For more information about the software modes, see "Software Description" on page 1-8.

NOTE

The first time you log into the Data Administration mode, log in using the user account "System Owner" and the password "1234". Before you log out, it is strongly recommended that the password is changed (see "Administrate the User List" on page 2-9).

Figure 2-1. Log into the Data Administration Mode (Isolera Dalton 2000 System with Assist Workflow Enabled Shown)

2.2 Administrate the Cartridge List

The software comes with a preconfigured list of cartridges and their settings. At the **Cartridges** tab, in the software's Data Administration mode, you may add other cartridges to this list, and select the cartridge types that will appear in the cartridge selection list when setting up a method in the Chemistry mode. It is also possible to edit and delete user defined cartridge types.

NOTE

The preconfigured Biotage cartridges cannot be edited or deleted. We recommend that you disable any preconfigured cartridges that will not be used to simplify finding the ones to use; see section 2.2.4 on page 2-3.

SNAP and SNAP Ultra 10g to 30g and ZIP and ZIP Sphere 5g to 45g cannot be used on Isolera LS. SNAP KP-Sil 750g, Flash 75M, and Flash 75L are not recommended for use on Isolera Prime, or Isolera Dalton 2000 when using the mass detector. SNAP KP-Sil 1500g is only recommended for use on Isolera LS.

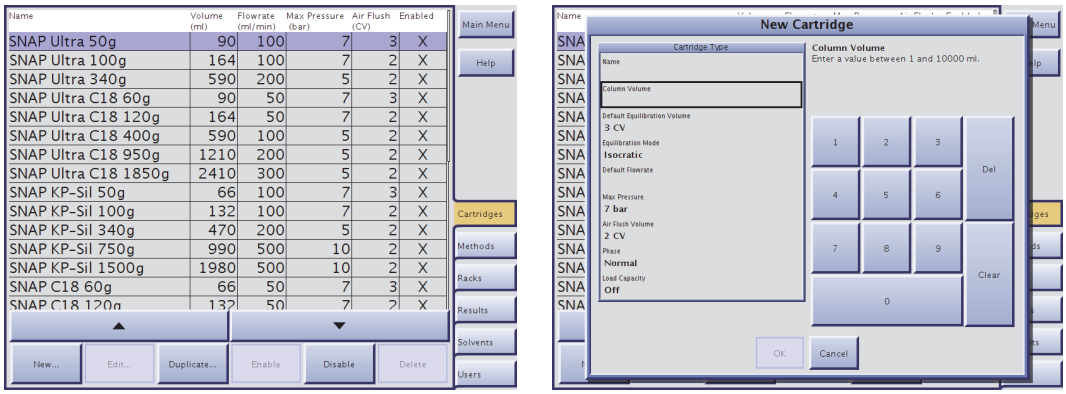


Figure 2-2. Cartridges Tab (Isolera LS Shown)

2.2.1 Add a Cartridge Type

1. Press **New...** or, if you want to base the new cartridge type on an existing one, select the cartridge and press **Duplicate...** at the **Cartridges** tab. The **New Cartridge** dialog opens.
2. Enter the cartridge settings. (A setting can be entered when the corresponding text box has been selected.) The cartridge parameters are:
   - The name of the cartridge type.
   - The column volume (CV) in ml.
   - The default equilibration volume in CV. This parameter is only used for isocratic equilibrations. Gradient equilibrations are fixed to 5 CV.
   - The equilibration mode, isocratic or gradient. Gradient equilibration will only be used when the percentage of the strongest solvent is above 10% in the initial solvent mix. When the percentage is less or equal to 10%, isocratic equilibration is used. Note that gradient equilibration is not applicable when using an Isolera Prime system.
   - The default flow rate in ml/min.
   - The maximum pressure in bar or psi (see "Set the Pressure Unit" on page 3-5). If the maximum pressure is exceeded during a purification, the run will be paused and the system has to be purged until an acceptable pressure level is reached.
   - The air flush volume in CV.
   - The suitable chromatography phase, normal or reversed.

- The approximate load capacity when the delta CV is 2.0. Note that only normal phase cartridges with a specified load capacity will be listed when setting up a method using the TLC to Linear Gradient editor or the TLC to Step Gradient editor.

3. To save the new cartridge type, press **OK**.

2.2.2 Delete an Unused Cartridge Type

1. Select the cartridge that you want to delete at the **Cartridges** tab.
2. Press **Delete**. The **Confirm Delete** dialog opens.
3. To confirm delete, press **Yes**.

2.2.3 Edit a Cartridge Type

1. Select the cartridge that you want to edit at the **Cartridges** tab.
2. Press **Edit...**. The **Edit Cartridge** dialog opens.
3. Edit the cartridge settings. (A setting can be edited when the corresponding text box has been selected.)
4. To save the changes, press **OK**.

2.2.4 Select Cartridge Types to List

1. If you want a cartridge type to appear when setting up a method in the Chemistry mode, select it at the **Cartridges** tab and press **Enable**.
2. If you do not want a cartridge type to appear when setting up a method in the Chemistry mode, select it at the **Cartridges** tab and press **Disable**.

2.3 Administrate the Method List

At the **Methods** tab, in the software's Data Administration mode, all saved methods are listed. You can export, import, and delete methods. It is also possible to set default methods, i.e. a list of methods that the users can choose from when setting up a new method at the **Method** tab in the Chemistry mode. (If the user wants to base a new method on a method that is not set as a default method, the user will have to browse for the method.)

NOTE

The preconfigured Biotage methods cannot be exported or deleted.

When using the Assist Workflow, the user will only be able to access methods that are saved in her/his method folder and contain all the necessary information to be run. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.

2.3.1 Delete Methods

You can either delete a single method or all methods of a user.

1. Select the owner of the method(s) in the user list at the **Methods** tab.
2. If you want to delete a single method, select the method and press **Delete Selected**.
3. If you want to delete all methods of the selected user, press **Delete All Listed**.
4. To confirm delete, press **Yes** in the **Confirm Delete** dialog.

2.3.2 Export Methods

You can export a single method, all methods of a user, or all saved methods to a USB memory device or to a share folder on your network (if file share has been set up; see page 3-9).

1. If you want to export a single method, select the owner of the method in the user list at the **Methods** tab and then select the method.
2. If you want to export all methods of a user, select the user at the **Methods** tab.
3. Press **Export...**. The **Export Methods** dialog opens.
4. Select the desired export option, **Selected Method**, **All For Selected User**, or **All**.
5. To save the method(s) to a USB memory device:
   a. Connect the memory device to the USB port located underneath the touch screen.
   b. Press **Export**.
6. To save the method(s) in the specified share folder, press **Export**. This button is only available if file share has been set up; see page 3-9.

The methods are saved at \biotage\isolera\methods\.

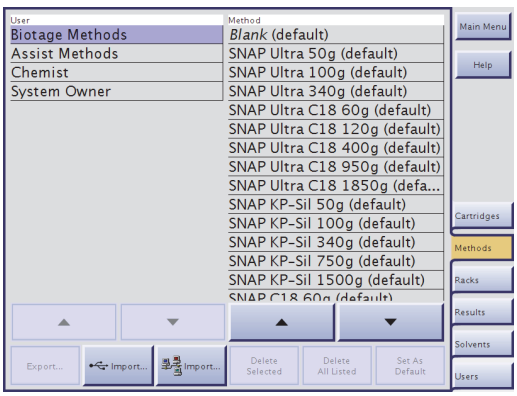


Figure 2-3. Methods Tab (Isolera LS Shown)

2.3.3 Import Methods

NOTE

If you import a method with a solvent, cartridge, rack, user, or project that is not available on the system, it will also be imported. The decision is based on the name of the solvent, cartridge, etc., and will not consider their settings.

You can either import a single method or all methods available on a USB memory device or in a share folder on your network (if file share has been set up; see page 3-9). The method(s) to import must be available at "USB":\biotage\isolera\methods or "share folder"\biotage\isolera\methods.

1. If you want to import the method(s) to a certain user, select the user at the **Methods** tab.
2. To import method(s) available on a USB memory device:
   a. Connect the memory device to the USB port located underneath the touch screen.
   b. Press **Import...**. The **Import Methods from USB** dialog opens.
3. To import method(s) available in the specified share folder, press **Import...**. The **Import Methods from Network** dialog opens.
4. To import a single method, select the method and then select **Selected Method** in the **Method(s) to Import** list.
5. To import all methods, select **All** in the **Method(s) to Import** list.

6. Select import destination. You can either save the method(s) to the user selected in step 1, **To Selected User**, or to the user defined in the method(s), **To Original Users**.
7. To import the method(s), press **OK**.

2.3.4 Set Default Methods

To set a method as a default method, i.e. add it to the list of methods that the users can choose from when setting up a new method at the **Method** tab in the Chemistry mode:

1. At the **Methods** tab, select the method that you want to add to the list of default methods.
2. Press **Set As Default**.

To remove a method from the list of default methods:

1. At the **Methods** tab, select the method that you want to remove from the list of default methods.
2. Press **Clear Default**.

2.4 Administrate the Rack List

The software comes with a preconfigured list of racks and their settings. At the **Racks** tab, in the software's Data Administration mode, you may add other racks to this list, and select the rack types that will appear in the rack selection list when setting up a method in the Chemistry mode. It is also possible to edit and delete user defined rack types.

NOTE

The preconfigured Biotage racks cannot be edited or deleted. We recommend that you disable any preconfigured cartridges that will not be used to simplify finding the ones to use; see section 2.4.4 on page 2-7.

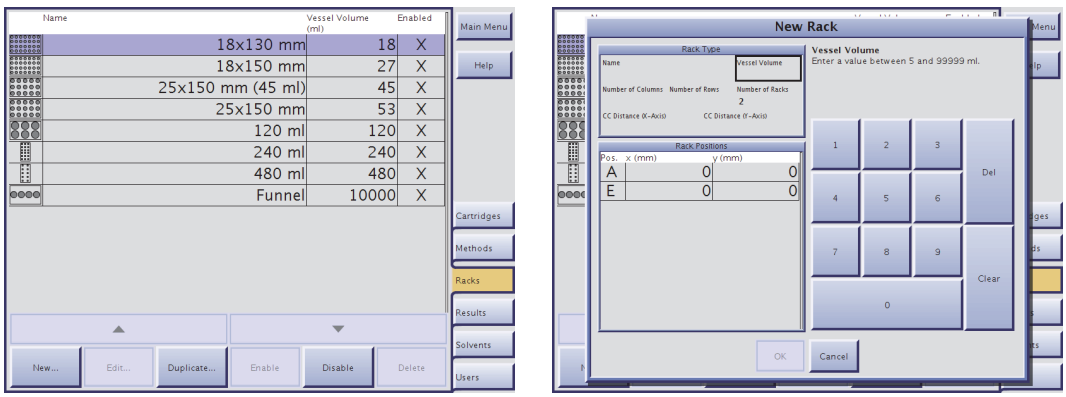
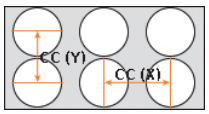


Figure 2-4. Racks Tab (Isolera LS Shown)

2.4.1 Add a Rack Type

1. Press **New...** or, if you want to base the new rack type on an existing one, select the rack and press **Duplicate...** at the **Racks** tab. The **New Rack** dialog opens.
2. Enter the rack settings. (A setting can be entered when the corresponding text box has been selected.) The rack parameters are:
   - The name of the rack type.
   - The vessel volume in ml.
   - The number of columns and rows of vessels in a rack. For example, the racks in Figure 2-5 have six (6) rows and eight (8) columns each.
   - The maximum number of racks that can be loaded onto a system with two collection trays. Can be either two or eight, i.e. one or four racks per tray.
   - The CC distance between vessel positions along the x-axis and the y-axis; see image below.

   - The x- and y-coordinates for the first vessel position of each rack on the collection area; see Figure 2-5.

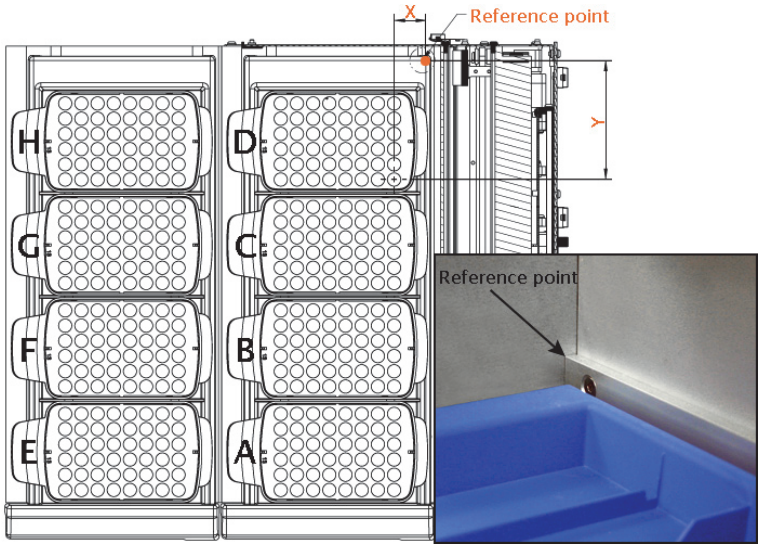


Figure 2-5. X- and Y-Coordinates for Rack Position D

3. To save the new rack type, press **OK**.

2.4.2 Delete an Unused Rack Type

1. Select the rack that you want to delete at the **Racks** tab.
2. Press **Delete**. The **Confirm Delete** dialog opens.
3. To confirm delete, press **Yes**.

2.4.3 Edit a Rack Type

1. Select the rack that you want to edit at the **Racks** tab.
2. Press **Edit...**. The **Edit Rack** dialog opens.
3. Edit the rack settings. (A setting can be edited when the corresponding text box has been selected.)
4. To save the changes, press **OK**.

2.4.4 Select Rack Types to List

1. If you want a rack type to appear when setting up a method in the Chemistry mode, select it at the **Racks** tab and press **Enable**.
2. If you do not want a rack type to appear when setting up a method in the Chemistry mode, select it at the **Racks** tab and press **Disable**.

2.5 Administrate the Result List

Each purification is stored as an individual record in the system's database. At the **Results** tab, in the software's Data Administration mode, you can export and delete result records and clean up the system's database.

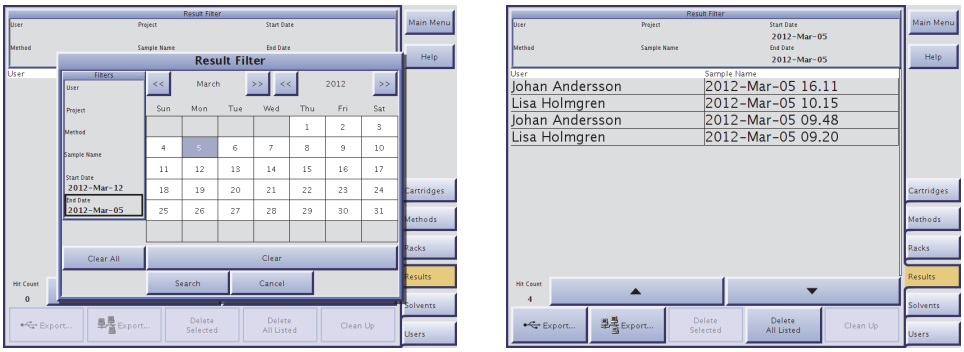


Figure 2-6. Results Tab

2.5.1 Export and Delete Result Records

You can either export a single or all listed records to a USB memory device or to a share folder on your network (if file share has been set up; see page 3-9). The records can be exported as CSV files (including all detector data), PDF files, XML files (including all raw data), or DATX files (including the mass detector data). CSV files can be imported into Microsoft Excel or other applications that can handle comma-delimited text files.

1. All records that fit the specified filter settings are listed at the **Results** tab. If a record that you want to export or delete is not listed, change the filter settings:
   a. Press the **Result Filter** field. The **Result Filter** dialog opens.
   b. Specify the search criteria. (If you want to list all result records, press **Clear All**.)
   c. To search, press **Search**. If there are records matching your filter settings, they are listed in chronological order at the **Results** tab. Note that the result filter is case sensitive.
2. To export a single or all listed records as a CSV file, PDF file, XML file, or DATX file(s):
   a. If you want to export the record(s) to a USB memory device, connect the memory device to the USB port located underneath the touch screen.
   b. If you want to export a single record, select it.
   c. Either press **Export...** to export the record(s) to the USB memory device connected in step 2a, or press **Export...** to export the record(s) to the specified share folder. The **Export Results** dialog opens.
   d. Select the desired export option, **Selected** or **All Listed**.
   e. Press **Export XML**, **Export CSV**, **Export PDF**, **Export DATX,** or **Export All**.
3. To delete a single or all listed records:
   a. To delete a single record, select it and press **Delete Selected**.
   b. To delete all the listed records, i.e. delete all the records that fit the settings of the result filter, press **Delete All Listed**.
   c. To confirm delete, press **Yes** in the **Confirm Delete** dialog.

2.5.2 Clean Up the System's Database

If the **Clean Up** button is enabled, there are one or more purifications available in the database that cannot be displayed at the **Results** tab, likely due to power failure during a purification. To remove the purification(s), press **Clean Up**.

2.6 Administrate the Solvent List

The software comes with a preconfigured list of solvents and their settings. At the **Solvents** tab, in the software's Data Administration mode, you may add other solvents to this list, and select the solvents that will appear in the solvent selection list when setting up a method in the Chemistry mode. It is also possible to edit and delete user defined solvents.

The solvent parameters are:

- The solvent name.
- The solvent strength value, a value between 0 and 1.[1,2]
- The maximum speed at which the solvent can be drawn into the pump during the fill stroke, in ml/min. The valid range is 1 to 200 ml/min, or 50 to 500 ml/min when using an Isolera LS system. If this parameter is turned off, the system's maximum fill rate will be used (i.e. 200 ml/min or 500 ml/min).

1 Neue U. D. *HPLC Columns Theory, Technology, and Practice*, Wiley-VCH (1997).

2 Dean J. A. *Lange's Handbook of Chemistry*, 15th edition, McGraw-Hill (1999).

NOTE

The preconfigured solvents cannot be edited or deleted. We recommend that you disable any preconfigured solvents that will not be used to simplify finding the ones to use; see section 2.6.4 on page 2-9.

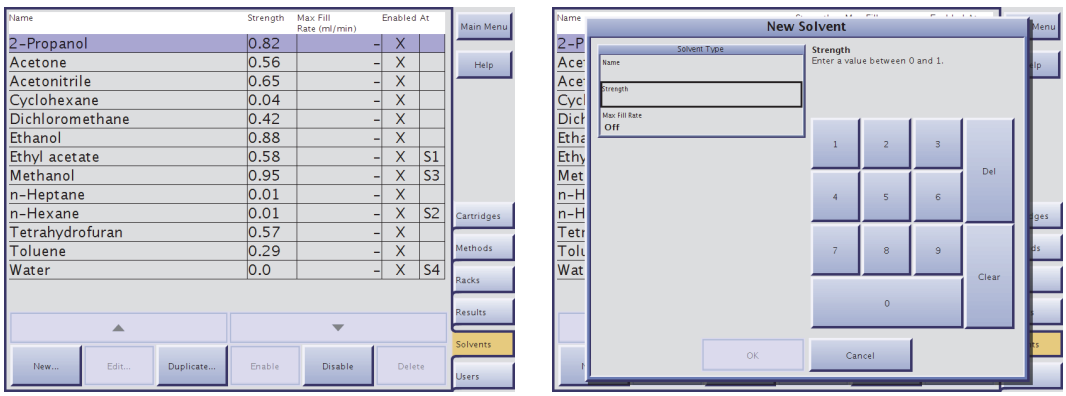


Figure 2-7. Solvents Tab (System with Four Solvent Inlets Shown)

2.6.1 Add a Solvent

1. Press **New...** or, if you want to base the new solvent on an existing one, select the solvent and press **Duplicate...** at the **Solvents** tab. The **New Solvent** dialog opens.
2. Enter a unique solvent name, the solvent strength, and the maximum fill rate (i.e the maximum speed at which the solvent can be drawn into the pump during the fill stroke) by selecting the corresponding text box. To turn off the maximum fill rate and use the system's maximum fill rate, select the **Max Fill Rate** text box and press **Off**.

To save the new solvent, press **OK**.

2.6.2 Delete an Unused Solvent

1. Select the solvent that you want to delete at the **Solvents** tab.
2. Press **Delete**. The **Confirm Delete** dialog opens.
3. To confirm delete, press **Yes**.

2.6.3 Edit a Solvent

1. Select the solvent that you want to edit at the **Solvents** tab.
2. Press **Edit...**. The **Edit Solvent** dialog opens.
3. Edit the solvent settings. (A setting can be edited when the corresponding text box has been selected.)
4. To save the changes, press **OK**.

2.6.4 Select Solvents to List

1. If you want a solvent to appear when setting up a method in the Chemistry mode, select it at the **Solvents** tab and press **Enable**.
2. If you do not want a solvent to appear when setting up a method in the Chemistry mode, select it at the **Solvents** tab and press **Disable**.

2.7 Administrate the User List

At the **Users** tab, in the software's Data Administration mode, you can add, edit, and delete user accounts. The user parameters are:

Name: The user name will be used in the various user name selection boxes as well as in the purification records. The user name is typically the person's name, initials, employee number, etc.

Password: It is possible to password-protect a user account. The password will be used when saving a method and, for users with system owner privilege, when entering the Data Administration and System modes.

E-mail: If the system is connected to your network and the Auto Send Reports option is enabled (see page 3-9), an e-mail with a result report will be sent to the e-mail address specified in the user's account when a purification has been completed. An e-mail will also be sent to the user when user interaction is required, e.g. when a rack has to be replaced, a solvent needs to be replenished (if monitoring is enabled), a waste reservoir needs to be emptied (if monitoring is enabled), and a leak is detected (if using an Isolera instrument tray with a solvent detector).

Privilege: A user can have chemist or system owner privilege:

- The chemist privilege gives the user access to the Chemistry mode, i.e. the user can set up and run purifications, and view results.
- The system owner privilege gives the user both the chemist privilege (see above) and access to the Data Administration mode (the user can administrate cartridge types, methods, rack types, results, solvents, and user accounts) and to the System mode (the user can change detector, network, reporting, and runtime settings, set the system clock, calibrate the fraction collector and internal detector, calibrate and vent or pump down the mass detector (optional), release stuck check valves, back up and restore the database, export the system configuration and logs, restore the system configuration, etc).
- **Assist Workflow Editor:** Set whether the user will get access to the basic or the advanced workflow editor when setting up a purification run in the Assist Workflow. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.

Performed Runs: The number of runs performed by the user.

2.7.1 Add a User Account

See the "Isolera™ Assist Quick Guide" (P/N 414767) for instructions on how to set up user accounts for the Assist Workflow.

1. Press **New...** or, if you want to base the new user account on an existing one, select the account and press **Duplicate...** at the **Users** tab. The **New User** dialog opens.
2. Enter a unique user name, the password, the e-mail address, the privilege, and the Assist Workflow editor by selecting the corresponding text box.
3. To save the new user, press **OK**.

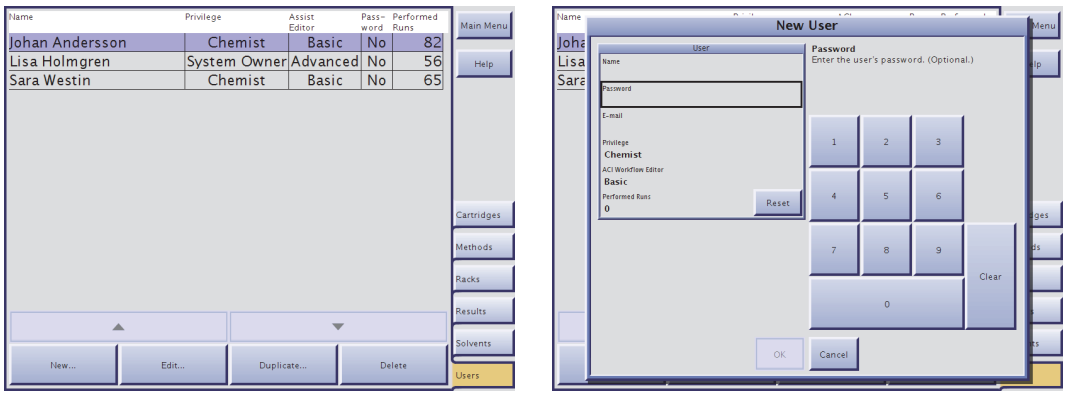


Figure 2-8. Users Tab

2.7.2 Change the Password or Privilege

1. Select the user account that you want to edit at the **Users** tab.
2. Press **Edit...**. The **Edit User** dialog opens.
3. Edit the user settings. (A setting can be entered when the corresponding text box has been selected.) If you change the user name, all methods and result records associated with the user will be updated.
4. To save the changes, press **OK**.

2.7.3 Delete a User Account

NOTE

All the results and methods of the user will also be deleted.

1. Select the user account that you want to delete at the **Users** tab.
2. Press **Delete**. The **Confirm Delete** dialog opens.
3. To confirm delete, press **Yes**.

2.7.4 Reset the Number of Performed Runs

1. Select the user account that you want to reset at the **Users** tab.
2. Press **Edit...**. The **Edit User** dialog opens.
3. Press **Reset** in the **User** field. The **Reset Performed Runs** dialog opens.
4. To confirm reset, press **Yes**.

Chapter 3

System Settings (System Mode)

WARNING

Before performing any procedures in this chapter, please read and observe the safety requirements in the "Isolera™ Installation and Safety" document (P/N 415796). Failure to follow those requirements may result in personal injury and/or equipment damage.

3.1 Log into the System Mode

You must log into the System mode to be able to enable the Assist Workflow, change detector, network, reporting, and runtime settings, set the system clock, calibrate the fraction collector and internal detector, calibrate and vent or pump down the mass detector (optional), release stuck check valves, clean the collect valve, back up and restore the database, export system logs, restore the system configuration, etc.

1. If you are not at the main menu, press **Main Menu** in the right-hand panel.
2. Press **System**. All user accounts with system owner privilege are listed in the **Select User** dialog.
3. Select your user name. If you have not been assigned a user name and/or system owner privilege, please contact your system supervisor.
4. Press **OK**. If your account is password-protected, the **Password** dialog opens.
   a. Enter your password using the keypad. For the purpose of security, password characters appear as asterisks.
   b. Press **OK**. If you have entered a valid password, the software is opened in the System mode.

For more information about the software modes, see "Software Description" on page 1-8.

NOTE

The first time you log into the System mode, log in using the user account "System Owner" and the password "1234". It is strongly recommended that the password for "System Owner" is changed; see "Administrate the User List" on page 2-9.

Figure 3-1. Log into the System Mode (Isolera Dalton 2000 System with Assist Workflow Enabled Shown)

3.2 Change Detector Settings

3.2.1 Enable or Disable the Internal Detector

1. Select the **Detector** tab in the right-hand panel.
2. To enable or disable the internal detector, select the **Enabled** text box in the **UV Detector** field and select **Yes** or **No**.

3.2.2 Enable or Disable the Mass Detector (Optional)

NOTE

The mass detector and Isolera Dalton Nanolink shall be unpacked and installed by an authorized Biotage service engineer. Should you need to move the mass detector, see the instructions in the "Isolera™ Dalton 2000 User Manual" (P/N 415730).

If the mass detector is not going to be used for a long period of time, disable it in the software and disconnect the mass detector and Isolera Dalton Nanolink from the system. Carefully follow the instructions in the "Move or Disconnect Biotage® Dalton 2000 and Isolera™ Dalton Nanolink" section in the "Isolera™ Dalton 2000 User Manual" (P/N 415730).

To enable the automatic connection of the mass detector at system startup and reconnect the mass detector and Isolera Dalton Nanolink, carefully follow the instructions in the "Connect Biotage® Dalton 2000 and Isolera™ Dalton Nanolink" section in the "Isolera™ Dalton 2000 User Manual" (P/N 415730).

To enable or disable sample introduction using an Atmospheric Solids Analysis Probe (ASAP), select the **ASAP** text box in the **Mass Detector** field and select **Enabled** or **Disabled**.

To change the maximum mass range to be used, select the **Mass Range** text box in the **Mass Detector** field and select **Normal (m/z 90-2000)** or **Full (m/z 10-2000)**.

3.2.3 Connect and Enable an External Detector (Optional)

NOTE

The external detector and the tube assembly must be able to handle a maximum dispense rate of 200 ml/min, or 500 ml/min when using an Isolera LS system, and a maximum pressure of 10 bar.

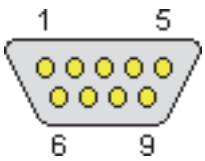
As some external detectors have flow rate limitations (e.g. ELSD), the flow stream may need to be split so that the majority of the flow is directed through the internal detector and a proportion diverted to the external detector, or the dispense rate (i.e. the speed at which liquid is pushed into the tubing of the external detector) must be limited (see step 7 below). Please contact the external detector manufacturer for flow rate specifications.

The external ELSD-A120 from Biotage shall be unpacked and installed by an authorized Biotage service engineer. Should you need to move the detector, see the instructions in the "Biotage® ELSD-A120 Getting Started Guide" (P/N 414124).

It is not possible to enable an external detector when using Biotage Dalton 2000.

To connect and enable an external detector:

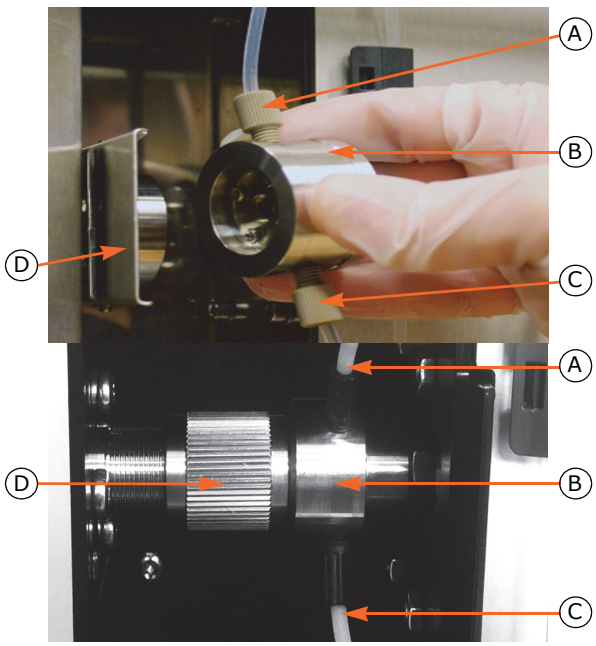
1. Connect the external detector (with an analog output signal from 0 V up to a maximum of 5 V) to the **EXT UV** port at the rear of the system using a male DE-9 connector:



Pin	Connection
1	Signal 0-5 V, referenced to pin 2
2	Ground
3-9	Not connected

Figure 3-2. Male DE-9 Connector

2. Connect the internal and external flow cells in series or by using a splitter, or move the internal detector's inlet and outlet tubing (see Figure 3-3) to the external flow cell. Ensure that you use as short extra tubing as possible. For more information, please refer to the manufacturer of the external detector. **Tip!** If the flow cells are connected in series and the pressure exceeds 10 bar (i.e. the run is aborted), retry using a splitter.
3. Select the **Detector** tab in the right-hand panel.
4. Select the **Enabled** text box in the **External Detector** field and select **Yes**.
5. Select the **Signal Range** text box and enter the maximum voltage of the signal from the external detector. The valid range is 1 mV to 5000 mV. During the run, the system will convert 0 mV to 0 mAU and the maximum voltage to 6000 mAU.
6. Select the **Extended Flush Volume** text box and enter the amount of solvent that has to be added to the flushes due to the flow cell volume and/or the extra tubing associated with the external detector.



A	Flow Cell Outlet Tube
B	Flow Cell
C	Flow Cell Inlet Tube
D	Retaining Latch (Upper Picture) or Nut (Lower Picture)

Figure 3-3. Internal Detector Flow Cell for Max 200 ml/min

7. Select the **Max Dispense Rate** text box and enter the maximum speed at which liquid can be pushed into the tubing of the external detector during the dispense stroke. The valid range is 1 to 200 ml/min except for when using an Isolera LS system, then the valid range is 50 to 500 ml/min. If this parameter is turned off, the system's maximum dispense rate will be used (i.e. 200 ml/min or 500 ml/min).

3.2.4 Disable an External Detector

To disable the external detector:

1. Select the **Detector** tab in the right-hand panel.
2. Select the **Enabled** text box in the **External Detector** field and select **No**.

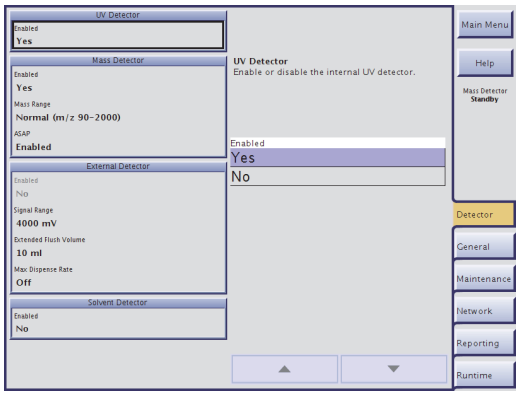


Figure 3-4. Detector Tab (Isolera Dalton 2000 Shown)

3.2.5 Enable or Disable the Solvent Detector

An Isolera instrument tray with a solvent detector can be used for safe, unattended operation. The solvent detector is standard on Isolera LS and optional on all other Isolera system platforms.

To enable the solvent detector:

1. For installation instructions, see the "Moving an Isolera™ system" section in the "Isolera™ Installation and Safety" document (P/N 415796).
2. Select the **Detector** tab in the right-hand panel.
3. Select the **Enabled** text box in the **Solvent Detector** field and select **Yes**.

To disable the solvent detector:

1. Select the **Detector** tab in the right-hand panel.
2. Select the **Enabled** text box in the **Solvent Detector** field and select **No**.

3.3 Change General Settings

3.3.1 Set the Date and Time

Setting the date and time correctly ensures an accurate date and time stamp for your result records and can help you search for specific records.

1. Select the **General** tab in the right-hand panel.
2. Edit the date and time settings in the **System Clock** field by selecting the corresponding text box.
3. To apply your new settings, press **Apply**.
4. For the settings to take effect, restart the system:
    a. Press **Main Menu**.
    b. Press **Shut Down** and then **Yes** to confirm.
    c. If using an Isolera Dalton 2000 system, the **Mass Detector** dialog opens. Press **OK**.
    d. When the message saying that it is safe to turn off the system appears on the screen, turn off the system. The power switch is located underneath the touch screen.
    e. Turn on the system.

3.3.2 Enable or Disable Mouse Pointer

When connecting a mouse to one of the USB ports at the rear of the system, you need to enable the mouse pointer. Note that some versions of the system have a **MOUSE** port and only one USB port at the rear of the system.

1. Select the **General** tab in the right-hand panel.
2. To enable or disable the mouse pointer, select the **Enabled** text box in the **Mouse Pointer** field and select **Yes** or **No**.

3.3.3 Set the Pressure Unit

1. Select the **General** tab in the right-hand panel.
2. Select the **Pressure Unit** text box and select the unit to be used by the system, **bar** or **psi**.

3.3.4 Set the Language Used in the Chemistry Mode

1. Select the **General** tab in the right-hand panel.
2. Select the **Language** text box and select the language to be used in the software's Chemistry mode.

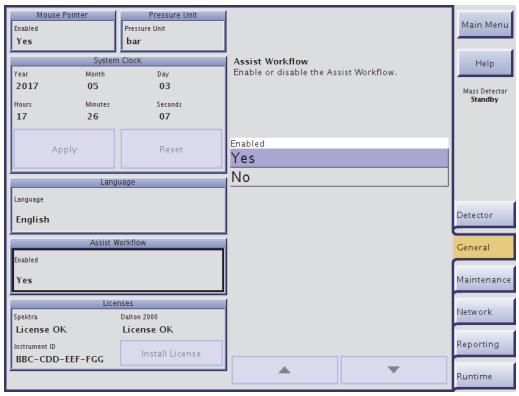


Figure 3-5. General Tab

3.3.5 Enable or Disable the Assist Workflow

The Assist Workflow is a streamlined and simplified way to set up and run purifications. When the Assist Workflow is enabled, it can be accessed by pressing **New** in the main menu. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.

1. Select the **General** tab in the right-hand panel.
2. To enable or disable the Assist Workflow, select the **Enabled** text box in the **Assist Workflow** field and select **Yes** or **No**.
3. For the setting to take effect, restart the system:
   a. Press **Main Menu**.
   b. Press **Shut Down** and then **Yes** to confirm.
   c. When the message saying that it is safe to turn off the system appears on the screen, turn off the system. The power switch is located underneath the touch screen.
   d. Turn on the system.

3.3.6 Install an Isolera™ Spektra or Dalton 2000 Software License

Isolera Spektra: To enable the λ-all detection mode, the baseline correction option, and the TLC to Step Gradient editor, you need an Isolera Spektra license. To purchase an Isolera Spektra license, please contact your local representative. Note that the license is not available for Isolera Prime.

Dalton 2000: When purchasing Biotage Dalton 2000, the mass detector is supplied with a Dalton 2000 software license. The license includes all the functionality of the Isolera Spektra software upgrade package (see above) as well as support for Biotage Dalton 2000.

To install a license:

1. Save the license file in a "biotage/isolera/" directory on a USB memory device.
2. Connect the memory device to the USB port located underneath the touch screen.
3. Select the **General** tab in the right-hand panel.
4. Press **Install License** in the **Licenses** field.
5. If the license is valid, *License OK* appears in the **Advanced Features** text box.
6. Remove the USB memory device.
7. Restart the system:
    a. Press **Main Menu** and then **Shut Down**.
    b. When the message saying that it is safe to turn off the system appears on the screen, turn off the system. The power switch is located underneath the touch screen.
    c. Turn on the system.

3.4 Configure a Network Connection

With your system connected to the network, it is possible to:

- Print purification reports to a network printer with postscript support; see "Set Up a Printer and Auto Print of Reports" on page 3-8.
- Receive a report by e-mail when a purification has been completed; see "Set Up Auto Send of Reports" on page 3-9.
- Receive an e-mail when user interaction is required during a purification, e.g. when the system run out of empty collection vessels.
- Make your reports and methods available to other users by saving them in a share folder on your network; see "Set Up File Sharing and Auto Save of Reports" on page 3-9.
- Back up your database and save the system configuration and logs in a share folder on your network; see "Back Up and Restore the System's Database" and "Export the System Configuration and Logs" on page 3-14.

With the system connected to your network, it is also possible to perform the following tasks through a standard web browser (through the Isolera Remote Viewer feature):

- Create a new method. If desired, the method can be based on a previous purification found in the Results view.
- Check the status of the system and the progress of a purification.
- Instruct the system to start and stop collecting fractions by clicking the **Collect All** button (✓ = fractions are collected).*
- Instruct the system to switch to a new collection vessel by clicking the **New Fraction** button.*
- Instruct the system to start and end an isocratic segment by clicking the **Hold** button (✓ = isocratic hold is enabled).*
- Instruct the system to pause the purification in progress by clicking the **Pause** button. For safety reasons, it is only possible to pause and not resume a run from your office.*
- Instruct the system to set the current UV level to 0 (zero) AU by clicking the **UV Zero** button.*
- View, print, and export purification results.

* You must be logged in as the user defined in the purification run.

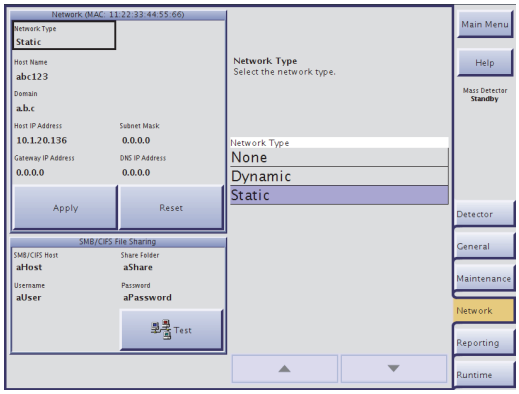


Figure 3-6. Network Tab

To configure the network connection:

1. Connect a shielded category 5 TP cable to the **ETHERNET** port.
2. Select the **Network** tab in the right-hand panel.
3. Select the **Network Type** text box and select **Dynamic** or **Static**.
4. Edit the network settings in the **Network** field. For more information, contact your IT administrator. (To reset changes that have not been applied, press **Reset**.)
5. To apply your new settings, press **Apply**.
6. For the network configurations to take effect, restart the system:
    a. Press **Main Menu**.
    b. Press **Shut Down** and then **Yes** to confirm.
    c. When the message saying that it is safe to turn off the system appears on the screen, turn off the system. The power switch is located underneath the touch screen.
    d. Turn on the system.

To access the Isolera Remote Viewer:

1. Enter the URL *http://MACHINENAME* in a web browser (where MACHINENAME is the hostname or the host IP address entered in the **Network** field above). **Tip!** You can also find the hostname and host IP address in the **About** dialog at the main menu.
2. Press **ENTER** and the Isolera Remote Viewer web page is loaded. The page is automatically updated every five seconds.

3.5 Change Report Settings

3.5.1 Set Up a Printer and Auto Print of Reports

If you want reports to be automatically printed when a purification has been completed, select the **Auto Print Reports** text box at the **Reporting** tab and select **Yes**.

NOTE

If connecting a network printer, only printers with postscript support can be used with the system.

To connect a USB printer to the system:

1. Connect your printer to one of the USB ports at the rear of the system.
2. Turn the printer on.
3. Select the **Reporting** tab in the right-hand panel.
4. Select the **Printer Type** text box and select **Local**.
5. Select the **Printer Model** text box. The **Printer Setup Wizard** opens.
6. Read and follow the instructions that appear on the screen. **Tip!** If the Isolera system does not have a matching printer driver for the connected printer, select **Generic** in the wizard's manufacturer list and check if there is a printer protocol available that is supported by your printer (check the printer user manual).
7. When you have completed the printer setup using the wizard, select the **Paper Size** text box and then select the desired paper format.
8. To test the connection, press **Test** in the **Printer** field. If a test page is printed, the connection is working properly.

To connect a network printer with postscript support to the system:

1. Configure the network connection; see instructions on page 3-6.
2. Select the **Reporting** tab in the right-hand panel.
3. Select the **Printer Type** text box and select **Network**.
4. Enter the correct **Network Printer Name** and **Network Printer Port** by selecting the corresponding text box. Contact your IT administrator for more information.
5. Select the **Paper Size** text box and then select the desired paper format.
6. To test the connection, press **Test** in the **Printer** field. If a test page is printed, the connection is working properly.

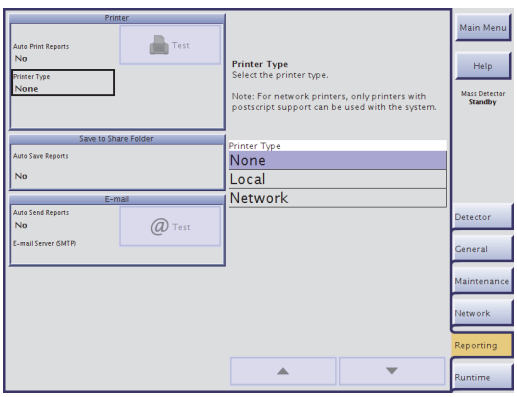


Figure 3-7. Reporting Tab

3.5.2 Set Up File Sharing and Auto Save of Reports

If the Auto Save Reports option is enabled, a report is saved in a share folder on your network when a purification has been completed. The report is saved as a PDF and/or a XML file (a text file including all raw data) and a SPECTRUM file (with the raw 3D UV spectrum). If using Biotage Dalton 2000, you will also get two DATX files (with the mass detector data) with the XML file.

1. Configure the network connection; see instructions on page 3-6.
2. Select the **Network** tab in the right-hand panel.
3. Enter the correct hostname, name of the share folder, user name, and password by selecting the corresponding text box in the **SMB/CIFS File Sharing** field. Contact your IT administrator for more information.
4. To test the connection, press **Test** in the **SMB/CIFS File Sharing** field.
5. If you want a report to be automatically saved in the specified share folder when a purification has been completed:
    a. Select the **Reporting** tab in the right-hand panel.
    b. Select the **Auto Save Reports** text box and select the desired report format, **PDF**, **XML** (text files including all raw data), or **PDF and XML**.

3.5.3 Set Up Auto Send of Reports

If the Auto Send Reports option is enabled, a report is sent to the e-mail address specified in the user's account when a purification has been completed. An e-mail will also be sent to the user when user interaction is required, e.g. when a rack has to be replaced, a chromatography solvent needs to be replenished (if monitoring is enabled), a waste reservoir needs to be emptied (if monitoring is enabled), and a leak is detected (if using an Isolera instrument tray with a solvent detector).

1. Configure the network connection; see instructions on page 3-6.
2. Select the **Reporting** tab in the right-hand panel.
3. Enter the outgoing e-mail server address by selecting the **E-mail Server (SMTP)** text box. For more information, contact your IT administrator.
4. Select the **Auto Send Reports** text box and select **Yes**.
5. To test the connection, press **Test** in the **E-mail** field. The **Test E-mail** dialog opens.
6. Enter your e-mail address and press **Send**. If you receive a test message, the connection is working properly.

3.6 Change Runtime Settings

3.6.1 Enable or Disable Audible Alarm

If the Audible Alarm option is enabled, an audible warning will sound whenever user interaction is required for the run to proceed. Examples of situations are: 1) rack change is needed, 2) leakage (if using an Isolera instrument tray with a solvent detector), 3) high pressure, 4) low solvent volume (if solvent monitoring is enabled), 5) full waste reservoir (if waste monitoring is enabled), 6) misalignment of the collection arm, 7) pump failure, 8) waste reservoir has to be reconnected (when using the bypass tray function), 9) the hold function has been active for 8 hours, and 10) high or low makeup flow pressure when using Biotage Dalton 2000.

1. Select the **Runtime** tab in the right-hand panel.
2. To enable or disable the audible alarm, select the **Audible Alarm** text box and select **Yes** or **No**.

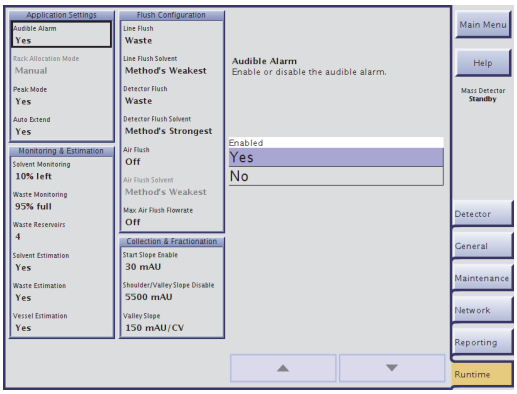


Figure 3-8. Runtime Tab (Isolera Four Shown)

3.6.2 Enable or Disable Automatic Rack Allocation

If automatic rack allocation is enabled, racks or vessels (depending on the selected method) are automatically allocated one at a time as they are needed. The collection area must therefore at all times be loaded with racks in all positions. Note that this feature is not available when using an Isolera Dalton 2000 system.

Two automatic rack allocation methods are available:

- **Automatic:** The system allocates racks in sequence, starting with rack A, then B, and so forth. With this allocation method, the collection tray can be shared by several runs with the same rack type but a single rack cannot be shared by several runs.
- **Automatic Vessel:** The system allocates vessels in sequence, starting with the first vessel in rack A, the second in rack A, and so forth. With this rack allocation method, racks can be shared by several runs but the collection tray cannot be shared by several users.

When enabling automatic rack allocation on an Isolera Four system, the following rules also apply:

- **All racks on the collection tray must be of the same type.** The rack type to be used is decided by the first purification that is queued up.
- **Allocation only when needed.** No rack or vessel allocation is performed when a purification is queued up. When the run is started racks or vessels are allocated one at a time as they are needed.
- **Always replace all racks when asked to.** If the system runs out of vessels, you will be asked to replace all racks in the collection area at the same time (even if some of the racks have been used by previous runs).

To enable or disable automatic rack allocation:

1. Select the **Runtime** tab in the right-hand panel.
2. Select the **Rack Allocation Mode** text box.
3. To enable automatic rack allocation, select **Automatic** or **Automatic Vessel**.
4. To disable automatic rack allocation, select **Manual**.

3.6.3 Enable or Disable Peak Mode

If Peak Mode is enabled, all fractions of a peak will have the same color in the status view (the Status tab in the Chemistry mode).

1. Select the **Runtime** tab in the right-hand panel.
2. To enable or disable Peak Mode in the status view, select the **Peak Mode** text box and select **Yes** or **No**.

3.6.4 Enable or Disable Auto Extend

If Auto Extend is enabled and the collection criteria are still met (remaining light absorption is detected or the mass signal is above the system's threshold) when the system nears the end of a purification, the system enters the Auto-Extend mode. This extends the gradient purification stage of the run with 25% of the total gradient length using the final conditions in the method.

1. Select the **Runtime** tab in the right-hand panel.
2. To enable or disable Auto Extend, select the **Auto Extend** text box and select **Yes** or **No**.

3.6.5 Enable or Disable Monitoring of Reservoirs

NOTE

If using an Isolera Dalton 2000 system, there is no monitoring of the makeup solvent and the waste reservoirs connected to the mass detector and Isolera Dalton Nanolink.

If the Solvent Monitoring and Waste Monitoring options are enabled, the system will issue a warning when it is time to replenish a chromatography solvent or empty a waste reservoir.

To enable solvent and/or waste monitoring:

1. Select the **Runtime** tab in the right-hand panel.
2. To track solvent levels, select the **Solvent Monitoring** text box and select the level when the processing shall be paused and refill requested.
3. To track waste levels, select the **Waste Monitoring** text box and select the level when the processing shall be paused and emptying requested.
4. If using system with four waste outlets, ensure to select the number of waste reservoirs to be used in the **Waste Reservoir** text box. The waste can either be pooled into a single waste reservoir (insert all four waste outlet tubes into a single waste reservoir) or collected in four waste reservoirs (insert each waste outlet tube into a single waste reservoir).
5. If you want an audible alarm to sound when it is time to replenish a chromatography solvent or empty a waste reservoir, check that the Audible Alarm option is enabled (see page 3-10).
6. In the Chemistry mode, enter the capacities and current fluid levels for the solvent and/or waste reservoirs; see "Set the Reservoir Volumes" on page 4-33. (For the system to be able to maintain a running calculation of fluid levels in each reservoir, the capacities and current fluid levels for the reservoirs must be entered each time you empty a waste reservoir or replenish a chromatography solvent.)

To disable solvent and/or waste monitoring:

1. Select the **Runtime** tab in the right-hand panel.
2. Select the **Solvent Monitoring** and/or **Waste Monitoring** text box and select **No**.

3.6.6 Enable or Disable Run Requirement Estimation

NOTE

If using an Isolera Dalton 2000 system, there is no estimation of the makeup solvent and the waste reservoirs connected to the mass detector and Isolera Dalton Nanolink.

The estimation feature is not available when the Assist Workflow is used by a user with the basic workflow editor enabled.

If you enable vessel, solvent, and waste estimation, you will be informed (when allocating the cartridge and rack positions) if a sufficient quantity of vessels is allocated, if a sufficient quantity of the correct solvent is present in each solvent reservoir, and if the waste reservoir has sufficient capacity for the run.

To enable or disable vessel, solvent, and/or waste estimation, select the corresponding text box and select **Yes** or **No**. If using a system with four waste outlets, ensure to select the number of waste reservoirs to be used in the **Waste Reservoir** text box. The waste can either be pooled into a single waste reservoir (insert all four waste outlet tubes into a single waste reservoir) or collected in four waste reservoirs (insert each waste outlet tube into a single waste reservoir).

3.6.7 Specify How Flushes Are Performed

At the end of a purification, i.e. after the gradient purification stage is completed, the system performs a:

- **Line Flush**: The system flushes the inlet line and the cartridge with the specified solvent or solvent mix.
- **Air Flush:** The system flushes the inlet line and the cartridge with air and a small amount of the specified solvent or solvent mix. The flush volume depends on the cartridge type. Air Flush is optional. This feature is not available with Isolera Prime.
- **Detector Flush**: The system flushes the internal detector and any external detector connected to the system with the specified solvent or solvent mix.

To change the flush settings:

1. Select the **Runtime** tab in the right-hand panel.
2. To enable or disable the line flush, select the **Line Flush** text box and select **Collect** (the line flush will be collected on the collection tray), **To Waste** (the line flush will be sent to waste), or **Off** (no line flush will be performed).
3. To enable or disable the detector flush, select the **Detector Flush** text box and select **Collect** (the detector flush will be collected on the collection tray), **To Waste** (the detector flush will be sent to waste), or **Off** (no detector flush will be performed).

NOTE

If the automatic line flush is disabled, ensure to prime the cartridge flow path before each run.

We recommend that the detector flush is kept enabled. A dirty detector flow cell has decreased transmissivity, which causes increased noise level, decreased response, and difficulties performing UV Zero.

4. To enable or disable air flush, select the **Air Flush** text box and select **To Waste** or **Off**. This feature is not available with Isolera Prime.
5. To change the solvent or solvent mix to be used for the line, detector, and/or air flush, select the corresponding text box and select the desired solvent or solvent mix:
   - **Method** – The system will flush with the weakest or strongest solvent used in the run.
   - **System** – The system will flush with the weakest or strongest solvent mounted on the system.
   - **Gradient** – The system will flush with the solvent mix used in the start or the end of the gradient. (Not available for air flush.)
   - **Channel x** – The system will flush with the solvent mounted on the specified channel (1, 2, 3, or 4); see "Assign Solvents to the Solvent Inlets" on page 4-33. Note that Isolera Prime has two solvent inlets while all other Isolera system platforms have four.

3.6.8 Configure Collection and Fractionation

Refer to the "Collection and Fractionation" appendix on page A-1 for details.

1. Select the **Runtime** tab in the right-hand panel.
2. Select the **Start Slope Enable** text box and enter the value when start slope is enabled.
3. Select the **Shoulder/Valley Slope Disable** text box and enter the level when shoulder and valley fractionation is disabled.
4. Select the **Valley Slope** text box and enter the level when valley fractionation occurs.

3.7 Maintenance

3.7.1 Export the System Configuration and Logs

If requested by Biotage® 1-Point Support™ to send in your system's log files, follow the instructions below. The system configuration and logs can be saved on a USB memory device connected to the USB port at the front of the system or in a share folder on your network (if file share has been set up; see page 3-9).

1. Select the **Maintenance** tab in the right-hand panel.
2. If you want to save the system configuration and logs on a USB memory device:
   a. Connect the memory device to the USB port located underneath the touch screen.
   b. Press **Export** in the **System Configuration and Logs** field.
3. If you want to save the system configuration and logs in the specified share folder, press **Export** in the **System Configuration and Logs** field.
4. The system configuration and logs are saved as a zip file at \biotage\isolera\logs\. Send the zip file to Biotage 1-Point Support; see "Contact Information" on page 7-1.

3.7.2 Restore the System Configuration

The system configuration contains all the settings available in the System mode except for the calibration of the mass detector (if used) and the UV detector (which is stored on the detector), the local printer (if connected), and the date and time.

NOTE

Only restore the system configuration when instructed to do so by Biotage. Do not restore the system configuration on your Isolera system using a system configuration for another Isolera system. If you have problem with the restore, please contact Biotage 1-Point Support.

To restore the system configuration e.g. after an update of the system's operating system:

1. Select the **Maintenance** tab in the right-hand panel.
2. Connect the USB memory device containing the zip file with the desired system configuration to the USB port located underneath the touch screen. Note that the zip file must be saved at \biotage\isolera\logs\ on the USB memory device.
3. Press **Restore Configuration** in the **System Configuration and Logs** field.
4. In the **Select Zip File** dialog, select the zip file and press **OK**. The **Restore from USB Device** dialog opens.
5. To confirm restore, press **Restore**.
6. When the **Restore Successful** dialog appears, press **OK**. The **Restart Required** dialog opens.
7. Press **OK** and restart the system:
   a. Press **Main Menu**.
   b. Press **Shut Down** and then **Yes** to confirm.
   c. If using an Isolera Dalton 2000 system, the **Mass Detector** dialog opens. Press **OK**.
   d. When the message saying that it is safe to turn off the system appears on the screen, turn off the system. The power switch is located underneath the touch screen.
   e. Turn on the system.
8. If you have a USB printer connected to the system, set up the printer as described in "Set Up a Printer and Auto Print of Reports" on page 3-8.
9. Verify that the calibration of the fraction collector is working properly. If the system needs to be recalibrated, see "Calibrate the Fraction Collector" on page 3-15.

3.7.3 Back Up and Restore the System's Database

To back up the database:

1. Select the **Maintenance** tab in the right-hand panel.
2. If you want to save the backup on a USB memory device:
   a. Connect a memory device to the USB port located underneath the touch screen.
   b. Press **Back Up** in the **Back Up and Restore Database** field.
3. If you want to save the backup in the specified share folder, press **Back Up** in the **Back Up and Restore Database** field. The backup file is saved at \biotage\isolera\backup\.

To restore the database:

1. Select the **Maintenance** tab in the right-hand panel.
2. If the backup file you want to use to restore the database is saved on a USB memory device (at \biotage\isolera\backup\):
   a. Connect the memory device to the USB port located underneath the touch screen.
   b. Press **Restore** in the **Back Up and Restore Database** field.
3. If the backup is available in the specified share folder (at \biotage\isolera\backup\), press **Restore** in the **Back Up and Restore Database** field.
4. In the **Select Backup File** dialog, select the backup file and press **OK**. The **Restore from USB Device/Share Folder** dialog opens.
5. To confirm restore, press **Restore**.

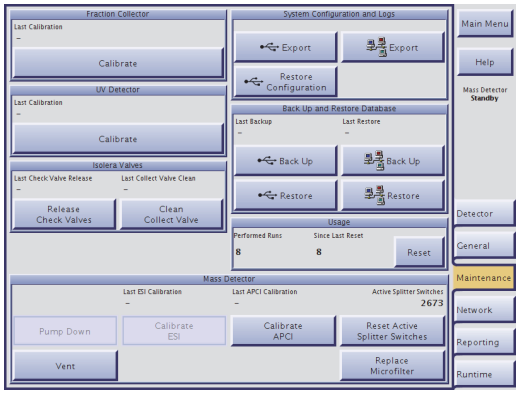


Figure 3-9. Maintenance Tab (Isolera Dalton 2000 Shown)

3.7.4 Calibrate the Fraction Collector

If the fraction collector needs to be recalibrated, use the following procedure.

1. Select the **Maintenance** tab in the right-hand panel.
2. Press **Calibrate** in the **Fraction Collector** field. Read and follow the instructions that appear on the screen.

3.7.5 Calibrate the Internal Detector

If the internal detector needs to be recalibrated (e.g. when a new detector lamp or flow cell has been installed), use the following procedure.

1. Select the **Maintenance** tab in the right-hand panel.
2. Press **Calibrate** in the **UV Detector** field. Read and follow the instructions that appear on the screen.

3.7.6 Perform an Intensity Scan of the Internal Detector

NOTE

The intensity scan feature is only available for detectors produced before December 2017.

If requested by Biotage 1-Point Support to perform an intensity scan of the internal detector, follow the instructions below.

1. Select the **Maintenance** tab in the right-hand panel.
2. Press **Intensity Scan** in the **UV Detector** field. Read and follow the instructions that appear on the screen. When the intensity scan has been completed successfully, the data is saved in a system log.
3. Export all system logs as described on page 3-13.
4. Send the system logs to Biotage 1-Point Support; see "Contact Information" on page 7-1.

3.7.7 Clean the Collect Valve

Dripping needle and/or inconsistent dispensing volumes can be signs of a dirty collect valve. Use the following procedure to clean the collect valve.

1. Select the **Maintenance** tab in the right-hand panel.
2. Press **Clean Collect Valve** in the **Valves** field. Read and follow the instructions that appear on the screen.

3.7.8 Release Stuck Check Valves

Low or inconsistent flow delivery volume and/or superimposed periodic UV or UV-VIS signals can be signs of sticking check valves. Use the following procedure to release stuck check valves.

1. Select the **Maintenance** tab in the right-hand panel.
2. Press **Release Check Valves** in the **Valves** field. Read and follow the instructions that appear on the screen.

3.7.9 View and Reset the Usage Statistics

Both the number of runs performed on the system since it was installed and the number of runs performed since the last reset are displayed in the **Usage** field at the **Maintenance** tab.

To reset the usage statistics:

1. Select the **Maintenance** tab in the right-hand panel.
2. Press **Reset** in the **Usage** field. The **Reset Performed Runs** dialog opens.
3. To confirm reset, press **Yes**.

3.7.10 Maintenance of Isolera™ Dalton 2000 Systems

If using an Isolera Dalton 2000 system, the following buttons are available in the **Mass Detector** field at the **Maintenance** tab:

- **Vent:** Only vent the mass detector when you want to 1) disconnect the mass detector to use Isolera One/Four without it or to move the system, 2) replace external tubing, or 3) clean the exterior of the system.
- **Pump Down:** If the mass detector is vented, it has to be pumped down for 30 minutes before it can be used.
- **Calibrate APCI/ESI:** The mass detector needs to be calibrated after a move or when a new ion source is used with the system. Only the calibration button for the installed ion source is enabled.
- **Reset Active Splitter:** The counter for active splitter switches should be reset after replacement of the rotor seal and stator face assembly.
- **Replace Microfilter:** Open a wizard to replace the microfilter.

For complete maintenance instructions for Isolera Dalton 2000 systems, please refer to the "Maintenance" section in the "Isolera™ Dalton 2000 User Manual" (P/N 415730).

Chapter 4

Operation (Chemistry Mode)

WARNING

Before performing any procedures in this chapter, please read and observe the safety requirements in the "Isolera™ Installation and Safety" document (P/N 415797). Failure to follow those requirements may result in personal injury and/or equipment damage.

4.1 Start Up the System

It is recommended that you start up the system in the following order:

1. Ensure that all liquid lines are connected correctly and securely; see the "Isolera™ Installation and Safety" document (P/N 415796).
2. If using an Isolera Dalton 2000 system:
   a. Ensure that the mass detector is in standby mode (green light). If the mass detector has been turned off, turn it on as described in the "Isolera™ Dalton 2000 User Manual" (P/N 415730).
   b. Turn on Isolera Dalton Nanolink using the power switch located at the rear.

WARNING

To avoid being struck by the collection arm, keep your hands out of range of the collection arm while the homing routine runs in step 3 below.

3. Turn on the Isolera system. The power switch is located underneath the touch screen. The collection arm moves through its homing routine and the system boots to the system's main menu.
4. To set up, control, monitor, and review a purification, press either **New** to use the Assist Workflow (see the "Isolera™ Assist Quick Guide" (P/N 414767) for more information) or **Chemistry**.

If you want to administrate cartridge types, methods, rack types, results, solvents, and user accounts, see "Log into the Data Administration Mode" on page 2-1.

If you want to change detector, network, reporting, and runtime settings, set the system clock, set the language used in the Chemistry mode, enable the λ-all detection mode, the TLC to Step Gradient editor (requires an Isolera Spektra or Dalton 2000 software license) and the Assist Workflow, calibrate the fraction collector, internal detector, and mass detector (optional), release stuck check valves, clean the collect valve, back up and restore the database, export the system configuration and logs, restore the system configuration, view and reset usage statistics, etc, see "Log into the System Mode" on page 3-1.

For more information about the software modes, see "Software Description" on page 1-8.

4.2 Shut Down the System

WARNING

Failure to perform an orderly system shutdown may result in user data corruption. If possible, avoid shutting down during a purification.

NOTE

If using an Isolera Dalton 2000 system, we recommend that you only shut down the mass detector when you want to 1) disconnect the mass detector to use the system without it or to move the system, 2) replace the external tubing, or 3) clean the exterior of the system. To shut down the mass detector, see the instructions in the "Isolera™ Dalton 2000 User Manual" (P/N 415730).

If using an Isolera Dalton 2000 system, do not turn off the roughing pump when the mass detector is on.

Use the following procedure to perform an orderly system shutdown:

1. If you are not at the main menu, press **Main Menu** in the right-hand panel.
2. Press **Shut Down** and then **Yes** to confirm.
3. If using an Isolera Dalton 2000 system, press **OK** in the **Mass Detector** dialog that opens.
4. When the message saying that it is safe to turn off the system appears on the screen, turn off the system. The power switch is located underneath the touch screen.
5. If using an Isolera Dalton 2000 system, turn off Isolera Dalton Nanolink using the power switch located at the rear and, if desired, unplug its power cord from the power outlet. Do not turn off or unplug the mass detector or roughing pump.
6. If desired, unplug the power cord for Isolera from the power outlet.

4.3 Control the UV Lamp

The UV lamp must be warmed up in order for the signal to be accurate and stable.

NOTE

The UV lamp should be turned on 7.5 minutes before starting a purification so the UV lamp can reach operating temperature.

The lamp(s)* is/are automatically turned on when:

- The Isolera system is switched on.
- The user enters the Chemistry mode (i.e. presses the **New** or **Chemistry** button in the main menu).
- The user starts a run (i.e. presses the ▶ button in the **Run Parameters** dialog). However, before starting the run, the software waits (approximately 7.5 minutes) for the UV lamp to warm up.

You can also turn on the internal detector lamp(s) manually by pressing **Turn Lamp On** in the right-hand panel. By doing this ahead of time, you can avoid the delay that is required if the software turns the lamp(s) on at the start of a run.

To prolong lamp life, the lamp(s) is/are turned off automatically if the system is idle (no purification is started and no user interaction is detected) for approximately two hours.

* If your system is equipped with an UV-VIS detector that was produced before December 2017, it has one UV lamp and one tungsten lamp.

Bypass the Automatic UV Lamp Warm-up Period

You can bypass the automatic UV lamp warm-up period by pressing **Skip Warm Up** in the right-hand panel. However, if the UV lamp has not reached operating temperature, purification results may not be optimal. Note that the **Skip Warm Up** button is disabled for the first 45 seconds of the UV lamp warm-up period.

4.4 Create, Open, and Edit Methods

NOTE

The Assist Workflow is a streamlined and simplified way to set up and run purifications. If you want to use the Assist Workflow, press New in the main menu. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.

4.4.1 Create or Open a Method

NOTE

If using the Assist Workflow, a user with the basic editor enabled will not have full functionality at the Method tab.

If you want to use the method wizard, see page 4-15. The wizard will guide you step-by-step through the setup of a purification with a gradient including up to three steps using two solvents.

When opening an old method in the Isolera 2.2 software or later, you may get the option to upgrade the method with a new default flow rate. If you accept for a method with a SNAP KP-Sil, SNAP Ultra (not C18), or ZIP Sphere cartridge, a gradient equilibration will be used if the percentage of the strongest solvent is above 10% in the initial solvent mix, and the equilibration length will be set to a fixed value. If you do not accept, you will not be able to change the cartridge type or the flow rate, and you will not be able to save any changes. Note that the method upgrade option is not applicable when using an Isolera Prime system.

1. To create a new method:
   c. In the chemistry mode, select the **Method** tab in the right-hand panel and press **New...**.
   d. If the system is configured to use default methods (see "Set Default Methods" on page 2-5), the **New Method** dialog opens. Select the default method that you want to base your method on and press **OK**. (If there is only one default method, it will be opened when you press **New**.)

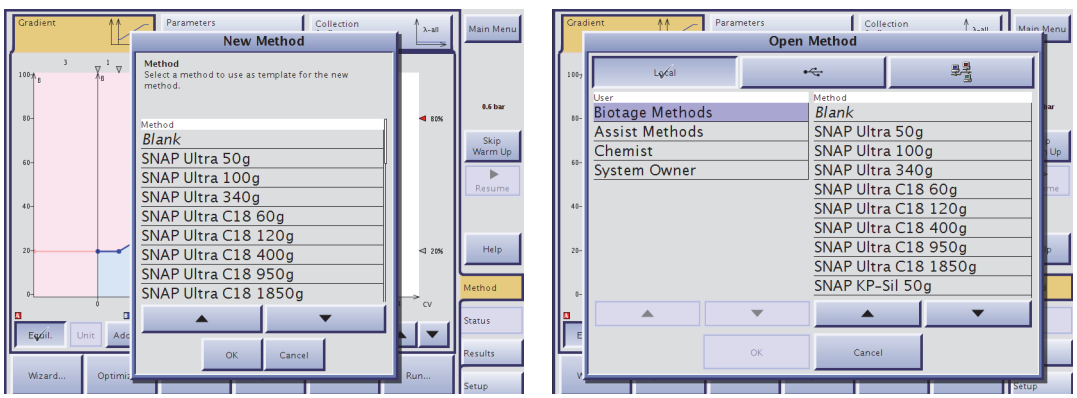


Figure 4-1. New Method and Open Method Dialogs (Isolera LS Shown)

2. To open and edit an existing method:
   a. Select the **Method** tab in the right-hand panel and press **Open...**.
   b. If the method is stored in the system's database, select the owner of the method in the **User** column in the **Open Method** dialog. All methods of the selected user are listed. To select one of the preconfigured Biotage methods, select the user "Biotage Methods".
   c. If the method is stored on a USB memory device, connect the device to the USB port located underneath the touch screen and press in the **Open Method** dialog.
   d. If the method is stored in the share folder on your network, press in the **Open Method** dialog. (If the button is disabled, no file sharing has been set up. See "Set Up File Sharing and Auto Save of Reports" on page 3-9.)
   e. Select the method and press **OK**.
3. To create a method from a previous purification:
   a. Select the **Results** tab in the right-hand panel.
   b. Select the run at the **Result Selection** tab and press **Create**. For information on how to find the desired run, see "Search for Records" on page 4-29. The method is opened at the **Method** tab and can be edited and/or saved. If desired, the system can help you to optimize the gradient to isolate one of the peaks and reduce the amount of solvent used. For instructions, see "Create a Gradient at the Gradient Tab" on page 4-4.

Create a Gradient at the Gradient Tab

NOTE

If you want to calculate the gradient from TLC data, see page 4-12.

Isolera Prime has two solvent inlets (A and B) while all the other Isolera system platforms have four (A, B, C, and D).

If using an Isolera Dalton 2000 system, always add an equilibration step of at least 3 CV to avoid clogging of the mass detector and Isolera Dalton Nanolink.

1. Select the **Gradient** tab.
2. The gradient can be set up using the gradient table and/or the gradient graph. To show or hide the gradient table, press the **Table** button (✓ = gradient table is shown).

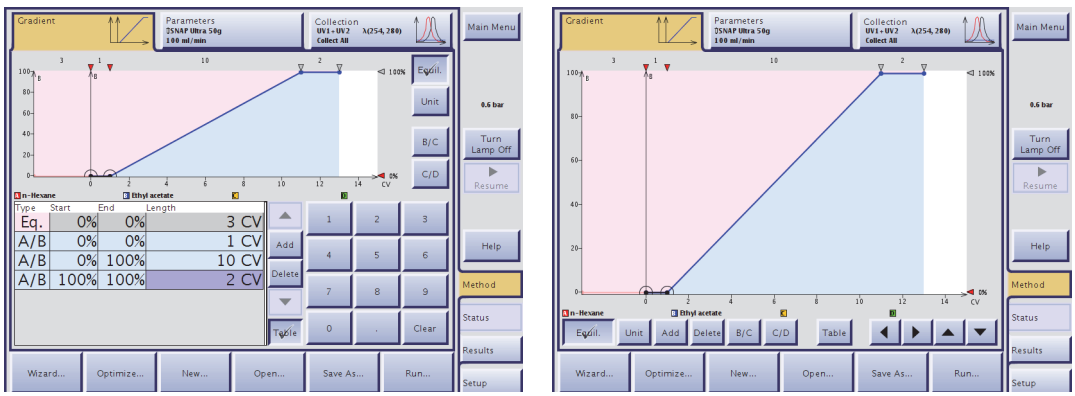


Figure 4-2. Gradient Tab With and Without Gradient Table (System With Four Solvent Inlets Shown)

3. In the graph, the X-axis indicates the length and the Y-axis indicates the percentage of Solvent B (if a blue gradient line), Solvent C (if a yellow gradient line), or Solvent D (if a green gradient line) in the solvent mix. To change the length unit, press the **Unit** button repeatedly until the desired unit appears; CV (Column Volumes), milliliter, or minutes. CV is the default unit.
4. If you want to equilibrate the cartridge before the run, press **Equil.** (✓ = equilibration is enabled). 2 to 5 CV are recommended for full equilibration. Note that the equilibration length is fixed when gradient equilibration is used. SNAP KP-Sil, SNAP Ultra (not C18), and ZIP Sphere cartridges also have a fixed equilibration length for isocratic equilibration.

   If gradient equilibration is enabled for the selected cartridge, it is used when the percentage of the strongest solvent is above 10% in the initial solvent mix. When less or equal to 10%, isocratic equilibration is used. See Figure 4-3.

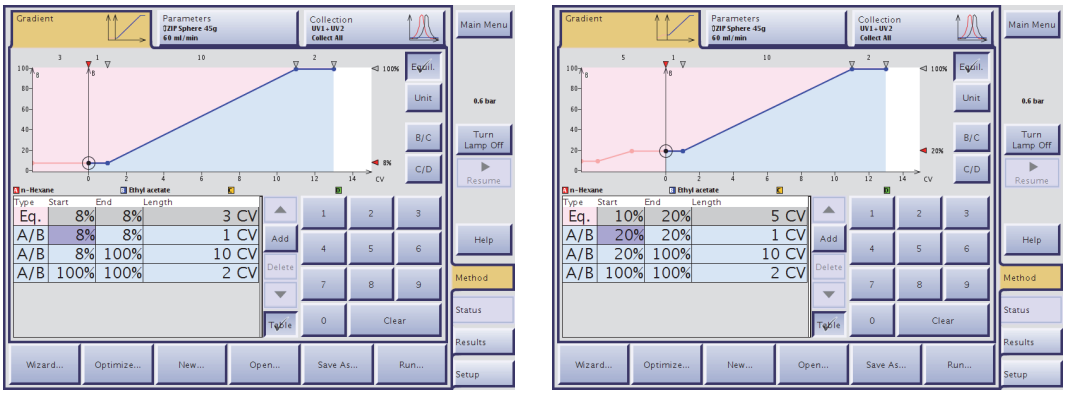


Figure 4-3. Linear or Gradient Equilibration Depending on the Solvent Strength (System With Four Solvent Inlets Shown)

5. To add a gradient segment, select a segment in the gradient table or the graph (see Figure 4-4) and press **Add**. The new segment will be added after the selected segment.
6. To add a gradient segment with Solvent B (■) and Solvent C (■) and/or with Solvent C and Solvent D (■), press **B/C** and/or **C/D**. The new segment will be added at the end of the gradient.
7. To delete a gradient segment, select it in the gradient table or the graph (see Figure 4-4) and press **Delete**.
8. To increase or decrease the length of a gradient segment or change the solvent mix:
   - Using the gradient graph: Drag the segment or node to the desired position (with 0.5 CV and 5% resolution). To fine tune (with 0.1 CV and 1% resolution), select a segment or node (see Figure 4-4) and use the ◀ and ▶ buttons to increase or decrease the length of a segment and the ▲ and ▼ buttons to increase or decrease the solvent percentage. The fine tuning buttons are only available when the gradient table is not shown.
   - Using the gradient table: Select the value you want to change and enter the desired value using the keypad.

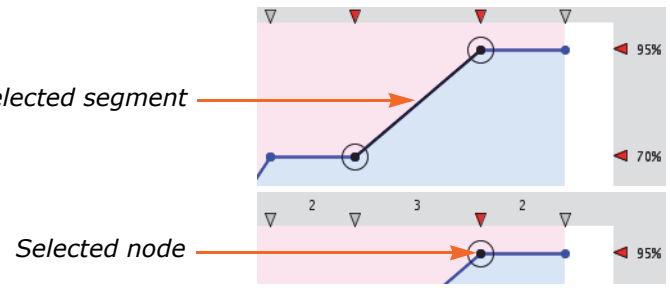


Figure 4-4. Selected Gradient Segment and Node

Specify the Method Parameters at the Parameters Tab

1. Select the **Parameters** tab.
2. Select the **User** text box and select your user name. If you have not been assigned a user name, please contact your system supervisor.
3. Select the **Cartridge Type** text box and select the cartridge type to be used. If you do not want to use the default (recommended) flow rate and/or equilibration flow rate for the cartridge type, select the corresponding text box and enter the desired flow rate. The maximum flow rate that can be used depends on:
   - the cartridge and any other accessories used in the setup,
   - the maximum fill rate(s) defined for the used solvent(s) in the Data Administration mode, and
   - the maximum dispense rate of the system (100 ml/min when using Isolera Dalton 2000 with the mass detector, 200 ml/min when using Isolera Prime, Isolera One, Isolera Four, or Isolera Dalton 2000 with only the internal UV detector, 500 ml/min when using Isolera LS) or, if using an external detector, the maximum dispense rate defined for the external detector in the System mode.

WARNING

Do not exceed recommended flow rates for flash cartridges (this information can be found in the flash cartridge documentation) nor allow flow at higher rates than specified through any Samplet, dry load vessels, or pre-cartridges that you may be using. This can increase the risk of static build-up.

Read and follow the safety precautions against static electricity in the "Isolera™ Installation and Safety" document (P/N 415796) that is supplied with the system.

If using an Isolera Prime system: As a single-piston pump only delivers liquid during the dispense stroke, the maximum flow rate when using an Isolera Prime system is equal to (Max Fill Rate x Max Dispense Rate)/(Max Fill Rate + Max Dispense Rate). Examples: If the fill rates for the used solvents are 50 ml/min and 80 ml/min and you use a external detector with the maximum dispense rate of 100 ml/min, the highest flow rate that can be used is (50x100)/(50+100)=33 ml/min. If the external detector is disabled, the highest flow rate that can be used is (50x200)/(50+200)=40 ml/min.

WARNING

If small test tubes are to be used, ensure to adjust the flow rate so that no splashing will occur when fractions are collected.

4. Select the **Rack Type** text box and select the rack type to be used; see Table 4-1 on page 4-8 for guidance. If you do not want to use the default max fraction volume for the vessel type and/or the default dispensation order (S-pattern), select the corresponding text box and change the setting. The software prevents you from entering a fraction volume that will overflow the vessels.
5. Select the solvents to be used by selecting the corresponding text box in the **Solvents** field. (They should be the solvents determined by the TLC procedure.) To show all solvents, press **Show All**. To only show the solvents connected to the system, press **Show Connected**. Note that the solvent percentages are specified at the **Gradient** tab.

NOTE

For reversed-phase purification with methanol and water, it is strongly recommended to premix the water with 5% of methanol and degass either through vacuum or sonication. Degassing of protic solvent blends decreases out-gassing of entrapped air during gradient elution, which will impact gradient performance and flow rates.

If automatic rack allocation is enabled, all racks on the collection tray must be of the same type. When using a system with four cartridge positions, the rack type to be used is decided by the first purification that is queued up. Any purification queued up after that must use the same rack type. To change rack type, wait until all present runs are finished and then remove them at the Status tab. Note that a user with the basic workflow editor enabled cannot queue runs when using the Assist Workflow.

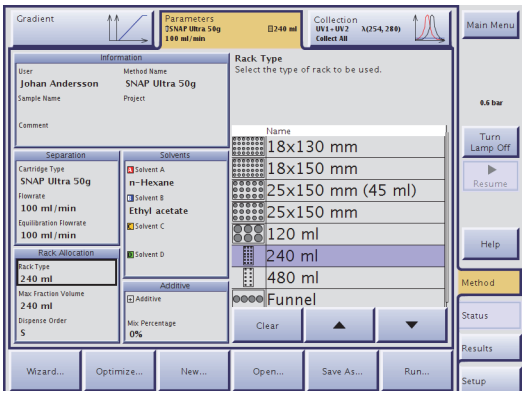


Figure 4-5. Parameters Tab (Isolera LS Shown)

Optional Method Parameters

- **Method Name:** If you want to save the method, select the **Method Name** text box and enter a method name. If you are editing an existing method and save it under a new name, the original method remains unaltered under its original name.
- **Sample Name:** If left blank, the sample name will be auto-generated when the purification is performed. To enter a sample name, select the **Sample Name** text box.
- **Project:** If you want the method to be associated with a project, select the **Project** text box and select or create the project.
- **Comment:** If you want to enter a comment, select the **Comment** text box.
- **Additive:** If you want to use a fixed percentage of additive during the purification, select the additive to be used (by selecting the **Additive** text box) and the percentage of the additive in the solvent mix (by selecting the **Mix Percentage** text box). Not available with Isolera Prime.

Rack Selection

Based on 13 CV gradient in Collect All mode (with slope and valley fractionation disabled), the following apply in the rack selection table on page 4-8:

Requires one (1) rack of this type.

Requires multiple racks of this type, but no rack change during the run. See the number of racks required in the table.

Rack change required during the run. See the number of racks required in the table.

Not recommended.

Note that:

- Isolera Prime can only be used with one collection tray.
- The maximum collection volume for Isolera LS can be increased from 9.6 liters up to 320 liters by using a funnel rack kit from Biotage. The funnel rack kit comes with two racks (16 positions each) and a cart with wheels that holds the Isolera LS system and the collection vessels. For more information, please contact your local representative.

Table 4-1: Rack Selection Table

System with One Collection Tray

Rack Type	Racks per Tray	Volume per Tray (ml)	Cartridge Type (CV in ml**)								
			10g* (15/17)	25g* (33/45)	50g (66/90)	100g (132/164)	340g (470/590)	75M§ (528)	750g§ (990)	75L§ (1056)	
13x100 mm†	4	432 x 4			2	2/3	4/5				
16x100 mm†	4	490 x 4			2	2/3	4/5				
18x130 mm†	4	504 x 4			2	2/3	4/5				
16x150 mm†	4	770 x 4				2	3				
18x150 mm	4	756 x 4				2	3				
25x150 mm	4	795 x 4‡				2	3				
120 ml	4	720 x 4				2	3				
240 ml	1	4320						2	2		
480 ml	1	4800						2	2		

System with Two Collection Trays¶

Rack Type	Racks per Tray	Volume per Tray (ml)	Cartridge Type (CV in ml**)								
			10g* (15/17)	25g* (33/45)	50g (66/90)	100g (132/164)	340g (470/590)	75M§ (528)	750g§ (990)	75L§ (1056)	1500g# (1980)
13x100 mm†	4	432 x 4		2	2/3	4/5	15/18	16			
16x100 mm†	4	490 x 4		2	2/3	4/5	13/16	15			
18x130 mm†	4	504 x 4		2	2/3	4/5	13/16	14			
16x150 mm†	4	770 x 4			2	3	8/10	9			
18x150 mm	4	756 x 4			2	3	9/11	10			
25x150 mm	4	795 x 4‡			2	3	8/10	9			
120 ml	4	720 x 4			2	3	9/11	10			
240 ml	1	4320					2	2	3	4	6
480 ml	1	4800					2	2	3	3	6

* Cannot be used on Isolera LS.
† Not recommended for use on Isolera LS.
‡ When using 53-ml test tubes.
§ Not recommended for use on Isolera Prime or Isolera Dalton 2000 when using the mass detector.
¶ Not available with Isolera Prime. Two collection trays can be used if the system is upgraded to Isolera One or Four.
# Not recommended for use on Isolera Prime, Isolera One, Isolera Four, or Isolera Dalton 2000.
** When two column volumes are listed, the first is for SNAP KP-Sil and the second is for SNAP Ultra.

Specify Collection Parameters at the Collection Tab

1. Select the **Collection** tab.
2. Select the signal to be used for collection by selecting **Collect** in the appearing **Signal Usage** list (see the image below). Available detection signals are:
   - **TIC:** The system uses the mass detector signal to monitor and collect based on mass-to-charge ratio (m/z). Collection and fractionation is based on the sum of all m/z signals in the selected m/z range. Background noise from the chromatography solvents, makeup solvent, and the mass detector itself is automatically removed from the TIC signals. Fractionation occurs on threshold, valley, and volume for both positive and negative ionization. Possible collection parameter is Collect All. (Only available on Isolera Dalton 2000.)
   - **XIC:** The system uses the mass detector signal to monitor and collect based on mass-to-charge ratio (m/z). Collection and fractionation is based on the sum of the m/z-ratio +/- 0.6 for up to four different ions. Each XIC signal has its own threshold, which is automatically set based on the noise level at the start of the run. The finishing threshold level for XIC is 10% of the highest value of the peak or the start threshold, whichever is higher. Fractionation occurs on threshold and volume. Possible collection parameters are Collect All and End Run After Peak. (Only available on Isolera Dalton 2000.)
   - **λ-all:** The system uses average absorbance within a user-defined wavelength range for collection and fractionation. Possible collection and fractionation parameters are Collect All, Start Threshold, and Valley (enable/disable in System mode). (Only available on systems with an Isolera Spektra or Dalton 2000 software license installed.)
   - **UV1 and UV2:** The system uses one or two wavelength signals for collection and fractionation. Possible collection and fractionation parameters are Collect All, Start Threshold, Slope, Shoulder, and Valley (enable/disable in System mode).
   - **External:** Control the collection and fractionation using an external detector (e.g. Biotage ELSD-A120). Possible collection and fractionation parameters are Collect All and Start Threshold. Note that the external detector has to be enabled in system mode to be displayed at the Collection tab.

   If no signal is used for collection and fractionation, the collection has to be started and stopped by the user. Automatic fractionation only occurs on volume.
3. Signals that are not used for collection and fractionation can be used for monitoring; select **Monitor** in the appearing **Signal Usage** list (see the image below).

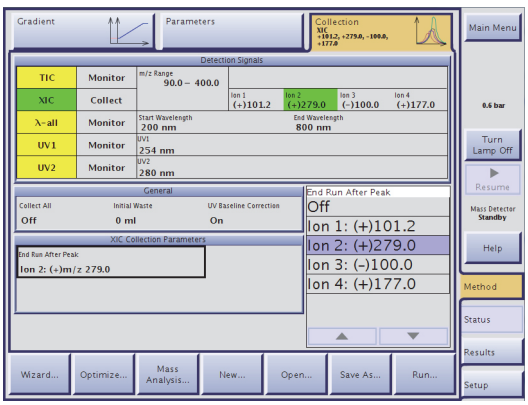


Figure 4-6. Collection Tab (Isolera Dalton 2000 Shown)

4. Select the collection parameters available for the selected detection signal. Refer to the tables below for guidance. For more information; see the "Collection and Fractionation" appendix on page A-1.

Table 4-2: General Collection Parameters

Parameter	Description
Collect All	Used to collect the entire run. This option can be toggled during the run to manually stop and start collection. Fractionation based on volume and collection signal(s) will still occur.
Initial Waste	Delay collection until a specified volume has been delivered to the waste.
UV Baseline Correction	If you want the system to perform UV baseline correction, turn on the **UV Baseline Correction** option. (Only available on systems with an Isolera Spektra or Dalton 2000 software license installed.) When this option is turned on, the gradient run is preceded by a light absorbance detection phase. During this phase, the UV absorbance of the used chromatography solvents is measured at all wavelengths that will be used for collection and fractionation (UV1, UV1+UV2, or λ-all). The measurement results in a baseline containing the maximum absorbance of the solvent. During the gradient run, the baseline is subtracted from the signal.

Table 4-3: λ-All Collection and Fractionation Parameters

Parameter	Description
Start/End Wavelength	The shortest/longest wavelength to be included in the λ-all signal. The range is 200 to 400 nm (UV detector) or 200 to 800 nm (UV-VIS detector).
Start Threshold	Used to collect samples with an absorbance exceeding the set absorbance threshold.

Table 4-4: TIC and XIC Collection and Fractionation Parameters

Parameter	Description
m/z Range	The used mass-to-charge range. Default range is 90 to 2000 but by reducing the range, the signal may be improved. The maximum range can be expanded by a setting in the System mode.
Ion 1-4	The mass-to-charge ratio(s) of the ion(s) used for detection, collection, and fractionation. The values must be within the set m/z Range and can be either entered manually or identified using the mass analysis wizard (see "Analyze the Sample" below). Positive or negative ionization can be selected for each ion individually.
End Run After Peak	When this option is turned on, the run is automatically ended once a sufficiently large peak of the selected ion has been collected. If the peak is too small, the run will continue. Only available when collecting on XIC.

Table 4-5: UV Collection and Fractionation Parameters

Parameter	Description
UV1 and UV2	The wavelengths to be used for channel 1 (**UV1**) and channel 2 (**UV2**). The range is 200 to 400 nm (UV detector) or 200 to 800 nm (UV-VIS detector).
Start Threshold	Used to collect samples with an absorbance exceeding the set absorbance threshold.
Slope	Collection is based on the start slope of the peaks. Refer to the "Collection and Fractionation" appendix on page A-1 for details.

Analyze the Sample (Only Isolera™ Dalton 2000)

A chemical sample, containing one or more components and dissolved in suitable solvent(s), can be injected into the system to identify the m/z-ratio(s) for the substance(s) of interest.

NOTE

When using ASAP, the results may differ from the run, as any potential makeup solvent adducts will not be produced.

Reducing the mass range will improve the signal-to-noise ratio.

1. If injecting the sample into the liquid injection valve on the mass detector, prepare the sample as follows:
   a. Make at least 0.5 ml diluted solution of your sample, approximately 0.01 mg/ml in LC-MS grade acetonitrile or your makeup solvent.
   b. Filter (0.5 µm or less pore size) your sample carefully to avoid clogging.
2. If introducing the sample into the mass detector using the optional ASAP probe, prepare the sample as follows:
   a. Dip the probe tip into a liquid or dissolved sample, or scratch a solid sample using the probe tip.
   b. Carefully wipe away any excess of sample from the probe tip using a clean, lint-free tissue.
3. Press **Mass Analysis...** at the **Method** tab. This opens the Mass Analysis Wizard. Follow the on-screen instructions.
4. When the analysis is completed, copy the identified m/z-ratio(s) and range to the current method by pressing **Save to Editor and Close** in the last step of the wizard. The spectra will be included in the archive report.

Save and/or Run the Method

1. If you want to save the method and reuse it in the future, press **Save As**. The **Save Method As** dialog opens.
2. Select the user name and enter the method name by selecting the corresponding text boxes.
3. To save the method in the system's database, press **Save**. If your user account is password-protected, the **Password** dialog opens. Enter your password, using the keypad, and press **OK**.
4. To save the method on a USB memory device, connect the device to the USB port located underneath the touch screen and press **Save**.
5. To save the method in a share folder on your network, press **Save**. (If the button is disabled, no file sharing has been set up. See "Set Up File Sharing and Auto Save of Reports" on page 3-9.)
6. To run a purification using the (saved or unsaved) method, see "Prepare and Run a Purification" on page 4-17.

NOTE

You do <u>not</u> have to save your method to be able to run it.

4.4.2 Calculate Gradient from TLC Data

Use the TLC to Linear Gradient editor or the TLC to Step Gradient editor (requires an Isolera Spektra or Dalton 2000 software license) to calculate a purification gradient and get cartridge and sample load recommendations.

NOTE

When using the Assist Workflow, the TLC editors are built into the workflow and cannot be accessed from the Method tab by a user with the basic workflow editor enabled. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.

TLC to Linear Gradient

The software's TLC to Linear Gradient editor is used to enter the following parameters obtained from a TLC procedure:

- R_f values for the compound of interest and the closest eluting compounds on the leading and trailing sides of the compound of interest.
- TLC solvents and their percentages.

The software uses these values to calculate a purification gradient and recommend cartridge type for your sample mass or vice versa.

1. Select the **Method** tab in the right-hand panel.
2. Press **Optimize...** and if the **Optimize...** dialog opens, press **Open TLC to Linear Gradient Editor**. The TLC to Linear Gradient editor opens.
3. Select the two solvents to be used by selecting the **Solvent A** and **Solvent B** text boxes. Normally the solvent with the lower solvent strength is selected as Solvent A.
4. Select the **Solvent Conditions** text box and enter the percentage of Solvent B in the TLC solution.

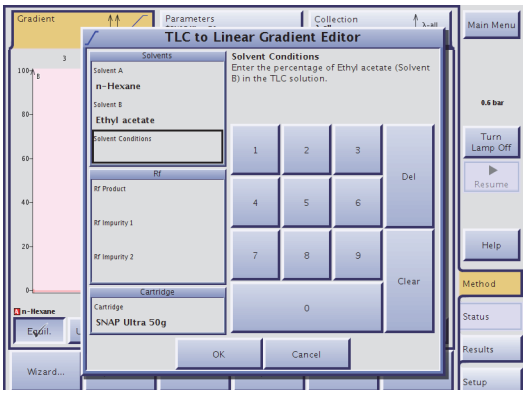


Figure 4-7. TLC to Linear Gradient Editor

5. Select the **Rf Product** text box and enter the R_f value of the product of interest.
6. Enter the R_f value of the impurity closest below and above the product in the **Rf Impurity 1** and **Rf Impurity 2** text boxes. A value for **Rf Impurity 1** is required.
7. Select the **Cartridge** text box and select a cartridge type with a loading capacity exceeding your crude sample mass.

8. To save the data, press **OK**. The TLC to Linear Gradient editor closes and the software calculates the purification gradient based on the TLC data and displays it at the **Gradient** tab.
   If gradient equilibration is enabled for the selected cartridge, it is used when the percentage of the strongest solvent is above 10% in the initial solvent mix. When less or equal to 10%, isocratic equilibration is used. Note that the equilibration length is fixed when gradient equilibration is used. SNAP KP-Sil, SNAP Ultra (not C18), and ZIP Sphere cartridges also have a fixed equilibration length for isocratic equilibration.
9. Set up the rest of the method parameters; see "Specify the Method Parameters at the Parameters Tab" on page 4-6 and "Specify Collection Parameters at the Collection Tab" on page 4-9.
10. To save the method and reuse it in the future, press **Save As**. See "Save and/or Run the Method" on page 4-11.
11. To run a purification using the (saved or unsaved) method, see "Prepare and Run a Purification" on page 4-17.

TLC to Step Gradient

The software's TLC to Step Gradient editor is used to enter the following parameters:

- The R_f value for each compound of interest, for each TLC plate.
- The percentage of the strongest solvent in the TLC solution, for each TLC plate.
- The crude sample mass.

The software uses these values to calculate a purification gradient and recommend a cartridge type.

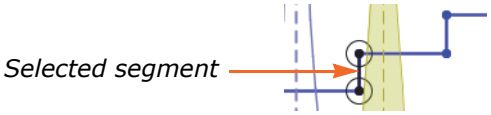
Note the following when using the TLC to Step Gradient editor:

- At least two TLC plates with R_f values greater than 0.04 are necessary to predict the mobility of the compounds.
- Accuracy of prediction can be reduced if using alcohols, such as methanol and ethanol, very volatile modifiers such as diethyl ether, or additives that permanently alter the properties of the silica.
- Internal delays due to the tubing is not accounted for in the predicted result. This is especially apparent when using the mass detector (only Isolera Dalton 2000) and/or small cartridges.
- Reversed-phase chromatography is currently not supported.
- Store the TLC plates in a dry and dark place.
- Use only freshly prepared TLC solutions.
- Slight variations in migration rates will be observed if samples are applied too near to the edge of the TLC plate. Apply samples at least 10 mm from the edge to avoid the edge effect.

To create a purification gradient using the TLC to Step Gradient editor:

1. Select the **Method** tab in the right-hand panel.
2. Press **Optimize...**. The **Optimize...** dialog opens.
3. Press **Open TLC to Step Gradient Editor**. The TLC to Step Gradient editor opens.
4. Enter the percentage of the strongest solvent in the TLC solution, for each TLC plate, by pressing the "0%" table headers.
   To add a plate, press **Add Plate...**.
   To delete a plate, select its table header and press **Delete Plate**. Note that two plates are required to predict the mobility of the compounds.
5. Enter the R_f value for each compound of interest, for each TLC plate, by pressing the corresponding table cells. Valid range is 0.05 to 0.95.
   To add a compound, press **Add Compound...**.
   To delete a compound, select its table header and press **Delete Compound**.
6. If desired, enter the name of each compound by pressing the compound table headers.

7. To view and modify (if desired) the predicted step gradient, select the **Gradient and Peaks** tab.
   - To exclude e.g. the last compound, select the second last compound by pressing on its label and then press **End Gradient At Selected Peak**.
   - To modify the outcome, drag a peak (by dragging the compound label) or a gradient segment to the desired position.



8. If desired, select the compound of interest by pressing on its label and press **Set Peak of Interest**.
9. When satisfied with the gradient, select the **Cartridge Selection** tab.
10. Select the **Sample Mass** text box and enter the crude sample mass.
11. Select the **Cartridge** text box and select a cartridge type with a loading capacity exceeding your crude sample mass. If a peak of interest has been set (see step 8), the load capacity is calculated according to the smallest ΔCV between the peak of interest and the peak before and after. If no peak of interest has been set, the load capacity for each cartridge type is calculated according to the smallest ΔCV that is required to separate all peaks.
12. To close the TLC to Step Gradient editor and open a method containing the purification gradient at the **Method** tab, press **OK**.

    If gradient equilibration is enabled for the selected cartridge, it is used when the percentage of the strongest solvent is above 10% in the initial solvent mix. When less or equal to 10%, isocratic equilibration is used. Note that the equilibration length is fixed when gradient equilibration is used. SNAP KP-Sil, SNAP Ultra (not C18), and ZIP Sphere cartridges also have a fixed equilibration length for isocratic equilibration.
13. Set up the rest of the method parameters; see "Specify the Method Parameters at the Parameters Tab" on page 4-6 and "Specify Collection Parameters at the Collection Tab" on page 4-9.
14. To save the method and reuse it in the future, see "Save and/or Run the Method" on page 4-11.
15. To run a purification using the (saved or unsaved) method, see "Prepare and Run a Purification" on page 4-17.

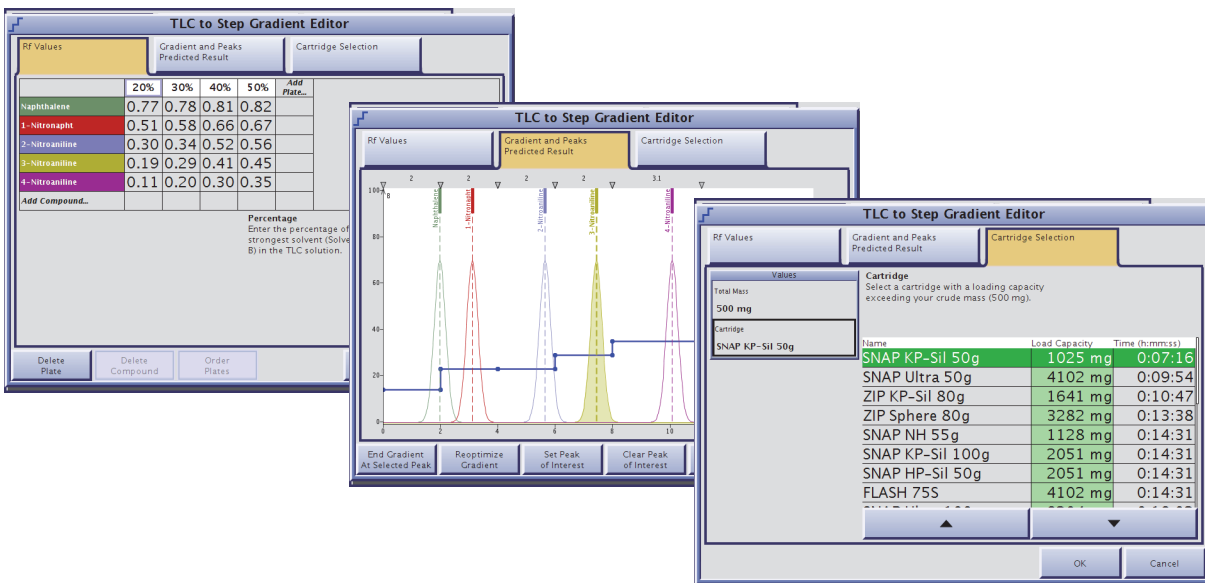


Figure 4-8. TLC to Step Gradient Editor (Isolera LS Shown)

4.4.3 Create a Method Using the Method Wizard

The method wizard will guide you step-by-step through the setup of a purification with a gradient including up to three steps using two solvents.

1. Select the **Method** tab in the right-hand panel.
2. Press **Wizard...**. The method wizard opens.

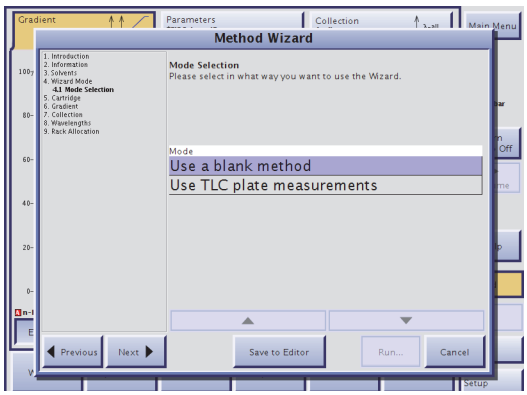


Figure 4-9. Method Wizard

3. Read and follow the instructions that appear on the screen. (See Table 4-1 on page 4-8 for guidance in selecting rack type.)
4. When you are pleased with the method, press **Save to Editor**. The method is opened at the **Method** tab and can be edited and/or saved.

   If gradient equilibration is enabled for the selected cartridge, it is used when the percentage of the strongest solvent is above 10% in the initial solvent mix. When less or equal to 10%, isocratic equilibration is used. Note that the equilibration length is fixed when gradient equilibration is used. SNAP KP-Sil, SNAP Ultra (not C18), and ZIP Sphere cartridges also have a fixed equilibration length for isocratic equilibration.
5. If you want to save the method and reuse it in the future, press **Save As**. See "Save and/or Run the Method" on page 4-11.
6. To run a purification using the method, press **Run...**.
7. Prepare the system as described on page 4-17.
8. Proceed with step 5 in "Run a Purification" on page 4-20.

4.4.4 Gradient Optimization

Linear gradients can be automatically optimized by converting them to step gradients which reduces solvent use.

NOTE

Gradients that are optimized on an Isolera LS system cannot be run on another Isolera system platform due to the different system volumes used for the calculation, and vice versa.

Clear separation between the peaks is required to receive a relevant result.

When using the Assist Workflow, the optimize feature is built into the workflow and cannot be accessed from the Results tab by a user with the basic workflow editor enabled. See the "Isolera™ Assist Quick Guide" (P/N 414767) for more information.

1. Select the **Results** tab in the right-hand panel.
2. Select the run at the **Result Selection** tab. For information on how to find the desired run, see "Search for Records" on page 4-29.
3. Press **Optimize...**. The **Gradient Optimization** dialog opens.

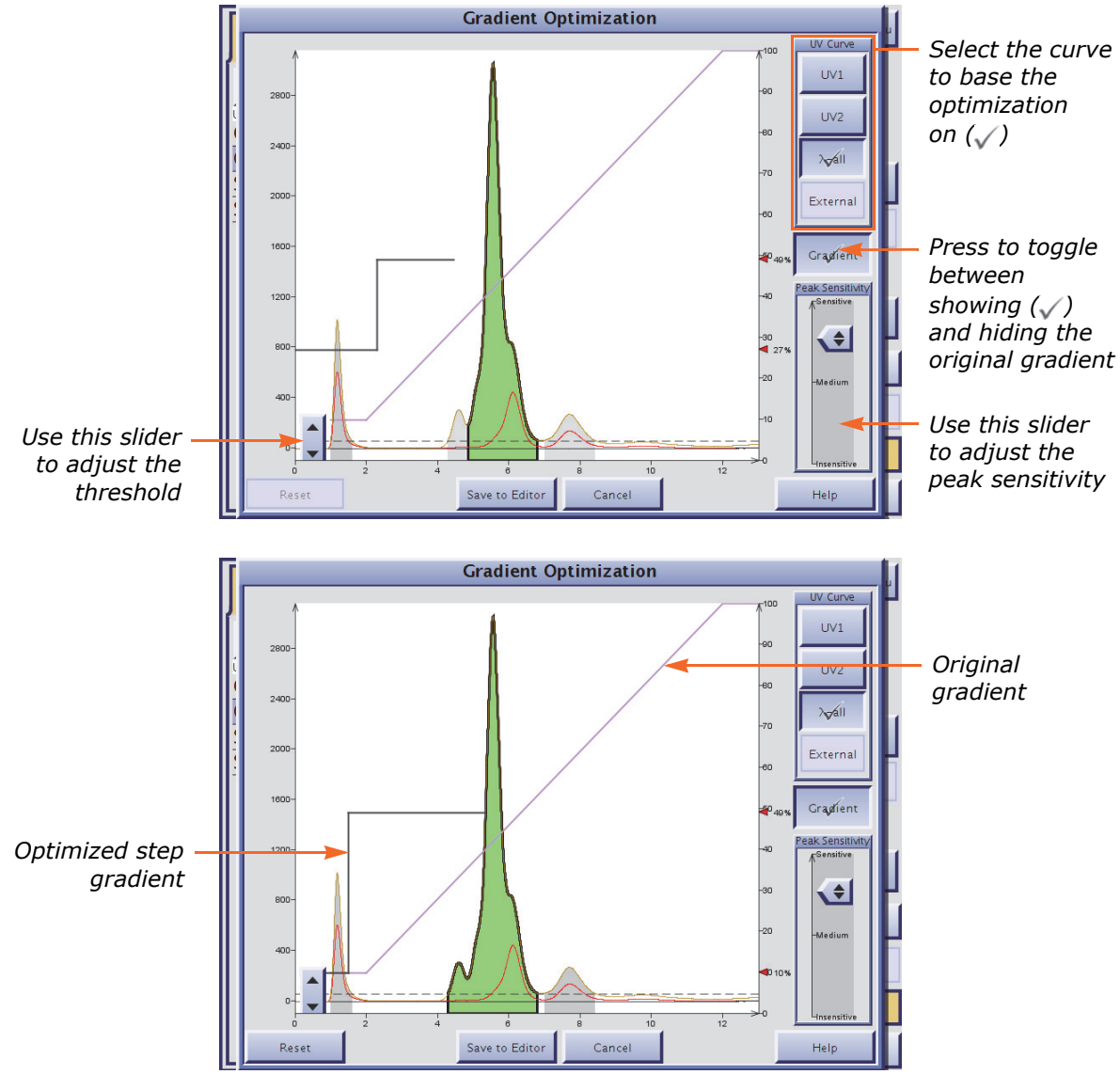


Figure 4-10. Gradient Optimization Dialog

4. To show the actual peaks, adjust the peak sensitivity and/or the threshold using the sliders (see Figure 4-10).
5. Select/press the peak that you want to collect. The suggested step gradient is displayed.
6. When you are pleased with the result, press **Save to Editor**. The method is opened at the **Method** tab and can be edited and/or saved.

4.4.5 Create a Method Using a Web Browser

See "Access the Isolera™ Remote Viewer" on page 4-36.

4.5 Prepare and Run a Purification

4.5.1 Prepare the System for a Run

1. If no equilibration is to be performed and an internal dry loading technique is to be used (see page 1-5), load the sample.
2. Load the cartridge and collection rack(s) that you want to use.
3. If you want to use the sample loading pump (only available on Isolera LS), see "Prepare the Sample Loading Pump for a Run (Only Isolera™ LS)" on page 4-17.
4. Ensure that a sufficient quantity of the correct solvent is present in each solvent reservoir and that the waste reservoir has sufficient capacity for the run. To assign solvents and set reservoir capacities and current levels, see page 4-33.
5. If you need to prime the system due to change of solvent or air bubbles, see page 4-34. Note that all solvent inlets must be primed with solvent.
6. If using the mass detector (only Isolera Dalton 2000), ensure that a sufficient quantity of the correct makeup solvent is present and that its inlet line is primed (see page 4-34).
7. To turn on the internal detector lamp(s) in advance, press **Turn Lamp On** in the right-hand panel (in the software).

NOTE

The UV lamp should be turned on approximately 7.5 minutes before operation so it can warm up and stabilize.

If automatic rack allocation is enabled, the collection area must at all times be loaded with racks in all positions.

All solvent inlets must be primed with solvent to achieve the specified pump performance.

Prepare the Sample Loading Pump for a Run (Only Isolera™ LS)

NOTE

To avoid the risk of cross-contamination and decreased pump performance, it is recommended that the peristaltic pump tube is discarded after each run.

The system is delivered with two kinds of peristaltic pump tubes (PharMed and Fluran). Ensure that the used peristaltic pump tube is compatible with the solvents and sample to be used; see "Chemical Resistance of the Peristaltic Pump Tube (Isolera™ LS)" on page 4-19.

Samples to be loaded using the sample load pump must be fully dissolved. Particulates in the sample may cut or weaken the peristaltic pump tube causing a sample leak.

Only Isolera LS is equipped with the sample loading pump.

To empty the pump tubing of liquid and mount a new peristaltic pump tube:

1. Place the pump's outlet line into a waste reservoir.
2. Select the **Setup** tab in the right-hand panel (in the software's chemistry mode) and then the **Sample Loading Pump** tab.
3. Press **Start** to start the pump and draw air into the inlet line.
4. When the pump tubing is empty, press **Stop**.

5. Remove the inlet and outlet tubing by removing the two screws; see Figure 4-11.

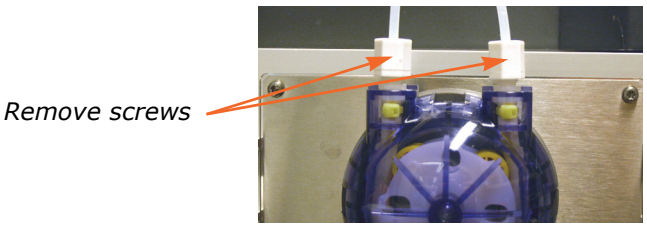


Figure 4-11. Remove the Inlet and Outlet Tubing

6. Remove the pump by turning it counterclockwise and then pulling it out; see Figure 4-12.
7. Replace the tube inside the pump. Ensure that the new tube is compatible with the solvents and sample to be used in the next run; see "Chemical Resistance of the Peristaltic Pump Tube (Isolera™ LS)" on page 4-19.
8. Remount the pump and the inlet and outlet tubing. If necessary, replace the inlet and outlet tubing.

Figure 4-12. Remove the Sample Loading Pump

To reuse the peristaltic pump tube used in the previous run (not recommended – see the note above), flush and fill the tubing with a solvent suitable for the next purification:

1. Place the pump's inlet line into the solvent of your choice.
2. Place the pump's outlet line into a waste reservoir.

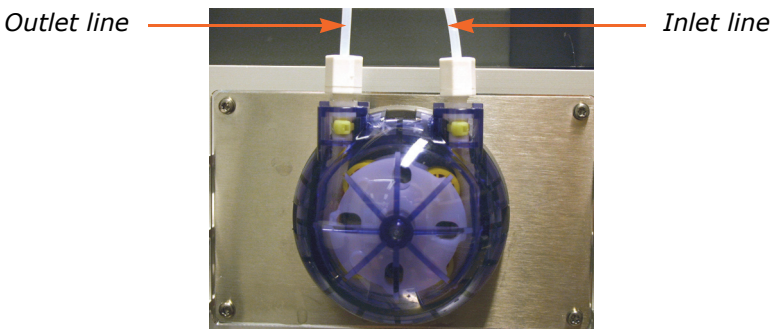


Figure 4-13. Sample Loading Pump

3. Select the **Setup** tab in the right-hand panel (in the software) and then the **Sample Loading Pump** tab.
4. To start the pump, press **Start**. Adjust the flow rate using the **Speed** slider.
5. When you are done, press **Stop**.

6. Connect the pump's outlet line to the cartridge's inlet.
7. Place the pump's inlet line into the sample.

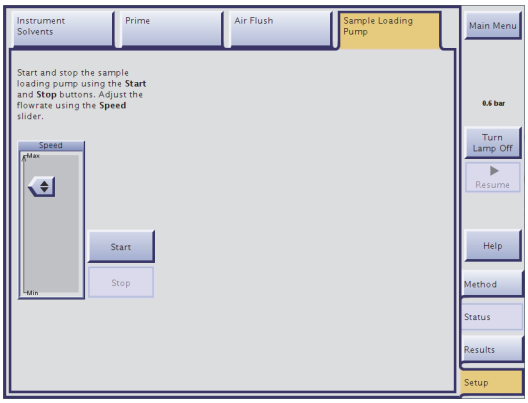


Figure 4-14. Sample Loading Pump Tab

Chemical Resistance of the Peristaltic Pump Tube (Isolera™ LS)

The Isolera LS system is delivered with two kinds of peristaltic pump tubes. Ensure that the used pump tube is compatible with the solvents and sample to be used.

NOTE

To avoid the risk of cross-contamination and decreased pump performance, it is recommended that the peristaltic pump tube is discarded after each run. If you still want to reuse the peristaltic pump tube, clean the tube as soon as possible as described in section 5.8.1 on page 5-7.

Solvent	PharMed, P/N 412480	Fluran, P/N 412481
Acetetic acid	Yes	No
Acetone	Limited	No
Acetonitrile	Yes	No
Ammonia	Yes	Yes
Dichloromethane	No	Yes
Ethanol	Yes	Yes
Ethyl acetate	Limited	No
Formic acid	Yes	Yes

Solvent	PharMed, P/N 412480	Fluran, P/N 412481
n-Heptane	Limited	Yes
n-Hexane	No	Yes
Isopropanol	Limited	Yes
Methanol	Yes	Yes
Pyridine	Limited	Limited
Toluene	No	Yes
Triethylamine	No	Yes
Water	Yes	Yes

Limited: The peristaltic pump tube must be discarded after each run.

The chemical resistance was tested in room temperature as follows: The peristaltic tube was 1) weighed, 2) submerged in solvent for three hours, 3) dried and visually inspected, 4) submerged for another three hours, 5) dried, visually inspected, and weighed, and 6) dried for 24 hours and weighed.

4.5.2 Run a Purification

WARNING

If you at any time during a purification need to pause the system, press the Pause button in the right-hand panel (in the software). The collection arm returns to its home position (the inner right corner) and the system is paused. Note that the system is pressurized when it is paused. To resume operation, press the Resume button.

Do not exceed recommended flow rates for flash cartridges (this information can be found in the flash cartridge documentation) nor allow flow at higher rates than specified through any Samplet, dry load vessels, or pre-cartridges that you may be using. This can increase the risk of static build-up.

Read and follow the safety precautions against static electricity in the "Isolera™ Installation and Safety" document (P/N 415796) that is supplied with the system.

1. Prepare the system as described on page 4-17.
2. Open or create a new method, see "Create, Open, and Edit Methods" on page 4-3.
3. To run a purification using the displayed method at the **Method** tab, press **Run...**. The **Run Parameters** and (if estimation is enabled) the **Estimation** dialog open. To enable or disable estimation, see "Enable or Disable Run Requirement Estimation" on page 3-12.
4. In the **Run Parameters** dialog:
   a. Select/press the rack position(s) to be used (✓).
   b. If using a system with four cartridge positions, select/press the cartridge position to be used (✓).
   c. If the run is to be performed without equilibration (not recommended when using the mass detector or when the percentage of the strongest solvent is above 10% in the initial solvent mix and you are using a SNAP KP-Sil, SNAP Ultra (not C18), or ZIP Sphere cartridge on all Isolera systems except Isolera Prime) and you want to inject a liquid sample into the cartridge, you can either use a syringe or the sample loading pump (only available on Isolera LS).

      To use the sample loading pump: Press **Load Sample** and read and follow the instructions in the **Load Sample** dialog. When you are done, press **Close** and connect the cartridge inlet tubing to the cartridge's inlet. If you want to reuse the peristaltic pump tube in the next run (not recommended), clean the tube with methanol as soon as possible. Note that the sample loading pump can be cleaned while a purification is in progress. For more information and instructions, see page 5-7.

NOTE

When using an Isolera Four system with automatic rack allocation enabled, several runs (using the displayed method) can be queued up simultaneously by selecting two or more cartridge positions in the Run Parameters dialog. Note that a user with the basic workflow editor enabled cannot queue runs when using the Assist Workflow.

5. In the **Estimation** dialog (if present), ensure that a sufficient quantity of the correct solvent is present in each solvent reservoir, the waste reservoir has sufficient capacity for the run, and the sufficient number of vessels have been allocated.
6. To start or queue up* the purification, press ▶**Equilibrate/Gradient** (see Figure 4-15). The **Status** tab is selected.

7. When the equilibration step (if included) is finished:
   a. Load your sample; see "Sample Loading Methods for Biotage® SNAP and Biotage® SNAP Ultra Cartridges" on page 1-5. If a liquid sample is used, press **Load Sample...** at the **Status** tab to enable sample injection. The **Load Sample** dialog opens. Inject your sample into the cartridge inlet Luer fitting using a syringe. For ideal loading, do not apply high manual pressure with the syringe. To load a sample using the sample loading pump (only available on Isolera LS), read and follow the instructions in the **Load Sample** dialog. When you have finished loading the sample, press **Close** and connect the cartridge inlet tubing to the cartridge's inlet.
   b. Start or queue up* the gradient run by pressing ▶**Gradient** at the **Status** tab.

   Before the run is started, the system performs a UV Zero using the gradient's initial solvent mix. If using an Isolera Dalton 2000 system, the system also flushes the mass detector and Isolera Dalton Nanolink tubing with the makeup solvent and then measures the conditions for the mass detector (thresholds and baselines for the XIC and TIC signals). Note that a gradient run where collection and fractionation is based on light absorption is not started until the UV lamp is sufficiently warmed up.

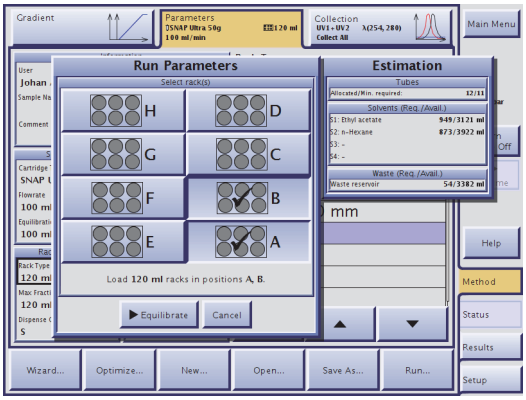
* Purifications can be queued up when using a system with four cartridge positions. Note that a user with the basic workflow editor enabled cannot queue runs when using the Assist Workflow.

NOTE

When using a system with four cartridge positions, queued equilibrations take priority over queued gradient runs.

Isolera One EXP and Isolera LS

Rack positions for 120 ml racks:



Isolera Four EXP

Rack positions for 16x100 mm racks:

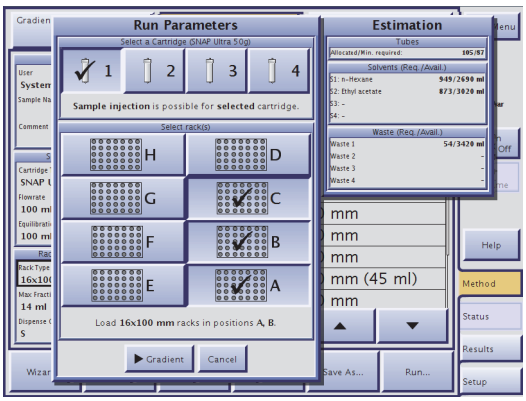


Figure 4-15. Run Parameters and Estimation Dialogs

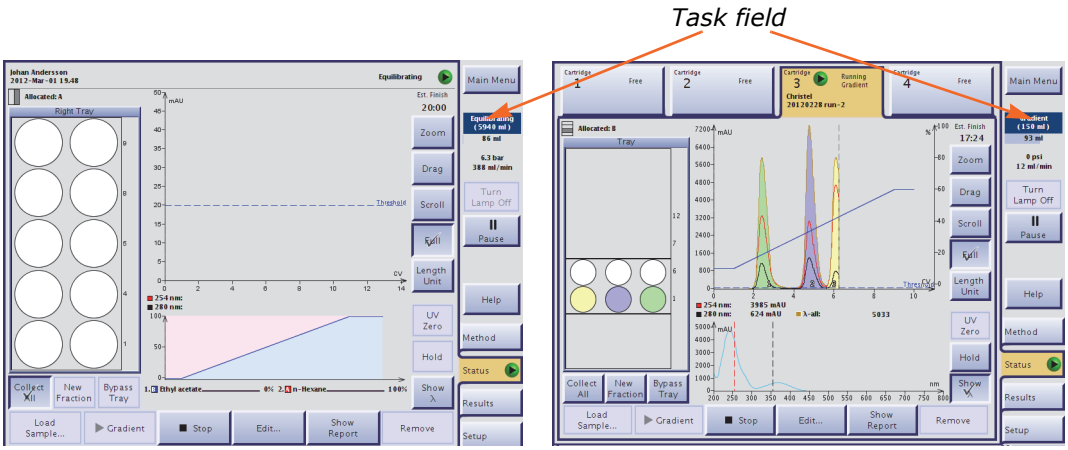


Figure 4-16. Status Tab (Isolera LS and Isolera Four EXP with Isolera Spektra License Shown)

Replace and Allocate Racks During the Run

If more fractions are to be collected than can fit in the allocated rack(s), the system pauses, the collection arm returns to the home position (the inner right corner), and the **Load New Racks** dialog opens. Replace the rack(s) according to the dialog and press the **Resume** button to resume the run. The collection is resumed in the first vessel in the lowest lettered rack. For example, if racks B and C are allocated for the run, the collection is resumed in rack B in vessel 1.

You can at any time allocate free rack positions by pressing **Edit...** at the **Status** tab. (This is not possible if automatic rack allocation is enabled.)

NOTE

When using an Isolera Four system with automatic rack allocation enabled, you have to replace all racks in the collection area at the same time when asked to (even if some of the racks have been used by previous runs).

Auto-Extend Mode

If the collection criteria are still met when the system nears the end of a purification, the system enters the Auto-Extend mode (if enabled in the system mode). This extends the gradient purification stage of the run with 25% of the total gradient length using the final conditions in the method.

Line Flush, Decompression, and Detector Flush

After the gradient purification stage is finished, the system performs a line flush (if enabled), air flush (if enabled), system decompression, and a detector flush (if enabled). To enable or disable flushes, specify whether enabled flushes are collected or not, and specify which solvents to use for the flushes, see “Specify How Flushes Are Performed” on page 3-12. Note that the Air Flush feature is not available with Isolera Prime.

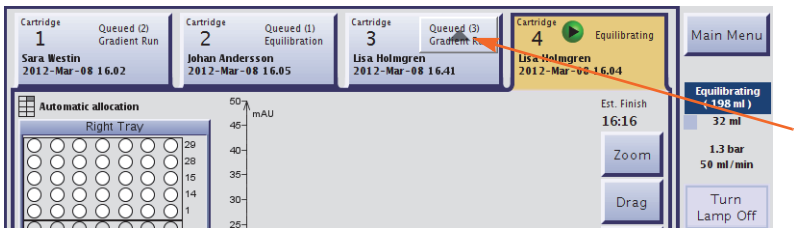
4.6 Change the Processing Order

When using a system with four cartridge positions, it is possible to change the processing order. To move up a purification in the queue, press the ▲ button at the **Status** tab; see Figure 4-17 below.

NOTE

Queued equilibrations take priority over queued gradient runs.

Runs cannot be queued up when the Assist Workflow is used by a user with the basic workflow editor enabled.



If you press this button, the run on cartridge 3 will be performed prior to the run on cartridge 1

Figure 4-17. Change the Processing Order

4.7 Monitor and Control a Purification

4.7.1 Monitor the Purification in Progress

Select the **Status** tab in the right-hand panel. While a purification is running, one graph displays the programmed gradient and the other a dynamic chromatogram. If an Isolera Spektra or Dalton 2000 software license has been installed on your system, an absorbance spectrum for the whole detector range can be viewed in the gradient view by pressing the **Show λ** button. The gradient is then displayed in the chromatogram.

You can adjust the chromatogram view by using the following buttons:

- **Zoom:** When enabled (✓), you can select a region of the chromatogram to magnify (zoom in).
- **Drag:** When enabled (✓), you can drag the chromatogram to the desired position.
- **Scroll:** When enabled (✓), the present reading +1 CV and -3 CV is shown on the horizontal axis.
- **Full:** When enabled (✓), all readings on the horizontal axis are shown.
- **Length Unit:** Toggle the horizontal axis unit between CV, minutes, and milliliter.
- **UV Zero:** Used to set the current UV level to 0 (zero) AU. This button is not available when the Assist Workflow is used by a user with the basic workflow editor enabled.

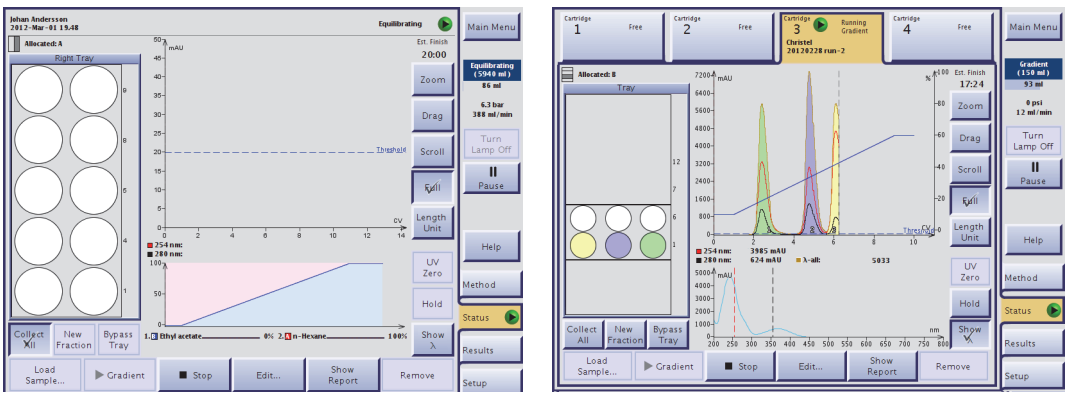


Figure 4-18. Status Tab (Isolera LS and Isolera Four EXP with Isolera Spektra License Shown)

Cartridge Status and Color Legends

See page 1-15 for a description of the colors used in the tray overview, chromatogram, gradient graph, and absorbance spectrum (requires an Isolera Spektra or Dalton 2000 software license).

See page 1-14 for a list of the different statuses of a cartridge.

4.7.2 Bypass the Automatic UV Lamp Warm-up Period

You can bypass the automatic UV lamp warm-up period by pressing **Skip Warm Up** in the right-hand panel. However, if the UV lamp has not reached operating temperature, purification results may not be optimal. Note that the **Skip Warm Up** button is disabled for the first 45 seconds of the UV lamp warm-up period.

4.7.3 End Initial Waste and Start Collecting Fractions

If you want to end an Initial Waste phase prematurely and start collecting fractions, press **Start Collect** at the **Status** tab.

4.7.4 Collect Through the Waste Channel

If you want to start collecting fractions through the waste channel, press the **Bypass Tray** button at the **Status** tab during the gradient run (✓ = collection through waste is enabled). You will be requested to connect a collection vessel to the waste channel and enter the max collection volume of the vessel. You can at any time switch to a new collection vessel by pressing **New Fraction** or stop collecting through the waste channel and go back to collecting on the tray by pressing the **Bypass Tray** button.

Fractions collected through the waste channel are colored in magenta in the chromatogram and numbered W1, W2, and so on.

The **Bypass Tray** button is not available when the Assist Workflow is used by a user with the basic workflow editor enabled.

4.7.5 Start and End an Isocratic Segment

You can at any time during the gradient run start an isocratic segment by pressing the **Hold** button (✓ = isocratic hold is enabled). End the segment by pressing the **Hold** button again. This button is is not available when the Assist Workflow is used by a user with the basic workflow editor enabled.

4.7.6 Control a Manual Collection

If you have set up a purification with manual collection, you can instruct the system to start and stop collecting fractions by pressing the **Collect All** button at the **Status** tab (✓ = fractions are collected). The system will fill each collection vessel according to the defined max fraction volume. You can at any time switch to a new collection vessel by pressing **New Fraction**.

4.7.7 Edit and Manually Extend a Purification

If the collection criteria are still met when the system nears the end of a purification, the system enters the Auto-Extend mode (if enabled in the system mode). This extends the gradient purification stage of the run with 25% of the total gradient length using the final conditions in the method.

To edit and manually extend a purification:

1. Select the **Status** tab in the right-hand panel.
2. Press **Edit...**. The system is paused and the **Edit Method** dialog opens.
3. Edit the method settings.
4. To save the changes and resume the operation (if the system was paused), press **OK**.
5. If you extended a finished run or edited a gradient run that was not started or queued up*, press ▶ **Gradient** to start (or queue up*) the run. Note that if you extend a run on a system with four cartridge positions and UV Zero has been performed on another cartridge in between, the system will perform a UV Zero.

NOTE

When using a system with four cartridge positions, queued equilibrations take priority over queued gradient runs.

* Purifications can be queued up when using a system with four cartridge positions. Note that a user with the basic workflow editor enabled cannot queue runs when using the Assist Workflow.

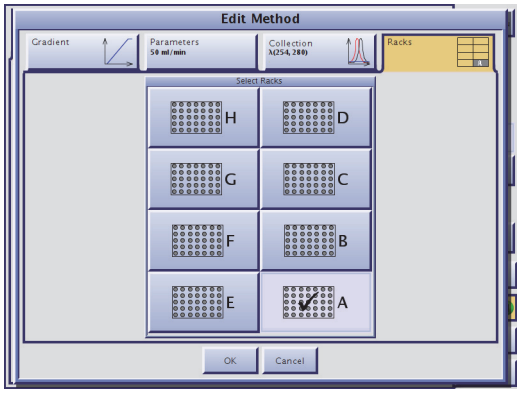


Figure 4-19. Edit Method Dialog

4.7.8 Pause, End, or Abort a Purification

WARNING

Keep your hands out of range of the collection arm until it has stopped moving (with the collection arm in the inner right corner).

The system is pressurized when it is paused.

Pause and Resume a Purification

1. To pause a purification in progress, press **Pause** in the right-hand panel (in the software). The collection arm returns to its home position (the inner right corner) and the system is paused.
2. To resume the run from the point at which it was paused, press **Resume** in the right-hand panel.

End or Abort a Purification

1. Select the **Status** tab in the right-hand panel.
2. If the purification is in progress:
   a. Press ■ **Stop**. The collection arm returns to its home position (the inner right corner), the system is paused, and the **Stop** dialog opens.
   b. If you want the system to perform all enabled flushes and line purges, press **End**.
   c. If you do not want the system to perform the enabled flushes and purges, press **Abort**. To release any remaining pressure in the cartridge, press **Purge...** in the **Purge** dialog that opens. (If there are queued runs*, the processing of the queue is started by pressing **Resume** in the right-hand panel.)
3. If the purification is not started, i.e. the equilibration is finished but the gradient run is not started or the run is queued up*, press **Remove**.

* Purifications can be queued up when using a system with four cartridge positions. Note that a user with the basic workflow editor enabled cannot queue runs when using the Assist Workflow.

4.8 Unload a Purification

When the purification is finished (check that the cartridge's status is "Finished" at the **Status** tab), unload the cartridge and rack(s) as described below.

1. If necessary, empty the cartridge of remaining solvents and pressure by using the air flush and purge features at the **Setup** tab. See "Flush a Cartridge with Air" below and "Purge a Cartridge" on page 4-28. Note that the Air Flush feature is not available with Isolera Prime.
2. If using a system with four cartridge positions and the system is processing, press **Pause**.
3. Unload the cartridge. To avoid leakage, plug the cartridge's inlet and outlet fittings and couple the inlet and outlet tubes together.
4. Unload the rack(s) used by the run. Allocated racks are listed at the **Allocated** heading at the **Status** tab.
5. If using an Isolera Four system with automatic rack allocation enabled, load new, empty racks of the same type.
6. To clear the cartridge and rack positions in the software, press **Remove**.
7. To resume processing of queued runs*, press **Resume...**.

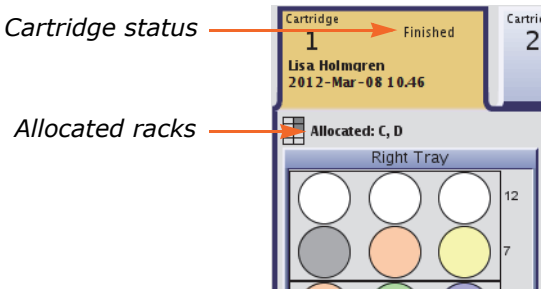


Figure 4-20. Cartridge Status and Allocated Racks (Isolera Four EXP Shown)

* Purifications can be queued up when using a system with four cartridge positions. Note that a user with the basic workflow editor enabled cannot queue runs when using the Assist Workflow.

4.8.1 Flush a Cartridge with Air

NOTE

It is not possible to flush a cartridge with air when using an Isolera Prime system.

Empty the cartridge of remaining solvents using the Air Flush feature. If you want the system to automatically perform an air flush at the end of a purification, enable the Air Flush option (see "Specify How Flushes Are Performed" on page 3-12).

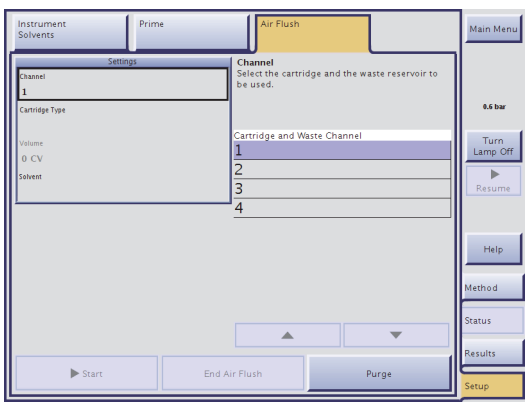


Figure 4-21. Air Flush Tab (Isolera Four Shown)

To manually instruct the system to perform an air flush:

1. Select the **Setup** tab in the right-hand panel.
2. Select the **Air Flush** tab.
3. If using a system with four cartridge positions, select the **Channel** text box and select the cartridge to be flushed and the waste reservoir to be used.
4. Ensure the correct cartridge type is displayed in the **Cartridge Type** text box. To change, select the **Cartridge Type** text box and select the cartridge type mounted on the system.
5. If you want to change the default flush volume, select the **Volume** text box.
6. For the pump to work properly, a small amount of solvent has to be used. By default the system will flush with the weakest solvent used in the run. To change, select the **Solvent** text box and select the desired solvent.
7. Ensure that a sufficient quantity of the selected solvent is present in the solvent reservoir (1% of the air flush volume is used) and that the waste reservoir has sufficient capacity for the air flush.
8. To start flushing, press ▶**Start**.

4.8.2 Purge a Cartridge

Any remaining pressure after a purification can be released using the purge feature.

1. Select the **Setup** tab in the right-hand panel.
2. Select the **Air Flush** tab.
3. If using a system with four cartridge positions, select the **Channel** text box and select the cartridge to be purged and the waste reservoir to be used.
4. Ensure that a waste reservoir is connected to the waste channel.
5. To purge, press **Purge**. The **Purge** dialog opens.
6. When the pressure has been reduced to an acceptable level, press **End** in the **Purge** dialog.

4.9 Access Result Records

NOTE

When using the Assist Workflow, a user with the basic workflow editor enabled cannot save records or reports in a share folder on the network or view reports for records that are saved on a USB memory device or on the network.

4.9.1 Search for Records

Purifications that are processed on the system are stored as individual records in the system's database. Possible search criteria for these records are 1) user name, 2) project name, 3) method name, 4) sample name, and 5) date when the purification was run.

To search for records in the system's database:

1. Select the **Results** tab in the right-hand panel.
2. Select the **Result Selection** tab.
3. If Local is not selected (✓), press **Local**.
4. Press the **Result Filter** field. The **Result Filter** dialog opens.
5. Specify the filter settings. Note that the result filter is case sensitive. (If you want to list all result records, press **Clear All**.)
6. To search, press **Search**. If there are records matching your search criteria, they are listed in chronological order. If more records are found than can be displayed at one time, use the ▲ and ▼ buttons to scroll through the records.

To search for records on a USB memory device or on the network:

1. To search records stored on a USB memory device, connect the device to the USB port located underneath the touch screen and press .
2. To search records stored in the share folder on your network, press . (If the button is disabled, no file sharing has been set up. See "Set Up File Sharing and Auto Save of Reports" on page 3-9.)
3. Use the ▲ and ▼ buttons to scroll through the records.

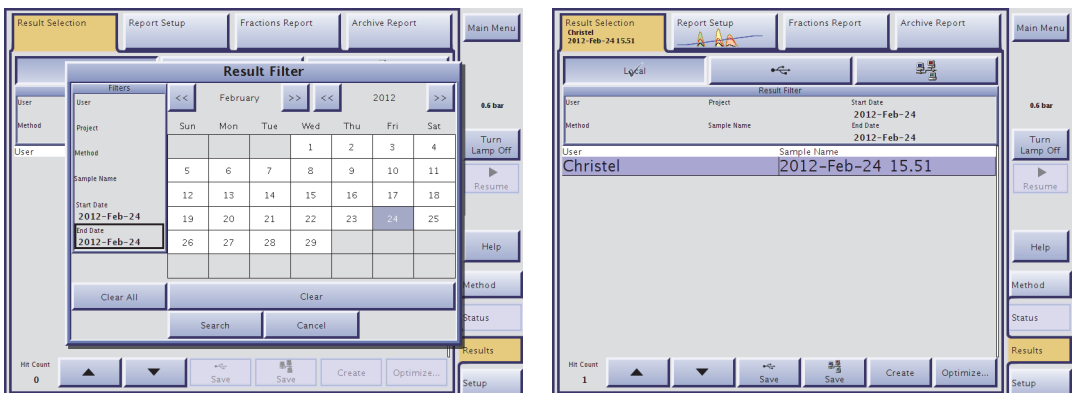


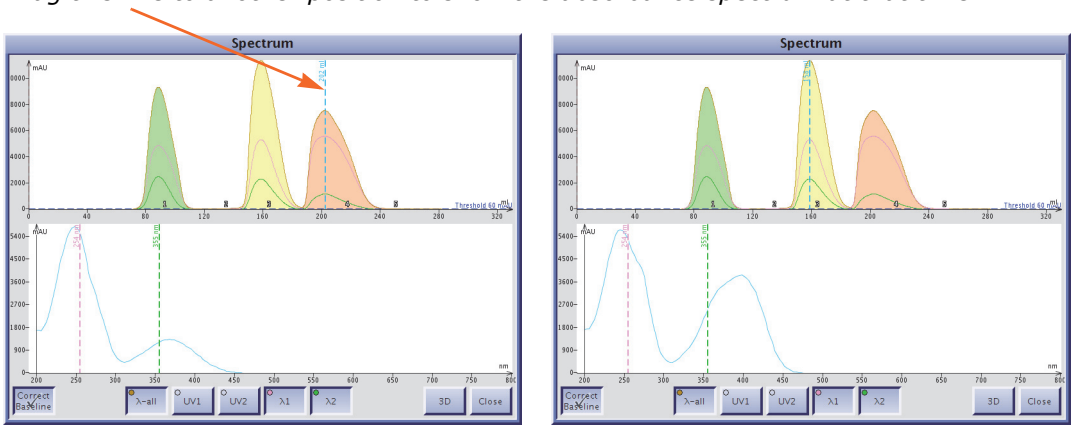
Figure 4-22. Search for Records

4.9.2 View, Print, and Save Reports on a USB Memory Device or the Network

Two result reports are available for each purification: 1) an archive report with the spectra from the mass analysis (if performed, only on Isolera Dalton 2000), purification details, a chromatogram, a 3D absorbance spectrum for the whole detector range (requires an Isolera Spektra or Dalton 2000 software license), and TLC data (if entered in the TLC to Step Gradient editor) and 2) a fraction report. These reports can be printed to a network printer with postscript support or a local USB printer and saved as PDF files on a USB memory device or in a share folder on your network.

1. Select the **Results** tab in the right-hand panel.
2. Select the desired record at the **Result Selection** tab. (If the record is not listed, see "Search for Records" above.) An archive report for the selected record is available at the **Archive Report** tab and a fraction report is available at the **Fraction Report** tab.
3. To view 2D and 3D absorbance spectra for the whole detector range (requires an Isolera Spektra license or Dalton 2000 software):
   a. Press **λ...** at the **Report Setup** tab. The **Spectrum** dialog opens.
   b. To enable or disable UV baseline correction, press **Correct Baseline**. When enabled (✓), the maximum solvent absorbance spectrum is subtracted from the signal presented by the internal detector.

Drag this line to another position to show the absorbance spectrum at that time.



Drag one or both of these lines to other wavelengths to show the chromatogram at those wavelengths.

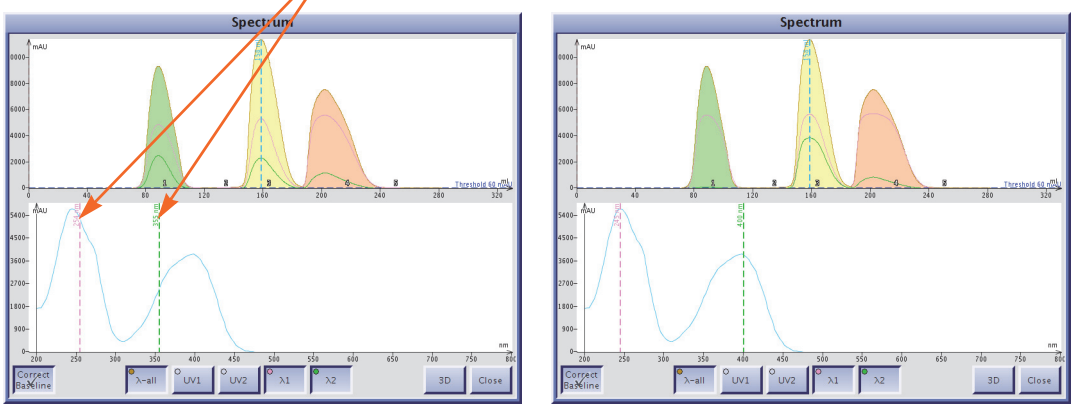


Figure 4-23. 2D Absorbance Spectrum

c. To show a 3D absorbance spectrum instead, press 3D. Drag the 3D spectrum to change the perspective, see the examples below in Figure 4-24. Note that the way it looks here is the way it will look in the archive report.

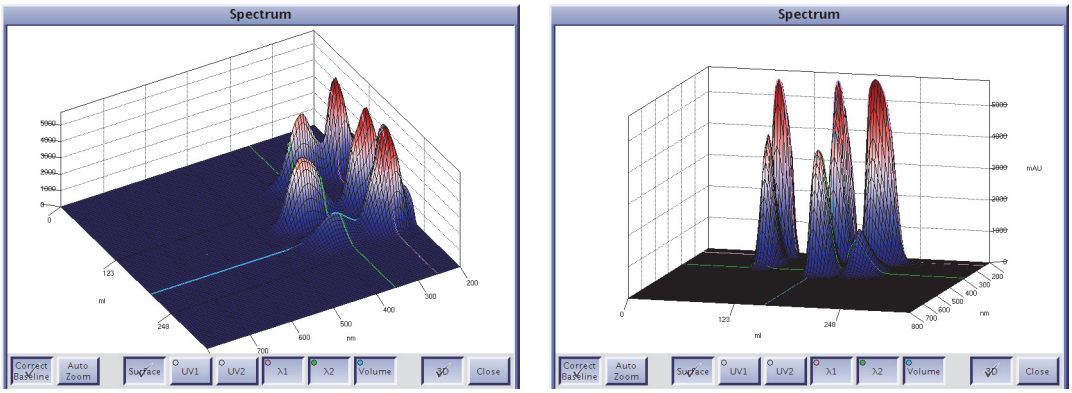


Figure 4-24. 3D Absorbance Spectrum in Different Perspectives

4. To view and analyze mass detector data (only Isolera Dalton 2000), press **m/z...** at the **Report Setup** tab. The **Mass Spectrum** dialog opens.
5. To modify the fraction and archive reports, select the **Report Setup** tab. The following buttons can be used to modify the reports:
   - **Gradient:** When enabled (✓), the programmed gradient is shown in the chromatogram.
   - **TIC:** When enabled (✓), the TIC is shown in the chromatogram. This button is only available for runs that have been performed using the mass detector.
   - **Reset Zoom:** Reset the zoom to default.
   - **Zoom:** When enabled (✓), you can select a region of the chromatogram to magnify (zoom in). This is useful when the chromatogram displays many fractions close together.
   - **Drag:** When enabled (✓), you can drag the chromatogram to the desired position.
   - **Select:** When enabled (✓), you can select and deselect fractions in the chromatogram. (Fractions can at any time be selected and deselected in the tray overview.)
   - **Select All:** Select all fractions.
   - **Deselect All:** Deselect all fractions.
   - **λ...:** Modify how the 3D absorbance spectrum is presented in the archive report. See step 3 above.
   - **Length Unit:** Change the horizontal axis (the x-axis) unit by pressing the button repeatedly until the desired unit appears; CV, minutes, or milliliter.

   If there was one or more rack changes during the run, or the run was performed on a system with two collection trays with allocated racks on both trays, toggle between the racks by pressing the ◀ and ▶ buttons.
6. To print the archive or fraction report to the (local or network) printer connected to the system, press **Print** at the corresponding tab. (If the button is disabled, no printer has been installed. See "Set Up a Printer and Auto Print of Reports" on page 3-8.)
7. To save the selected report as a PDF file on a USB memory device:
   a. Connect a USB memory device to the USB port located underneath the touch screen.
   b. Press **Save**. The report is saved at "USB":\biotage\isolera\reports.
8. To save the selected report as a PDF file in a share folder on your network, press **Save**. The report is saved at "share folder"\biotage\isolera\reports\. (If the button is disabled, no file sharing has been set up. See "Set Up File Sharing and Auto Save of Reports" on page 3-9.)

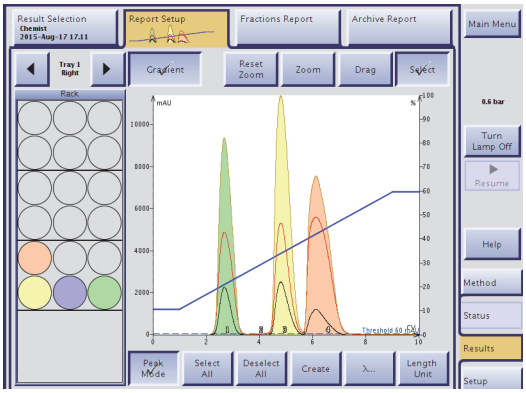


Figure 4-25. Report Setup Tab (with Isolera Spektra License)

Color Legend

The colors used at the **Report Setup** tab are:

- = The vessel has not been used for the viewed purification.
- = The vessel is deselected.
- = The vessel is selected and contains flush liquid.
- , , , and = The vessel is selected and contains a fraction. (The vessel color corresponds with the fraction color in the chromatogram.)

If fractions are collected through the waste channel (i.e. the Bypass Tray mode is enabled), the fractions are colored in magenta in the chromatogram and numbered W1, W2, and so on.

4.9.3 Save Records on a USB Memory Device or the Network

Purifications that are processed on the system are stored as individual records in the system's database. The records can, if desired, be saved by the user as XML files (including all raw data) and SPECTRUM files (with the raw 3D UV spectrum) on a USB memory device or in a share folder on the network. If the run was performed using the mass detector (only Isolera Dalton 2000), you will also get DATX files containing the mass detector data. The XML files can be opened on any Isolera system with the same or a newer version of the software.

1. Select the **Results** tab in the right-hand panel.
2. Select the desired record. (If the record is not listed, see "Search for Records" on page 4-29.)
3. To save the record on a USB memory device, connect the device to the USB port located underneath the touch screen and press **Save**. The record is saved at "USB":\biotage\isolera\results.
4. To save the records in the share folder on your network, press **Save**. The record is saved at "share folder"\biotage\isolera\results\. (If the button is disabled, no file sharing has been set up. See "Set Up File Sharing and Auto Save of Reports" on page 3-9.)

4.10 View System Status and Results from Your Office

See "Access the Isolera™ Remote Viewer" on page 4-36.

4.11 Solvent and Waste Handling

4.11.1 Assign Solvents to the Solvent Inlets

When a purification is run, the software references the solvent assignments to determine which solvent inlets that are connected to the chromatography solvents used in the method.

1. Select the **Setup** tab in the right-hand panel.
2. Select the **Instrument Solvents** tab.

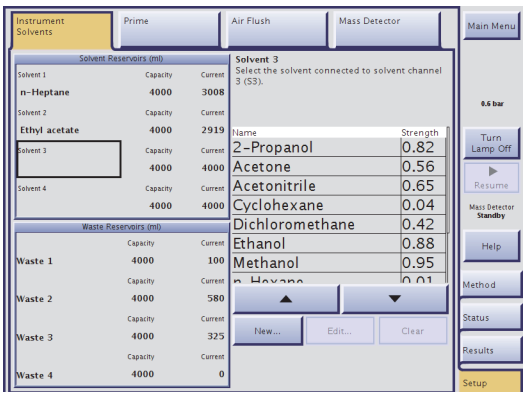


Figure 4-26. Setup Tab (Isolera Four with Mass Detector Shown)

3. Press one of the **Solvent** text boxes.
4. Select the solvent that you want to assign to the selected inlet. To scroll the list down or up, press ▼ and ▲. The software comes with a preconfigured list of solvents and their parameters. You may add other solvents to this list (press **New...**) and edit user defined solvents (select the solvent and press **Edit...**).
5. If solvent monitoring is enabled, select the **Capacity** text box and enter the capacity of the solvent reservoir.*
6. If solvent monitoring is enabled, select the **Current** text box and enter the current solvent level of the reservoir.*
7. Repeat step 3 through 6 for all solvent inlets.

* These values are used by the system to track reservoir capacity. To enable this feature, see "Enable or Disable Monitoring of Reservoirs" on page 3-11.

4.11.2 Set the Reservoir Volumes

If the monitoring of solvent and/or waste levels is enabled (see "Enable or Disable Monitoring of Reservoirs" on page 3-11), you have to enter the capacities and current fluid levels for the solvent and waste reservoirs each time you empty a waste reservoir or replenish a chromatography solvent.

1. Select the **Setup** tab in the right-hand panel.
2. Select the **Instrument Solvents** tab.
3. Enter the capacity of a reservoir by pressing the applicable **Capacity** text box.
4. Enter the current level of a reservoir by pressing the applicable **Current** text box.

4.11.3 Prime the System

Before you start a purification on your Isolera system, you might need to prime the solvent inlets to:

- Remove any air bubbles from the pump and the solvent inlets by flushing them with solvents.
- Empty the solvent inlets of solvents used in the previous purification and fill them with new solvents.

WARNING

Never prime the Isolera system without a cartridge mounted on the system or without the cartridge's inlet and outlet tubing coupled together. To avoid leakage, check all tubes and fittings before priming the system.

NOTE

All solvent inlets must be primed with solvent to achieve the specified pump performance.

To prime the system:

1. Ensure that a sufficient quantity of the solvent(s) you want to use is present in the solvent reservoir(s) and the waste reservoir has sufficient capacity for the prime.
2. Select the **Setup** tab in the right-hand panel.
3. Ensure that the solvents are assigned accurately to the solvent inlets at the **Instrument Solvents** tab. If the capacity and current volume values are available for the waste and/or solvent reservoirs (i.e. the monitoring of levels is enabled), check that the values are correct. To make changes, see "Assign Solvents to the Solvent Inlets" and "Set the Reservoir Volumes" on page 4-33.
4. Select the **Prime** tab.

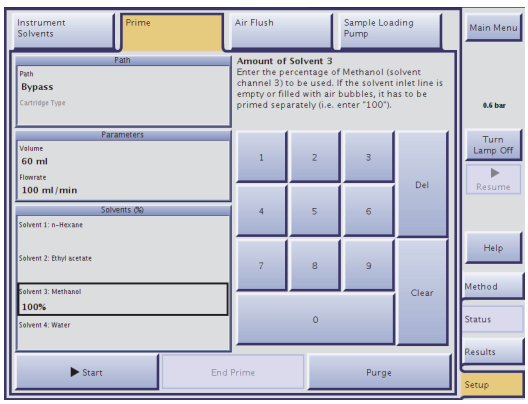


Figure 4-27. Prime Tab (Isolera Four Shown)

5. If using a system with four cartridge positions, select the **Channel** text box and select the cartridge position and waste reservoir to be used.
6. Select the **Path** text box and select the prime path. If you do not want to prime the cartridge, press **Bypass**.
7. If you selected the path **With Cartridge**, select the **Cartridge Type** text box and select the cartridge type mounted on the system.

8. Select the **Volume** text box and enter the total prime volume.
   If you want to fill a solvent inlet with a new solvent, we recommend that you prime with at least 20 ml, or 50 ml when using an Isolera LS system, of that solvent. If you also want to fill the liquid lines from the pump to the (selected*) waste valve with the same solvent, we recommend that you prime with at least 60 ml, or 100 ml when using an Isolera LS system.
9. Select the **Flowrate** text box and enter the flow rate. The maximum flow rate that can be used depends on:
   - the cartridge type mounted on the system, if you selected the **With Cartridge** path in step 6 above,
   - the maximum fill rate(s) defined for the used solvent(s) in the Data Administration mode, and
   - the maximum dispense rate of the system (200 ml/min, or 500 ml/min when using an Isolera LS system) or, if using an external detector, the maximum dispense rate defined for the external detector in the System mode.

   If using an Isolera Prime system: As a single-piston pump only delivers liquid during the dispense stroke, the maximum flow rate when using an Isolera Prime system is equal to (Max Fill Rate x Max Dispense Rate)/(Max Fill Rate + Max Dispense Rate). Examples: If the fill rates for the used solvents are 50 ml/min and 80 ml/min and you use a external detector with the maximum dispense rate of 100 ml/min, the highest flow rate that can be used is (50x100)/(50+100)=33 ml/min. If the external detector is disabled, the highest flow rate that can be used is (50x200)/(50+200)=40 ml/min.
10. Enter the percentage of each solvent connected to the system that shall be used in the prime. If using an Isolera LS system and a solvent inlet line is empty or filled with air bubbles, you should prime it separately. (If several solvents are listed and you only want to use one solvent, enter "100" for the solvent that you want to use and enter "0" or press **Clear** for the other solvent(s).)
11. To start priming, press ▶**Start**.

* When using a system with four cartridge positions.

Prime the Makeup Solvent Inlet (Only Isolera™ Dalton 2000)

1. Verify that a waste outlet tube is connected to the **WASTE** port on the right hand side of Isolera Dalton Nanolink and inserted into a waste reservoir.
2. In the chemistry mode, select the **Setup** tab in the right-hand panel.
3. Select the **Mass Detector** tab.
4. Press ▶**Start Prime**. A status field and progress bar in the right-hand panel indicates the progress.

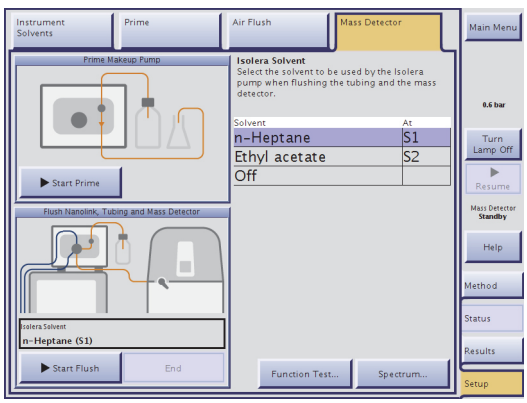


Figure 4-28. Mass Detector Tab (Isolera Dalton 2000 Shown)

4.12 Access the Isolera™ Remote Viewer

With the system connected to your network, it is possible to perform the following tasks through a standard web browser (through the Isolera Remote Viewer feature):

- Create a new method. If desired, the method can be based on a previous purification found in the Results view.
- Check the status of the system and the progress of a purification.
- Instruct the system to start and stop collecting fractions by clicking the **Collect All** button (✓ = fractions are collected).*
- Instruct the system to switch to a new collection vessel by clicking the **New Fraction** button.*
- Instruct the system to start and end an isocratic segment by clicking the **Hold** button (✓ = isocratic hold is enabled).*
- Instruct the system to pause the purification in progress by clicking the **Pause** button. For safety reasons, it is only possible to pause and not resume a run from your office.
- Instruct the system to set the current UV level to 0 (zero) AU by clicking the **UV Zero** button.*
- View, print, and export purification results.

* You must be logged in as the user defined in the purification run.

To access the Isolera Remote Viewer:

1. Enter the URL *http://MACHINENAME* in a web browser (where MACHINENAME is the hostname or the host IP address). The hostname and host IP address are available in the **About** dialog at the main menu.
2. Press **ENTER** and the Isolera Remote Viewer web page is loaded. The page is automatically updated every five seconds.

NOTE

If you need help accessing the Isolera Remote Viewer, contact your IT administrator.

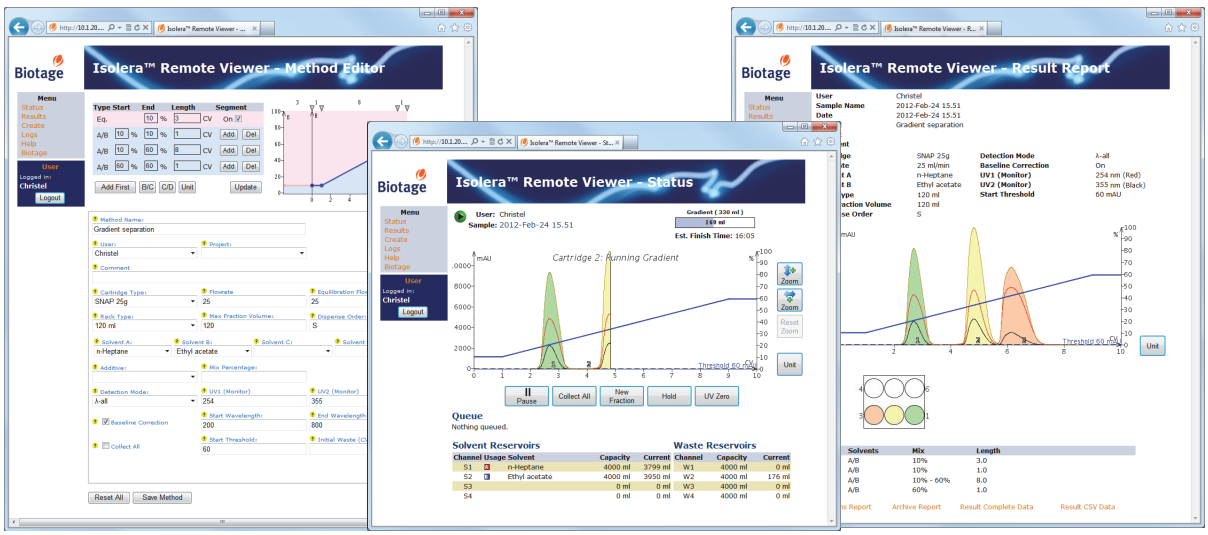


Figure 4-29. Isolera Remote Viewer (System with Four Solvent Inlets and Spektra License Shown)

Chapter 5 Maintenance

WARNING

Before performing any procedures in this chapter, please read and observe the safety requirements in the "Isolera™ Installation and Safety" document (P/N 415797). Failure to follow those requirements may result in personal injury and/or equipment damage. If the system has been damaged and does not function properly, shut it down immediately and contact Biotage 1-Point Support.

5.1 Contact Biotage® 1-Point Support™

If your system has been damaged and does not function properly, shut it down and contact Biotage 1-Point Support. See contact details on page 7-1.

5.2 Accessories

To order cartridges, please see the Biotage Product Catalog. The catalog can be downloaded at www.biotage.com. For a complete list of accessories, please contact your local representative.

5.2.1 All Isolera™ Systems

Accessory	Quantity	Part Number
Test tube rack, 18 x 130 mm (for 17.5 x 130 mm test tubes) Not recommended on Isolera LS.	4	412593
Test tube rack, 18 x 150 mm	4	411792
Test tube rack, 25 x 150 mm	4	411793
Bottle rack, 120 ml	4	411794
Bottle rack, 240 ml	1	411934
Bottle rack, 480 ml	1	411929
120 ml bottles	120	08742
240 ml bottles	84	08743
480 ml bottles	24	411935
Rack number guide for 18 x 130 mm and 18 x 150 mm racks	4	413176
Rack number guide for 25 x 150 mm rack	4	413175
Collection tray for Isolera racks	1	411853
Cartridge holder for SNAP and SNAP Ultra 50g to 120g	1	411923
Cartridge holder for SNAP and SNAP Ultra 340g to 400g	1	411924
Cartridge holder for ZIP and ZIP Sphere 80g	1	413303

Accessory	Quantity	Part Number
Cartridge holder for ZIP and ZIP Sphere 120g	1	413304
Cartridge holder nut	1	411340
Isolera instrument tray with solvent detector for systems with one collection tray (Prime, One, and Four systems)	1	412019
Isolera instrument tray with solvent detector for systems with two collection trays (EXP and LS systems)	1	412062

5.2.2 Isolera™ Prime, Isolera™ One, Isolera™ Four, and Isolera™ Dalton 2000 Systems

Accessory	Quantity	Part Number
Test tube rack, 13 x 100 mm	4	411789
Test tube rack, 16 x 100 mm	4	411790
Test tube rack, 16 x 150 mm	4	411791
Rack number guide for 13 x 100 mm rack	4	413178
Rack number guide for 16 x 100 mm and 16 x 150 mm racks	4	413177
Expanded bed upgrade	1	411926
Cartridge holder for SNAP and SNAP Ultra 10g to 12g	1	411922
Cartridge holder for SNAP and SNAP Ultra 25g to 30g	1	411776
Cartridge holder for ZIP and ZIP Sphere 5g and 10g	1	413092
Cartridge holder for ZIP and ZIP Sphere 30g	1	413302
Cartridge holder for ZIP and ZIP Sphere 45g	1	413303
Sample injection valve, 3-way with Luer adapters	1	411967
Biotage ELSD-A120, external evaporative light-scattering detector	1	ISO-ELSD-A120
Dalton 2000 APCI Source	1	IS-APCI-S01
Dalton 2000 ESI Source	1	IS-ESI-S01
Dalton ASAP Kit incl probe, capillary stand, and APCI source port	1	UP-ASAP-SNL

5.2.3 Isolera™ LS

Accessory	Quantity	Part Number
Sample pump tube, PharMed (limited use) Peristaltic pump tube, incl. connectors. See the chemical resistance properties on page 4-19.	1	412480
Sample pump tube, Fluran (limited use) Peristaltic pump tube, incl. connectors. See the chemical resistance properties on page 4-19.	1	412481
Sample pump tube, ChemSure Peristaltic pump tube, incl. connectors. Can be used with both non-polar and polar solvents.	1	412482
Cartridge holder for SNAP 750g and 1500g	1	412422
Male Luer fitting for SNAP 750g and 1500g	1	412537
Female Luer fitting for SNAP 750g and 1500g	1	412358
Sample injection valve, 3-way, large bore	1	413027
Funnel rack kit including two racks, portable cart, bottle board, positioning shafts (for the bottles), and solvent detector	1	FNRK-032

5.3 Spare Parts

A list of spare parts is available at www.biotage.com.

5.4 Clean the Exterior of the System

Regular cleaning of the touch screen, if performed properly, extends the touch screen life and protects it from contaminants that cause unnecessary wear. Always turn the system off before cleaning the screen.

WARNING

When cleaning the touch screen, use only non-ammonia based window cleaner and do not apply the liquid directly to the screen as this could damage electronic components.

NOTE

Avoid harsh cleaners and chemicals, and moisture getting into the system.

1. Shut down the system as described on page 4-2. If using an Isolera Dalton 2000 system, the mass detector also has to be shut down; see the "Isolera™ Dalton 2000 User Manual" (P/N 415730).
2. Disconnect the power cord(s) from the power outlet(s).
3. Clean the touch screen using a clean, non-abrasive, **dry** cloth. If this does not clean the screen properly, the cloth can be lightly dampened with a **non-ammonia based** window cleaner. After cleaning, wipe dry with a clean, non-abrasive cloth.
4. Clean the exterior surfaces of the Isolera system using a clean, lint-free cloth lightly dampened with water. If required, a small amount of mild soap may also be used. After cleaning, wipe dry with a clean, lint-free cloth.

5. If using an Isolera Dalton 2000 system, clean the exterior surfaces of the mass detector and Isolera Dalton Nanolink using a soft and clean cloth. The cloth can be dry or lightly dampened with 10% aqueous isopropyl alcohol. After cleaning, wipe dry with a clean, lint-free cloth.

5.5 Implement a Mobile Phase Change

To avoid faults during a mobile phase change, it is necessary to perform the change throughout the entire chromatographic system (i.e. in the reservoir, pump, injector, column, and detector). The procedure is to fill and flush the system, using the prime function (see page 4-34), with a series of mutually miscible solvents until a gradual change to the new mobile phase (solvent) is accomplished. If this procedure is not observed, precipitation may occur not only in the flow cell of the internal detector but also in other parts of the system. For example, to change from an organic mobile phase to aqueous solutions, it is necessary to flush the whole system with acetone or an alcohol (e.g. isopropanol).

5.6 Clean the Flow Cell of the Internal Detector

WARNING

Ultraviolet (UV) light can injure your eyes. Do not operate the Isolera system with the detector flow cell removed and/or the retaining latch/nut open.

NOTE

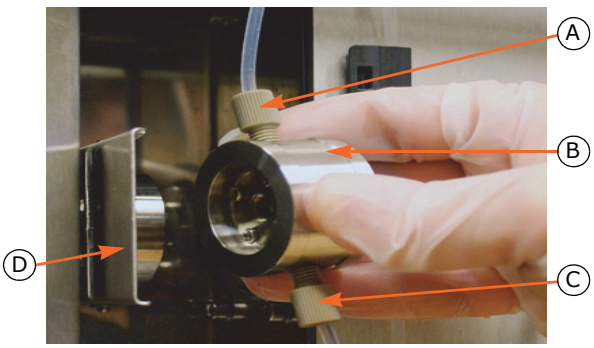
The retaining latch/nut should close with little effort. If the retaining latch/nut is difficult to close, the flow cell is probably misaligned.

Keep the detector flow cell clean and protect it from dust and chemical spills. Particular attention should be paid to preventing the flow cell from leaking.

A dirty cell has decreased transmissivity, which causes increased noise level, decreased response, and difficulties performing both auto and manual UV Zero functions.

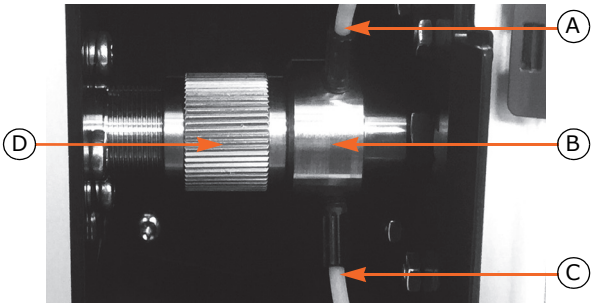
To clean the detector flow cell:

1. Shut down the system as described in on page 4-2.
2. Unlock the flow cell:
   a. To unlock the flow cell on detectors with a retaining latch (see Figure 5-1), hold one hand under the flow cell (to prevent it from falling) and then pull outward on the retaining latch.
   b. To remove the flow cell on detectors with a retaining nut (see Figure 5-2), hold the flow cell with one hand while loosening the retaining nut.



A Flow Cell Outlet Tube
B Flow Cell
C Flow Cell Inlet Tube
D Retaining Latch

Figure 5-1. Remove the Flow Cell on Detectors with a Retaining Latch (Isolera Prime, One, and Four Shown)



A Flow Cell Outlet Tube

B Flow Cell

C Flow Cell Inlet Tube

D Retaining Nut

Figure 5-2. Remove the Flow Cell on Detectors with a Retaining Nut

3. Disconnect the inlet and outlet tubing from the flow cell and visually inspect the cell for contamination.
4. Flush the flow cell with a series of miscible solvents using the injection maintenance kit supplied with the system. Select the solvents based on the contamination. It is possible to use both organic and inorganic solvents and diluted solutions of acids (e.g. H_2SO_4 or HNO_3 diluted with distilled water in a ratio of 1:20 to 1:10), unless they attack stainless steel, PTFE, or fused silica windows.
5. Visually re-examine cell windows for visible contamination. If contamination is still present, repeat step 4. If you are not able to remove the contamination, replace the flow cell and calibrate the detector (see "Calibrate the Internal Detector" on page 3-15).

Flow Cell	Used in
P/N 414204	Isolera One, Four and Prime detectors with a retaining latch.
P/N 414205	Isolera LS detectors with a retaining latch.
P/N 415625SP	Isolera One, Four and Prime detectors with a retaining nut.
P/N 415701SP	Isolera LS detectors with a retaining nut.

6. Reconnect the tubing to the flow cell.
7. Carefully insert the flow cell and close the retaining latch.

5.7 Clean or Release Valves

5.7.1 Clean the Pump Check Valves

If the last purification of the day is performed with a halogenated solvent (e.g. DCM), we recommend that you flush the pump check valves with methanol or a similar solvent. Use the following procedure to flush the pump check valves.

1. Place the solvent inlet line used with the halogenated solvent into methanol or a similar solvent.
2. Ensure that the waste reservoir has sufficient capacity for the flush.
3. Select the **Setup** tab in the right-hand panel.
4. Select the **Prime** tab.
5. If using a system with four cartridge positions, select the **Channel** text box and select the cartridge position and waste reservoir to be used.
6. Select the **Path** text box and press **Bypass**.
7. Ensure that the cartridge's inlet and outlet tubing are coupled together.
8. Select the **Volume** text box and enter the total flush volume. We recommend that you flush with at least 30 ml (Isolera Prime), 60 ml (Isolera One, Isolera Four, and Isolera Dalton 2000), or 100 ml (Isolera LS).
9. Select the **Flowrate** text box and set the flow rate to 100 ml/min (Isolera Prime), 200 ml/min (Isolera One, Isolera Four, and Isolera Dalton 2000) or 500 ml/min (Isolera LS).
10. Enter 100 percentage for the solvent inlet line used for methanol. Enter "0" or press **Clear** for the other solvents that are connected.
11. To start flushing the pump check valves, press ▶**Start**.

5.7.2 Release Stuck Check Valves

Low or inconsistent flow delivery volume and/or superimposed periodic UV signals can be signs of sticking check valves. Use the following procedure to release stuck check valves.

1. Log into the System mode; see page 3-1.
2. Select the **Maintenance** tab in the right-hand panel.
3. Press **Release Check Valves** in the **Valves** field. Read and follow the instructions that appear on the screen.

5.7.3 Clean the Collect Valve

Dripping needle and/or inconsistent dispensing volumes can be signs of a dirty collect valve. Use the following procedure to clean the collect valve.

1. Log into the System mode; see page 3-1.
2. Select the **Maintenance** tab in the right-hand panel.
3. Press **Clean Collect Valve** in the **Valves** field. Read and follow the instructions that appear on the screen.

5.8 Clean or Replace the Sample Loading Pump Tubing

NOTE

To avoid the risk of cross-contamination and decreased pump performance, it is recommended that the peristaltic pump tube is discarded after each run.

Only Isolera LS is equipped with the sample loading pump.

5.8.1 Clean the Sample Loading Pump Tubing

If you want to reuse the peristaltic pump tube (not recommended – see note above), clean the tube with sample solvent as soon as possible after the sample loading. Note that the sample loading pump can be cleaned while a purification is in progress.

1. Place the pump's inlet line into a reservoir with the sample solvent.
2. Place the pump's outlet line into a waste reservoir.

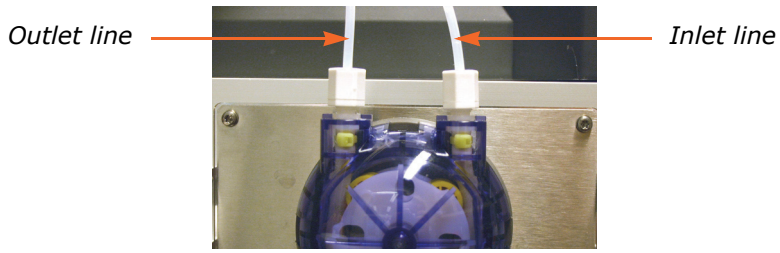


Figure 5-3. Sample Loading Pump

3. Select the **Setup** tab in the right-hand panel (in the software) and then the **Sample Loading Pump** tab.
4. To start the pump, press **Start**. Adjust the flow rate using the **Speed** slider.
5. After rinsing with solvent, continue flushing the sample loading pump with air for a minimum of one minute.
6. When you are done, press **Stop**.

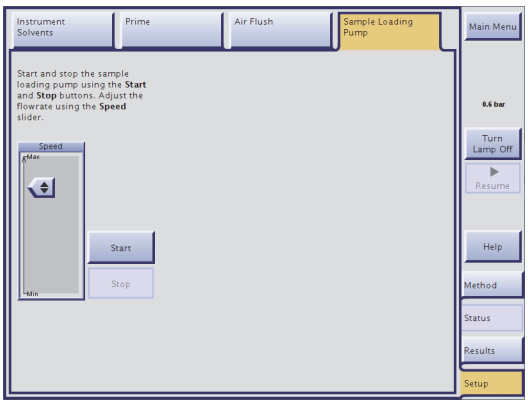


Figure 5-4. Sample Loading Pump Tab

5.8.2 Replace the Sample Loading Pump Tubing

See "Prepare the Sample Loading Pump for a Run (Only Isolera™ LS)" on page 4-17.

5.9 Sonicate Solvent Inlet Filters

A solvent inlet filter is installed on the end of each solvent inlet line. The solvent inlet filters protect the pump and cartridges from damage due to particulate contamination. These filters should be cleaned (sonicated) or replaced every 1000 hours of operation or every 12 months, whichever comes first (P/N 412720SP for Isolera Prime, One, Four, and Dalton 2000 systems, P/N 412628SP for Isolera LS systems, and 413460SP for Isolera Dalton Nanolink, which includes both solvent inlet lines and filters).

If using a system where the filters are not screwed onto the solvent inlet lines, we recommend that you replace both the inlet lines and the filters (P/N 413008SP for Isolera One and Isolera Four, and P/N 413017SP for Isolera LS, both kits include four inlet lines and four filters).

5.10 Leaks

WARNING

Follow all generally-accepted lab safety procedures and applicable laws and regulations.

Always follow local and national safety regulations and the solvent manufacturer's safety, handling, storage, and disposal recommendations; see solvent manufacturer's SDS sheets.

The Isolera system operates using electricity, which can introduce additional hazards with certain solvents if not properly connected, vented, or set up with recommended manufacturer approved settings.

Personnel working with or near the Isolera system must wear protective clothing, safety gear, and eye protection that have been approved by applicable local and national safety regulations.

Use only tubing, nuts, and ferrules supplied by Biotage.

5.10.1 Shut Down the System

If a leakage is observed, shut down the system as follows:

1. If the **Leak Detected** dialog is open, close it by pressing **Close**.
2. If a purification is in progress, press **Stop** at the **Status** tab and then **Abort** in the **Stop** dialog.
3. If a prime is in progress, press **End Prime** at the **Setup** tab.
4. If an air flush (started by the user) is in progress, press **End Air Flush** at the **Setup** tab. The Air Flush feature is not available with Isolera Prime.
5. If using a system with four cartridge positions, remove any queued purifications at the **Status** tab by pressing **Remove**.
6. Press **Main Menu** in the right-hand panel.
7. Press **Shut Down** and then **Yes** to confirm.
8. If using an Isolera Dalton 2000 system, the **Mass Detector** dialog opens. Press **OK**.
9. When the message saying that it is safe to turn off the system appears on the screen, turn off the system. The power switch is located underneath the touch screen.
10. If using an Isolera Dalton 2000 system, turn off Isolera Dalton Nanolink using the power switch located at the rear.

5.10.2 Internal Leakage

Internal leakage, due to e.g. worn pump seals or tube fittings, is drained through drain ports underneath the system. If using an Isolera Dalton 2000 system and the leakage is detected on the Isolera Dalton Nanolink tray, or via the drain tube connected to the tray, then the leak is likely located inside Isolera Dalton Nanolink.

If an internal leakage is observed:

1. Shut down the system as described in section 5.10.1.
2. Disconnect the power cord(s) from the power outlet(s).
3. Ensure that the leakage is not external; check all tubes and connections for leaks.
4. Contact Biotage 1-Point Support; see contact details on page 7-1.

5.10.3 External Leakage

External leakage may occur due to e.g. loose fittings or damaged tubing. Any leakage in the flow cell of the internal detector is drained via the drip sheet; see Figure 5-5 on page 5-9.

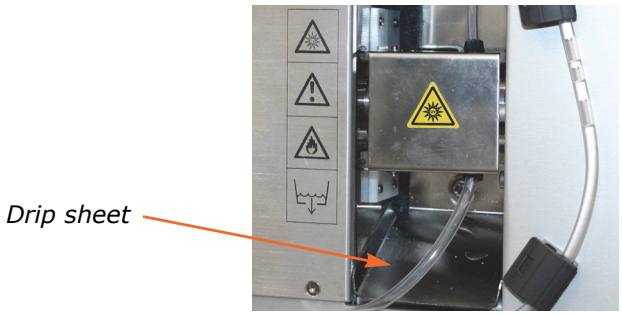


Figure 5-5. Drainage of Leakage from the Flow Cell of the Internal Detector (Isolera LS Shown)

If an external leakage is observed:

1. Shut down the system as described in section 5.10.1.
2. Disconnect the power cord(s) from the power outlet(s).
3. Remove the spillage using the appropriate safety precautions. In the event of leakage from a cartridge, allow all solvent vapor to dissipate before removing the cartridge. Do not wipe away any excess solvent from the cartridge surface as the process of wiping can generate additional static charge.
4. If using an Isolera instrument tray with a solvent detector, ensure that the solvent detector and tray (including the space underneath the internal detector) is cleaned and wiped dry.

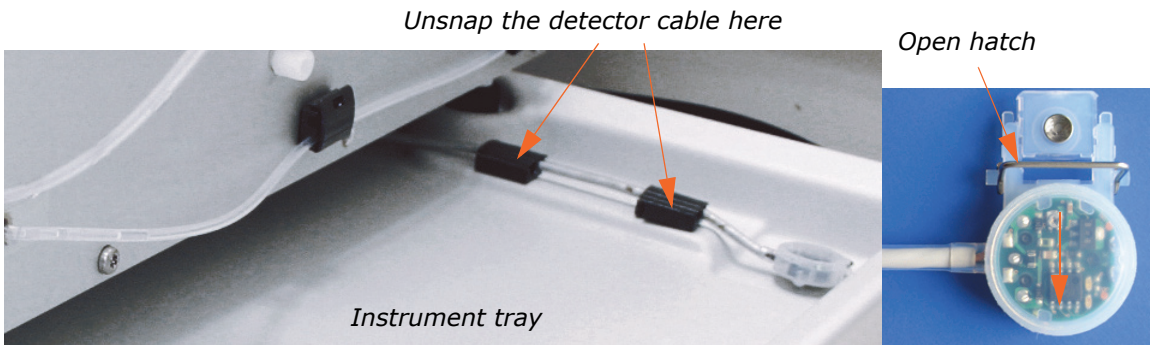


Figure 5-6. Isolera System with Solvent Detector

5. Check all external tubes and connections for leaks. Ensure that the tubes are assembled correctly; see "Assembling Tubes Correctly" on page 5-11. Use caution when tightening fittings to prevent stripped threads or crushed ferrules. Replace damaged tubing; a list of spare parts is available at www.biotage.com.
6. Ensure that the drain tube for the solvent tray is not damaged and is safely connected to the Drain port at the rear of the system. The other end shall be inserted into a separate waste reservoir.

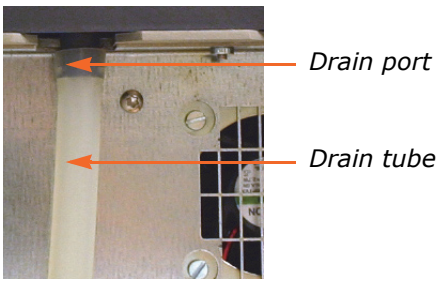


Figure 5-7. Drainage of Spillage on the Solvent Tray

WARNING

To avoid being struck by the collection arm, keep your hands out of range of the collection arm while the homing routine runs in step 10 below.

7. If using an Isolera Dalton 2000 system and the active splitter leaks from the stator ring, the rotor seal and stator face assembly the rotor seal and stator face assembly may be worn or damaged and in need of replacement. Replace the rotor seal and stator face assembly using the maintenance kit supplied by Biotage (P/N 413714SP). Instructions are provided in the kit. When done, reset the counter for active splitter at the **Maintenance** tab in the System mode.
8. Once you have located and taken care of the leakage, reconnect the system.
9. If using an Isolera Dalton 2000 system, turn on Isolera Dalton Nanolink using the power switch located at the rear.
10. Turn on the Isolera system. The collection arm moves through its homing routine and the system boots to the system's main menu.
11. Check all tubes and connections for leaks using the prime function; see "Prime the System" on page 4-34. Prime with water or another suitable solvent. If using an Isolera Dalton 2000 system, also check the tubing and connections of the mass detector and Isolera Dalton Nanolink by priming the makeup solvent inlet and then flushing the tubing with LC-MS grade acetonitrile or other makeup solvent as described in the "Isolera™ Dalton 2000 User Manual" (P/N 415730) supplied with Isolera Dalton Nanolink.
12. If the problem persists, press **End Prime**, shut down the system (see page 4-2), disconnect the power cord(s) from the power outlet(s), and contact Biotage 1-Point Support (see contact details on page 7-1).

5.10.4 Assembling Tubes Correctly

WARNING

Shut down the system before replacing any tubing.

Use only tubing, nuts, and ferrules supplied by Biotage.

All external tubing on the Isolera systems except for the tubing inside the collection arm can be replaced by the user; a list of spare parts is available at www.biotage.com. Verify that any refitted tubes are assembled correctly.

If using an Isolera system with flangeless tubing, please notice that proper sealing is only achieved with the ferrule oriented as shown in Figure 5-8, with the tapered portion of the ferrule facing **toward** the nut. The ferrule should be placed near the end of the tube as shown below.

Figure 5-8. Flangeless Tubing with Properly Oriented Ferrule

If using super flangeless tubing, ensure the ferrules are fitted onto the tube ends as described in the instructions supplied with the ferrules.

Use caution when finger tightening fittings to prevent stripped threads or crushed ferrules.

Isolera™ Dalton Nanolink (Isolera™ Dalton 2000 Systems)

Tubes should be inserted all the way and tube fittings/nuts tightened. Use caution when finger tightening fittings to prevent stripped threads or crushed ferrules.

To ensure proper sealing for tubing connected at the front of Isolera Dalton Nanolink, it is important to orient the ferrule with the tapered portion of the ferrule facing **away** from the nut. Carefully place the ferrule inside the fitting before fastening the nut.

5.11 Replace the Fuses

NOTE

There are no user-replaceable fuses in Biotage Dalton 2000.

WARNING

The system uses double-pole fusing (both sides of the line are fused). Fire hazard; always replace with the same type and rate fuses only. If the system is damaged or does not function properly, contact Biotage 1-Point Support for repair information (see contact details on page 7-1).

1. Shut down the system as described on page 4-2.
2. Disconnect the power cord from the power outlet.
3. Unplug the power cord from the rear of the system. (You will not be able to remove the fuse holder if the power cord is plugged in.)
4. Loosen the fuse holder by carefully prying under the notch on the left side of the holder with a small standard (flat blade) screwdriver; see Figure 5-9.

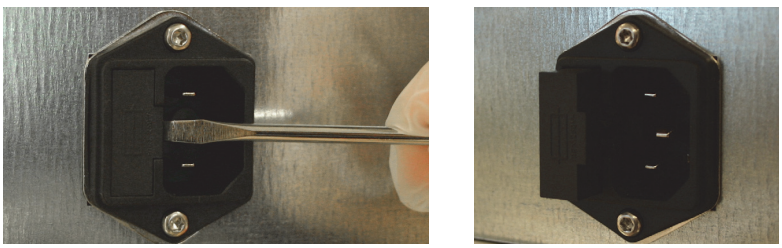


Figure 5-9. Loosen the Fuse Holder

5. Grasp the fuse holder with your fingers and pull it out of the system.

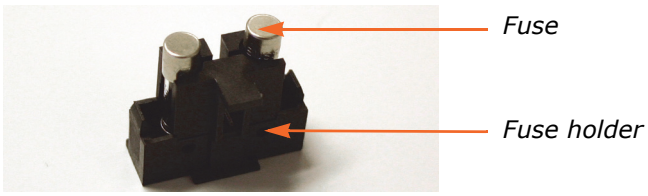


Figure 5-10. Replace the Fuses

6. Replace the two fuses with new fuses of the same type and rate, 4.0 TA/250 V (P/N 411916).
7. Put the fuse holder back in place.

5.12 Replace the Needle

1. Shut down the system as described on page 4-2.
2. Remove the malfunctioning needle.
3. Assemble the new needle, ferrule, and peek nut (P/N 411915 for Ø1.65 needle and P/N 412616 for Ø3.2 needle). Ensure that the needle is aligned with the end of the ferrule; see Figure 5-11.

Figure 5-11. Assemble the Needle, Ferrule, and Nut

4. Mount the needle on the collection arm. Ensure that the needle is touching the needle guide.

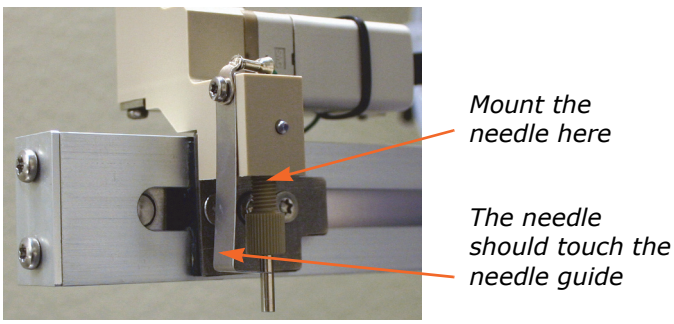


Figure 5-12. Mount the Needle on the Collection Arm

5.13 Replace the Internal Detector Lamp(s)

To replace the internal detector lamp(s) on systems produced before December 2017 (detectors with a retaining latch):

1. Shut down the system as described on page 4-2.
2. Disconnect the power cord from the power outlet.
3. Remove the internal detector service panel at the left side of the system using a Torx 10 screwdriver.
4. Loosen the screw locking the UV lamp in a vertical position (see Figure 5-13) using a Torx 20 screwdriver.

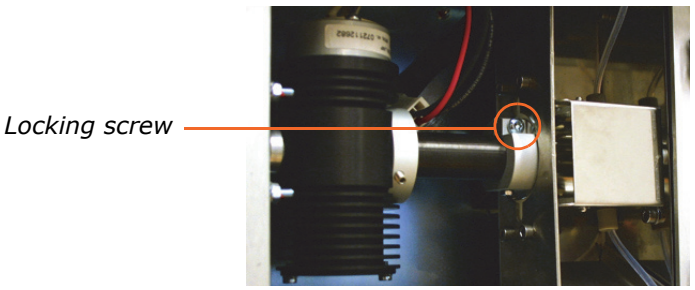


Figure 5-13. Loosen the Locking Screw (UV Detector Shown)

5. Turn the UV lamp into a horizontal position with the lamp socket facing you; see Figure 5-14.
6. Remove the two screws on the lamp socket (see Figure 5-14) using a Torx 10 screwdriver.

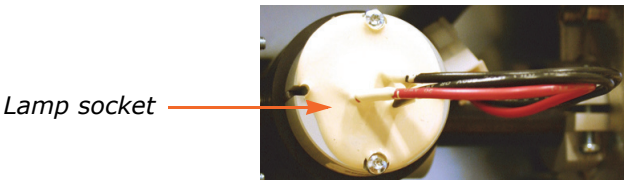


Figure 5-14. Turn the UV Lamp and Remove

7. Pull out the UV lamp and disconnect it from the power cable; see Figure 5-15.

NOTE

Do no touch the UV lamp glass.

Figure 5-15. Disconnect the UV Lamp

8. Mount a new UV lamp (P/N 09830). Ensure the power cable is positioned so that it does not get in contact with the fans at the left side of the UV lamp.
9. If using a UV-VIS detector, replace the tungsten lamp (P/N 412970):
   a. Loosen the screw holding the tungsten lamp in position using the wrench delivered with the system.
   b. Pull out the tungsten lamp and disconnect it from the power cable; see Figure 5-16.
   c. Mount a new tungsten lamp. Ensure the lamp is fully inserted and the power cable is positioned so that it does not get in contact with the fans at the left side of the UV lamp.

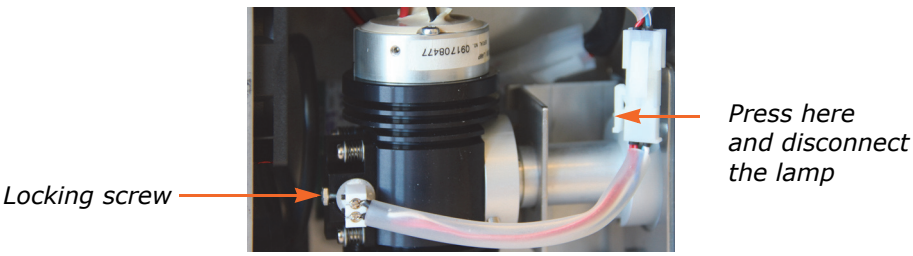


Figure 5-16. Disconnect the Tungsten Lamp

10. When you are done, put the service panel back in place.
11. Reconnect the system and turn it on.
12. When the main menu appears, log into the System mode (see page 3-1).

13. Ensure that the detector flow cell is clean (see page 5-4), then perform an intensity calibration:
    a. Select the **Maintenance** tab in the right-hand panel.
    b. Press **Calibrate** in the **UV Detector** field. Read and follow the instructions that appear on the screen.

To replace the internal detector lamp on systems produced in December 2017 or later (detectors with a retaining nut):

1. Shut down the system as described on page 4-2.
2. Disconnect the power cord from the power outlet.
3. Remove the internal detector service panel at the left side of the system using a Torx 10 screwdriver.
4. Remove the flow cell; hold the flow cell with one hand while loosening the retaining nut (see Figure 5-17).
5. Loosen the three screws locking the UV lamp in a vertical position using an angled Torx 20 screwdriver; see Figure 5-17.

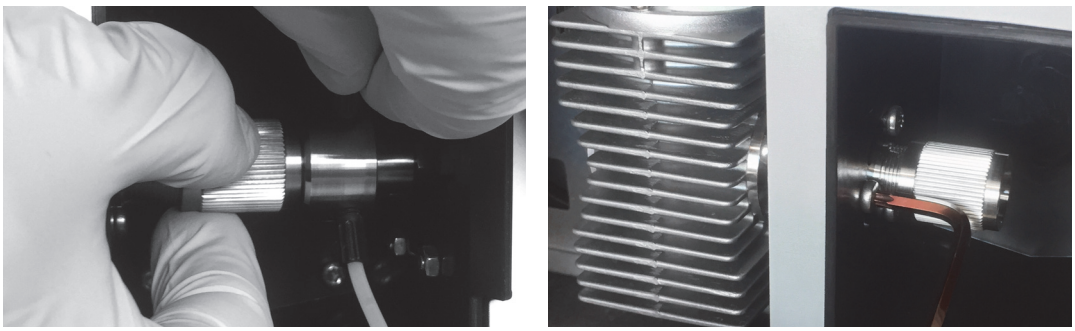


Figure 5-17. Loosen the Retaining Nut (Left) and the Locking Screws (Right)

6. Disconnect the power cable for the UV lamp; see Figure 5-18.
7. Turn the UV lamp into a horizontal position with the lamp socket facing you; see Figure 5-18.
8. Remove the two screws on the lamp socket (see Figure 5-18) using a Torx 10 screwdriver and pull out the UV lamp.

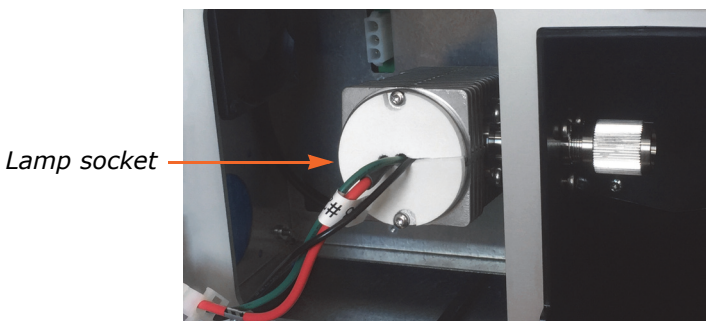


Figure 5-18. Remove the UV Lamp

9. Mount a new UV lamp (P/N 415599SP) and put the flow cell and service panel back in place by reversing the remove procedure in step 3 through 8. Ensure the power cable for the UV lamp is positioned so that it does not get in contact with the fan at the left side of the UV lamp.

NOTE

Do no touch the UV lamp glass.

10. When the system has been reassembled, reconnect the power cord to the power outlet and turn on the system.
11. When the main menu appears, log into the System mode (see page 3-1).
12. Ensure that the detector flow cell is clean (see page 5-4), then calibrate the detector:
    a. Select the **Maintenance** tab in the right-hand panel.
    b. Press **Calibrate** in the **UV Detector** field. Read and follow the instructions that appear on the screen.

Chapter 6 Troubleshooting

WARNING

Before performing any procedures in this chapter, please read and observe the safety requirements in the "Isolera™ Installation and Safety" document (P/N 415796). Failure to follow those requirements may result in personal injury and/or equipment damage. If the system has been damaged and does not function properly, shut it down immediately and contact Biotage 1-Point Support.

6.1 Contact Biotage® 1-Point Support™

If your problem persists, or for assistance at any time during troubleshooting, contact Biotage 1-Point Support. See contact details on page 7-1.

6.2 Accessories and Spare Parts

A condensed list of accessories is available on page 5-1. For a complete list, please contact your local representative.

A list of spare parts is available at www.biotage.com.

6.3 Fraction Collector-Related Problems

Problem	Possible Causes	Solutions
1. The collection arm does not position correctly over each collection vessel.	a. Misaligned rack(s).	Ensure that the rack(s) is/are aligned correctly.
	b. Wrong rack type selected in the software.	Ensure that you select the correct rack type when setting up a method; see "Prepare and Run a Purification" on page 4-17.
	c. Incorrect rack parameters. (Only if using a user-defined rack type.)	Ensure that the correct rack parameters have been entered for the used rack type; see "Administrate the Rack List" on page 2-5.
	d. The collection arm is obstructed.	Remove any obstacles that obstruct or restrict arm movement.
	e. Improper calibration of the collection arm.	Calibrate the collection arm; see "Calibrate the Fraction Collector" on page 3-15.
2. Dripping needle and/or inconsistent dispensing volumes.	Dirty collect valve.	Clean the collect valve with methanol or a similar solvent using the Collect Valve Clean Wizard; see page 3-16.

6.4 Pump-Related Problems

NOTE

If using highly volatile (i.e. high vapor pressure) solvents such as dichloromethane, reservoir elevation is strongly recommended. Use the solvent tray at the top of the system. It is also highly advisable to reduce the flow rate and, if possible, lower the ambient temperature. See "Solvent Specifications" on page 1-20 for the vapor pressure of different solvents.

Problem	Possible Causes	Solutions
1. Air bubbles moving through the cartridge outlet tubing in a steady stream during solvent delivery. Note that saturation of a cartridge takes a minimum of 3 CV.	a. Little or no solvent in the lines.	1. Replenish the solvent reservoir(s). 2. Ensure that all used solvent inlet lines are submerged in solvent; see "Prime the System" on page 4-34.
	b. One or more solvent inlet lines are loose.	Check fittings, loosen, and retighten. If using flangeless tubing, see "Assembling Tubes Correctly" on page 5-11. If using super flangeless tubing, ensure that the ferrules are fitted onto the tube ends as described in the instructions supplied with the ferrules.
	c. One or more solvent inlet filters are plugged.	Sonicate or replace the solvent inlet filters; see page 5-8. Re-circulating the solvent is not recommended. Particulate-free solvent is required. If the problem persists, the pump piston seals or the check valve seals may be worn. Please contact Biotage 1-Point Support; see contact details on page 7-1.
2. Low or inconsistent flow delivery volume and/or superimposed periodic detector signals.	a. One or more pump check valves are not functioning properly.	1. Flush the pump check valves with methanol or a similar solvent using the Check Valves Release Wizard; see page 3-16. 2. Check for leaks in the tubing or fittings; see "Leaks" on page 5-8. 3. If the problem persists, the pump piston seals or the check valve seals may be worn. Please contact Biotage 1-Point Support; see contact details on page 7-1.
	b. One or more pump check valves are not functioning properly due to particulates in the solvent.	1. Flush the pump check valves with methanol or a similar solvent using the Check Valves Release Wizard; see page 3-16. 2. If the problem persists, please contact Biotage 1-Point Support (see page 7-1).
	c. Solvent vaporization during refill stroke.	1. Elevate the solvent reservoir(s); use the solvent tray. 2. If possible, lower the ambient temperature. 3. Sonicate or replace the solvent inlet filters; see page 5-8. 4. Reduce the fill rate for the used solvents (in the Data Administration mode). Note that the **Max Fill Rate** parameter can only be changed for user-defined solvents. If using a preconfigured solvent, make a copy of it and then change the fill rate.

Problem	Possible Causes	Solutions
3. The pump does not deliver solvent.	a. No or insufficient solvent in the reservoir(s).	1. Replenish the solvent reservoir(s). 2. Ensure that all used solvent inlet lines are submerged in solvent; see "Prime the System" on page 4-34.
	b. Stuck pump check valve(s).	Flush the pump check valves with methanol or a similar solvent using the Check Valves Release Wizard; see page 3-16.
	c. Blockage in solvent line(s).	1. Sonicate or replace the solvent inlet filters; see page 5-8. 2. Flush the entire system with methanol or isopropanol at a low flow rate; see "Prime the System" on page 4-34.
	d. Leakage.	Check for leaks in the tubing or fittings; see "Leaks" on page 5-8.

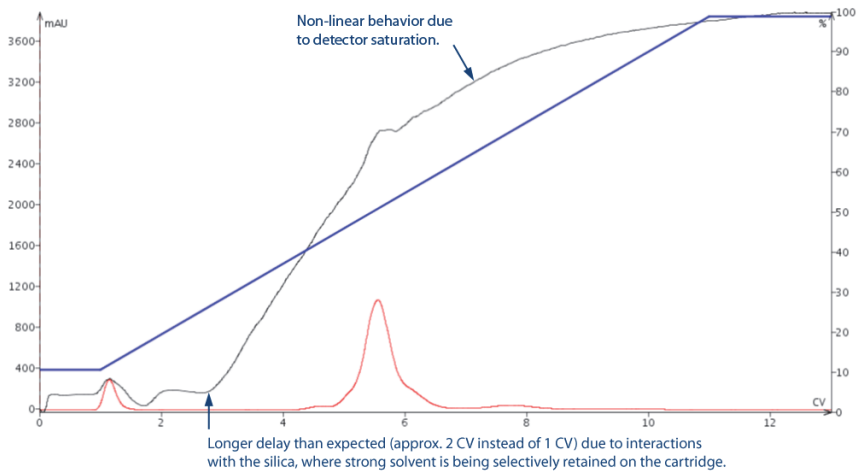
6.5 Internal Detector-Related Problems

Problem	Possible Causes	Solutions
1. Noise.	a. Dirty flow cell.	Clean the flow cell; see page 5-4.
	b. Flow cell incorrectly inserted.	Open the retaining latch and turn the flow cell upside down. **Note:** This only applies to detectors produced before December 2017.
2. Drifting baseline.	a. Dirty flow cell.	Clean the flow cell; see page 5-4.
	b. Solvent is high-absorbing at the selected wavelength(s).	Change the collection and fractionation wavelength(s) or turn the **UV Baseline Correction** option on in your method (if using a system with an Isolera Spektra or Dalton 2000 software license installed).
	c. Lamp(s) is/are defective.	Replace the lamp(s) and recalibrate the internal detector; see page 5-13. **Note:** If the UV lamp has been used for more than 2000 hours (detectors produced before December 2017) or 4000 hours (detectors produced in December 2017 or later), the message *The detector lamp has been used for x hours and should be replaced* appears at startup.
3. Missing peaks when using UV baseline correction.	Solvent(s) are high-absorbing over a wide range of wavelengths.	If expected peaks do not show when you have the **UV Baseline Correction** option turned on in your method and you are using solvents that are high-absorbing over a wide range of wavelengths (e.g. acetone or toluene), try performing the run without UV baseline correction.
4. The UV Detector Failure dialog appears during auto or manual UV Zero.	a. Dirty flow cell.	Clean the flow cell; see page 5-4.
	b. Highly absorbing solvent(s).	Choose less absorbing solvent(s).
	c. Sample in the flow cell.	Retry performing the UV Zero when there is no sample in the flow cell. If the problem persists, please contact Biotage 1-Point Support (see page 7-1).

6.6 Biotage® Dalton 2000 and Isolera™ Dalton Nanolink Problems

See the "Troubleshooting" section in the "Isolera™ Dalton 2000 User Manual" (P/N 415730).

6.7 Gradient Problems

Problem	Solutions
Low composition gradient is not correct.	When generating solvent mixtures where the composition contains less than 10% of one solvent: 1. Ensure that the solvent with the lowest percentage is pumped first, i.e. is mounted on a solvent inlet with a lower number than the solvent with the highest percentage. Use for example inlet S1 for the solvent with the lowest percentage and S2 for the other one. 2. If the problem persists, premix the solvent using the desired final % strong solvent in the weak solvent and use this as solvent B. Program the gradient from 0–100% B using the pre-mixed solvent B. See "Prepare and Run a Purification" on page 4-17.
The baseline drift is different from the programmed gradient.	Two factors contribute to altering the gradient as observed by the UV detector on the output side of the cartridge: 1. As it takes at least 1 CV for the solvent to pass through the cartridge and reach the UV detector, the initial front of the gradient will always be delayed by at least 1 CV compared to the programmed gradient. A longer gradient delay may be due to interactions with the silica, where strong solvent is being selectively retained on the cartridge. 2. The gradient as observed by the UV detector will at times decline and plateau before the programmed gradient does. This is due to the UV detector reaching saturation for the wavelengths absorbed by the strong solvent. Any increase in the concentration of strong solvent will not be registered as no light is penetrating the solvent at those particular wavelengths.  If using a system with an Isolera Spektra or Dalton 2000 software license installed, try performing the run with the **UV Baseline Correction** option turned on.

6.8 Leak Detected

See "Leaks" on page 5-8.

6.9 Overpressure Detected

Possible Causes	Solutions
1. Blockage due to kinked tubing.	a. Press **Purge...** in the alarm dialog. b. When the pressure has been reduced to an acceptable level, press **End** in the **Purge** dialog. c. Press **Pause** in the alarm dialog. d. Shut down the system; see page 4-2. If using an Isolera Dalton 2000 system, the mass detector also has to be shut down (see the "Isolera™ Dalton 2000 User Manual", P/N 415730). To be able to shut down the system, you have to abort the task in progress. e. Straighten or replace the kinked tubing. For more information, see "Assembling Tubes Correctly" on page 5-11. **WARNING** **To avoid being struck by the collection arm, keep your hands out of range of the collection arm while the homing routine runs in step f below.** f. Turn on the system as described on page 4-1. The collection arm moves through its homing routine and the system boots to the system's main menu.
2. The flow rate is too high for the purification or prime.	a. Press **Purge...** in the alarm dialog. b. When the pressure has been reduced to an acceptable level, press **End** in the **Purge** dialog. c. Press **Pause** in the alarm dialog. d. If the overpressure occurred during a prime, press **End Prime** at the **Prime** tab and start a new prime with a lower flow rate. e. If the overpressure occurred during an equilibration or a gradient run: 1. Press **Edit...** at the **Status** tab. The **Edit Method** dialog opens. 2. Lower the flow rate and press **OK**. 3. To resume the run, press **Resume** in the right-hand panel.
3. Blockage in the cartridge, tubing, or UV detector flow cell from precipitations. Note that there may be precipitation in areas not visible.	a. Press **Purge...** in the alarm dialog. b. When the pressure has been reduced to an acceptable level, press **End** in the **Purge** dialog. c. Press **Pause** in the alarm dialog. d. Shut down the system; see page 4-2. If using an Isolera Dalton 2000 system, the mass detector also has to be shut down (see the "Isolera™ Dalton 2000 User Manual", P/N 415730). To be able to shut down the system, you have to abort the task in progress. e. Visually inspect all tubing for precipitation. If found, remove and clean the tubing. For more information, see "Assembling Tubes Correctly" on page 5-11. f. Visually inspect the UV detector flow cell for precipitation. If found, clean the flow cell. For more information, see page 5-4. **WARNING** **To avoid being struck by the collection arm, keep your hands out of range of the collection arm while the homing routine runs in step g below.** g. Turn on the system as described on page 4-1. The collection arm moves through its homing routine and the system boots to the system's main menu. h. Prime the system using e.g. methanol; see page 4-34.

Chapter 7

Contact Information

Manufacturer

Biotage Sweden AB

Advion Inc. for Biotage Sweden AB (Biotage Dalton 2000)

Biotage Sweden AB

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E-mail: info@biotage.com
Website: www.biotage.com

Sales Offices and Distributors

Please visit www.biotage.com for contact information.

Technical Support

Call, fax, or e-mail Biotage 1-Point Support, or request support online (www.biotage.com).
See contact information on the back of this document or visit our website www.biotage.com.

When contacting Biotage 1-Point Support, please have the following information ready:

- Company name and contact information.
- Serial number of your Isolera system (see the product label at the rear of the system).
- A brief description of the symptoms or technical problems you are experiencing.

Appendix A

Collection and Fractionation

This appendix describes the details of the different collection and fractionation methods that are available for the Isolera system. It also contains information on how gradients are automatically extended.

A.1 Collection and Fractionation Methods

The Isolera system supports several collection and fractionation methods controlled by the internal detector, the mass detector (optional), or an external signal. The internal detector provides collection and fractionation methods based on one or two specific wavelengths, or on a continuous wavelength range. It is possible to combine these methods in a number of ways as described in section A.1.4.

A.1.1 Collection Methods

The following collection methods are supported:

- *Manual (Collect All)*: Everything is collected while Collect All is on. Collection can be started and stopped by the user.
- *Threshold*: Everything above a specified threshold level is collected. Note that the mass detector automatically calculates the collection threshold.
- *Slope*: Collection is started when the slope of the light absorption is above a specified limit. Collection is stopped when the absorption level is below the level when the collection was started. Note that the Slope collection mode is only available with the UV1 and UV2 detection signals.

A.1.2 Fractionation Methods

The following fractionation methods are supported:

- *Manual*: Manual fractionation.
- *Volume*: Fractionation when a test tube or bottle is full, based on the specified max fraction volume for the vessel type.
- *Valley*: Fractionation when a valley is detected in the light absorption curve or TIC signal.
- *Shoulder*: Fractionation when a shoulder is detected in the light absorption curve.
- *Threshold*: When more than one signal is used with threshold collection method, fractionation will occur every time one of the signals passes its threshold level.

A.1.3 Detector Signals

The Isolera system includes either a UV detector with the range 200-400 nm or a UV-VIS detector with the range 200-800 nm. (The UV-VIS detector is not available with Isolera Prime.) If using an Isolera One or Four system with a Dalton 2000 software license, it is also possible to connect a mass detector, Biotage Dalton 2000, to the system. It is also possible to connect an external detector to the system, e.g. Biotage ELSD-A120. The mass detector and external detector cannot be connected at the same time.

Depending on the detector in use, collection and fractionation are performed slightly different:

- *UV or UV-VIS detector:*
  1. *UV1 and UV2:* The system uses one or two wavelength signals for collection and fractionation. Possible collection and fractionation parameters are Collect All, Start Threshold, Slope, Shoulder, and Valley (enable/disable in System mode).
  2. *λ-all:* The system uses average absorbance within a user-defined wavelength range for collection and fractionation. Possible collection and fractionation parameters are Collect All, Start Threshold, and Valley (enable/disable in System mode). (Only available on systems with an Isolera Spektra or Dalton 2000 software license installed.)
- *Mass detector:*
  1. *TIC:* The system uses the mass detector signal to monitor and collect based on mass-to-charge ratio (m/z). Collection and fractionation is based on the sum of all m/z signals in the selected m/z range. Background noise from the chromatography solvents, makeup solvent, and the mass detector itself is automatically removed from the TIC signals. Fractionation occurs on threshold, valley, and volume for both positive and negative ionization. Possible collection parameter is Collect All.
  2. *XIC:* The system uses the mass detector signal to monitor and collect based on mass-to-charge ratio (m/z). Collection and fractionation is based on the sum of the m/z-ratio +/- 0.6 for up to four different ions. Each XIC signal has its own threshold, which is automatically set based on the noise level at the start of the run. The finishing threshold level for XIC is 10% of the highest value of the peak or the start threshold, whichever is higher. Fractionation occurs on threshold and volume. Possible collection parameters are Collect All and End Run After Peak.
- *External detector:* The system uses only the signal from the external detector for collection.

Signals that are not used for collection and fractionation can be used for monitoring.

If Collect All is on, fractionation based on volume and collection signal(s) will still occur. If no signal is used for collection and fractionation, the collection is started and stopped by the user. Volume based fractionation will still occur.

A.1.4 Collection and Fractionation Methods

The possible combinations of collection and fractionation methods are listed in Table A-1.

All collection methods fractionate on volume, which is when a test tube or bottle is full based on the specified max fraction volume for the vessel type. At any time, you can manually switch to a new collection vessel by pressing **New Fraction** at the **Status** tab.

Table A-1: Possible Combinations of Methods

Collection Method	Fractionation Method			
	Manual	**Volume**	**Valley**	**Shoulder**
Manual	X	X		
Threshold	X	X	X*	
Slope†	X	X	X	X

* Valley fractionation will not occur with XIC detection.

† The Slope collection mode is only available with the UV1 and UV2 detection signals.

Manual Collection

When using the Manual collection method, collection is started and stopped manually by pressing **Collect All,** or selecting **Collect All** in general collection parameters**.**

It is possible to combine the manual collection with the Threshold and/or the Slope collection method. This means that everything will be collected and fractionation will occur on the Start Threshold level and/or the Start Slope setting. Fractionation will also occur on detected valleys and on shoulders, if enabled.

Threshold

When using the Threshold collection method, the collection is started and stopped depending on the signal level(s) from the detector.

When more than one UV signal or m/z-ratios are specified, collection is started when one of the signals used for collection and fractionation reaches the specified Start Threshold level. Fractionations occur when another signal reaches the Start Threshold level and when one of the signals decreases below the its threshold level. When all signals decrease below their threshold level, the collection is stopped.

Slope

When using the Slope collection method, the collection is started depending on the calculated slope of the signal from the detector. The collection is stopped when the signal is below the absorption level when the collection was started.

When collecting on two UV wavelengths, collection is started when the calculated slope of one of the signals used for collection and fractionation reaches the specified Start Slope setting. Fractionations occur when the other signal's calculated slope reaches the Start Slope setting and then when one of the signals decreases below the absorption level detected when the collection was started. When both signals decrease below the level detected when the collection was started, the collection is stopped. The Slope collection method also enables the possibility to fractionate when shoulders are detected in the signal.

Note that the Slope collection mode is only available with the UV1 and UV2 detection signals.

A.1.5 Collection and Fractionation Parameters

Method Parameters

The method parameters are defined for each method and can be changed by the user. The following method parameters are available:

Table A-2: Method Parameters

Parameter	Description
Detection Signals	The basic configuration for collection and fractionation. The available options are [TIC | XIC | λ-all | UV1| UV2 | External]. The λ-all detection signal is only available on systems with an Isolera Spektra or Dalton 2000 software license installed. The TIC and XIC detection signals are only available with the optional mass detector (Biotage Dalton 2000). An external detector and the mass detector cannot be enabled in the software at the same time.
Start Wavelength	The shortest wavelength to be included in the λ-all signal. The range is 200 to 399 nm (UV detector) or 200 to 799 nm (UV-VIS detector). (Only available with the λ-all detection signal.)

Table A-2: Method Parameters

Parameter	Description
End Wavelength	The longest wavelength to be included in the λ-all signal. The range is 201 to 400 nm (UV detector) or 201 to 800 nm (UV-VIS detector). (Only available with the λ-all detection signal.)
m/z Range	The used mass-to-charge range. Default range is 90 to 2000 but by reducing the range, the signal may be improved. Note that the maximum range can be expanded by a setting in the System mode. (Only available with the TIC and XIC detection signals.)
Ion 1-4	The mass-to-charge ratio(s) of the ion(s) used for detection, collection, and fractionation. The values must be within the set m/z Range. Positive or negative ionization can be selected for each ion individually. (Only available with the TIC and XIC detection signals.)
End Run After Peak	When this option is turned on, the run is automatically ended once a sufficiently large peak of the selected ion has been collected. If the peak is too small, the run will continue. (Only available when collecting on XIC.)
Collect All	Used to collect the entire run. The setting is [On | Off]. This option can be toggled during the run to manually stop and start collection.
UV Baseline Correction	Used to remove the interference of the light absorbance of the used solvents from the absorbance signal during chromatography. (Only available on systems with an Isolera Spektra or Dalton 2000 license installed.) When this option is turned on, the gradient run is preceded by a short solvent light absorbance detection phase. During this phase, the light absorbance of the used solvents is measured at all wavelengths that will be used for collection and fractionation (UV1, UV1 and UV2, or λ-all). The measurement results in a baseline containing the maximum absorbance of the solvent system at each wavelength. During the gradient run, the baseline is subtracted from the signal from the detector. Only absorbance values above zero are reported, anything below that is reported as zero. In effect this automates the procedure of manually excluding parts of the spectrum from the summation. In a normal use case, peaks that have absorbance in the same wavelengths as the solvent are still detected because of absorbance above the baseline.
Start Threshold	Used to collect samples with a light absorbance exceeding the set absorbance threshold. The range is [Off | 0 to 6000 mAU]. For collection and fractionation based on mass detection, the threshold levels are calculated by the system and cannot be altered.
Initial Waste	Delay collection until a specified volume has been delivered to the waste. The range is [0 to 10000 ml]. If the gradient is defined in CV, then the initial waste is also defined in CV.
Slope Mode	Used to collect samples based on the slope of the signal. The range is [Off | Low Slope | Medium Slope | Custom Slope]. (Only available with the UV1 and UV2 detection signals.)
Start Slope	The value of the collect start slope. The range is [Off | 1 to 5000 mAU/CV]. (Only available with slope collection; see Slope Mode above.)
Shoulder Slope	The value of the fractionation shoulder slope. The range is [Off | 1 to 5000 mAU/CV]. (Only available with slope collection; see Slope Mode above.)

System Parameters

The system parameters are defined for all methods and can be changed (in the System mode) only by a user with system owner privilege. The following system parameters are available:

Table A-3: System Parameters

Parameter	Description
Start Slope Enable	The level where the parameter Start Slope is enabled. Below this level the Start Slope parameter is ignored. The range is [0 to 6000 mAU]. The default value is 30 mAU.
Shoulder/Valley Slope Disable	The level where the parameters Shoulder Slope and Valley Slope are disabled. Above this level the Shoulder Slope and Valley Slope parameters are ignored. The range is [0 to 6000 mAU]. The default value is 5500 mAU.
Valley Slope	The value of the fractionation valley slope. The range is [Off | 1 to 5000 mAU/CV]. The default value is 150 mAU/CV.
Mass Range	The maximum mass range to be used. The setting is [Normal (m/z 90-2000) | Full (m/z 10-2000)]. The default value is Normal.

A.2 Shoulder Slope and Valley Fractionation

A.2.1 Fractionation Using the Shoulder Slope Parameter

When the Shoulder Slope parameter is enabled (the range is 1 to 5000 mAU/CV), the Isolera system fractionates on rising and falling shoulders and double fractionates on valleys.

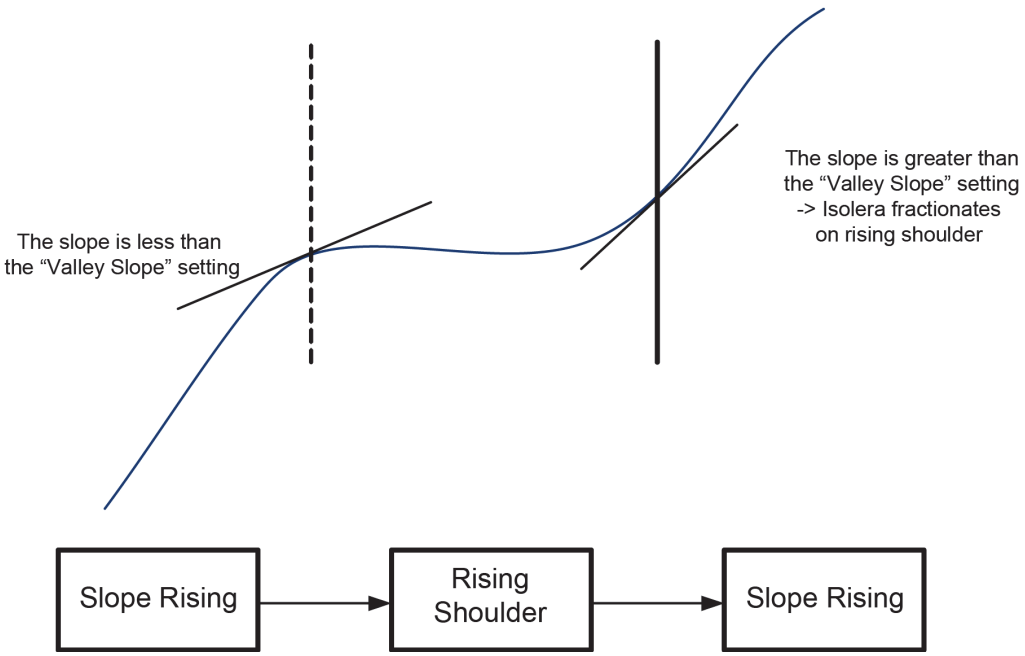


Figure A-1. Fractionation on Rising Shoulder

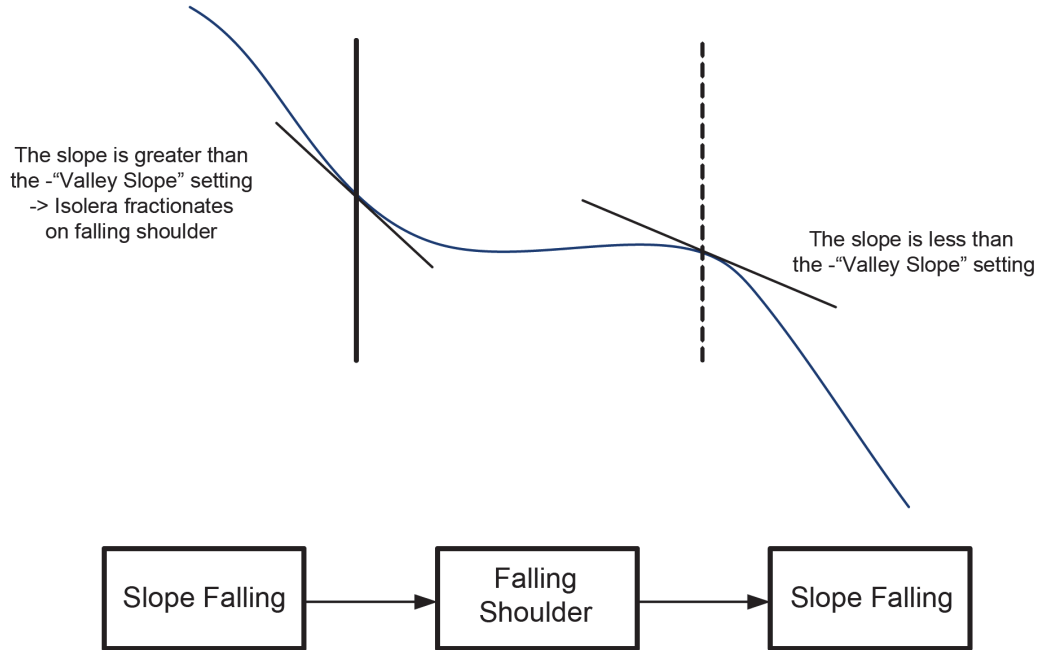


Figure A-2. Fractionation on Falling Shoulder

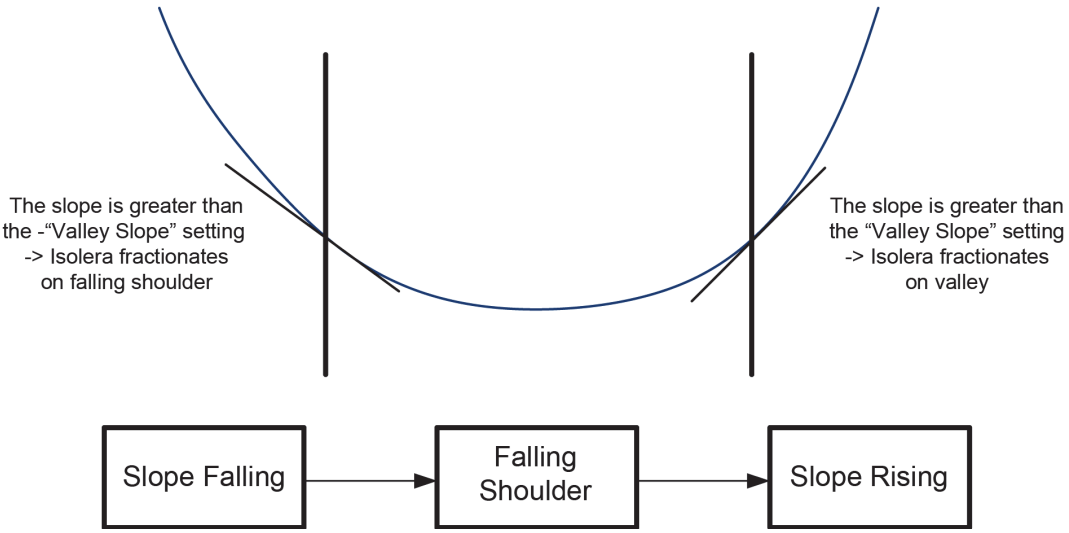


Figure A-3. Double Fractionation on Valley

A.2.2 Fractionation Using the Valley Slope Parameter

When the Valley Slope parameter is enabled (the range is 1 to 5000 mAU/CV) while the Shoulder Slope parameter is disabled (Off), the Isolera system only fractionates on valleys.

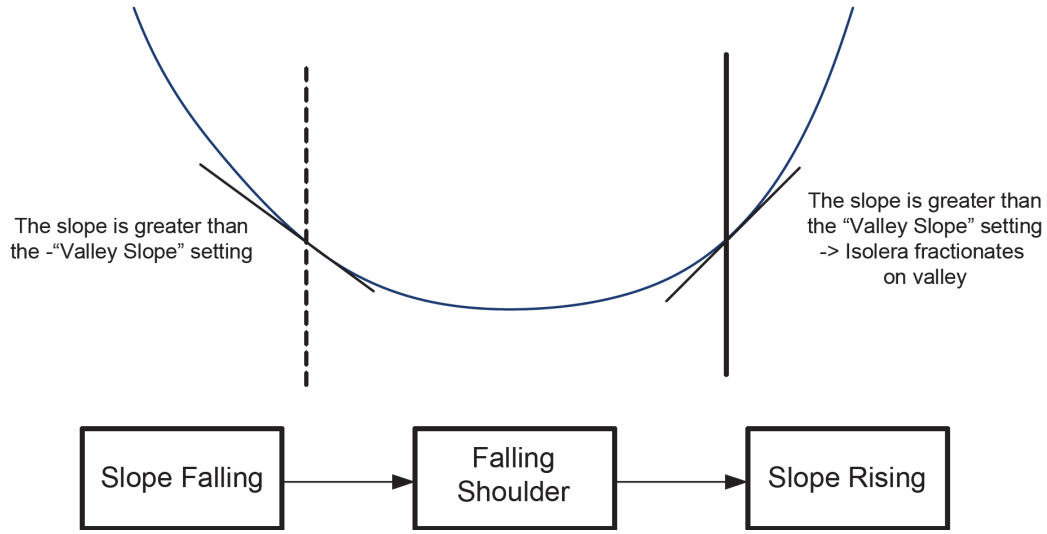
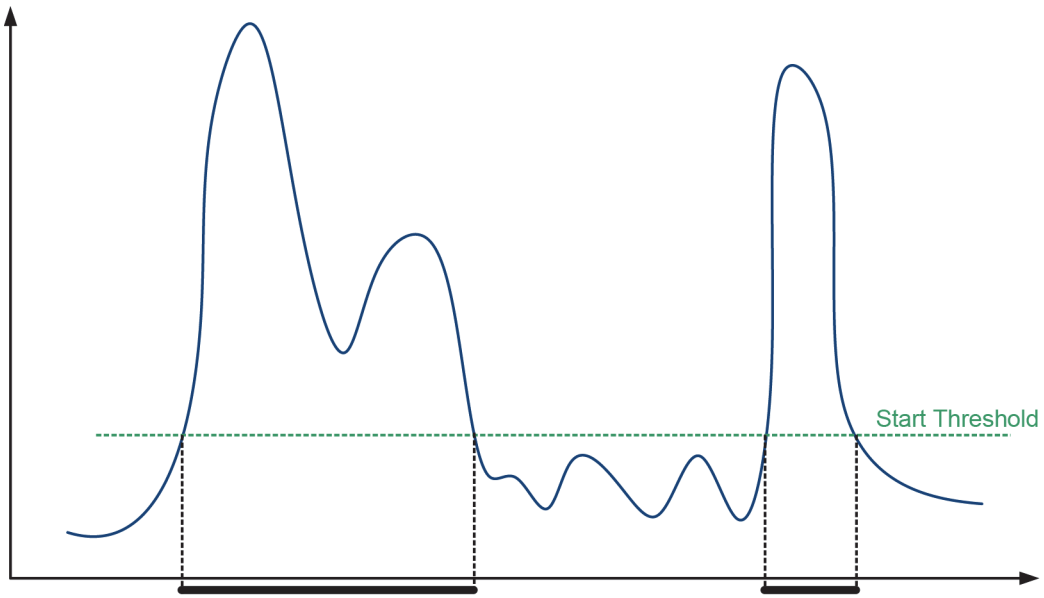


Figure A-4. Single Fractionation on Valley

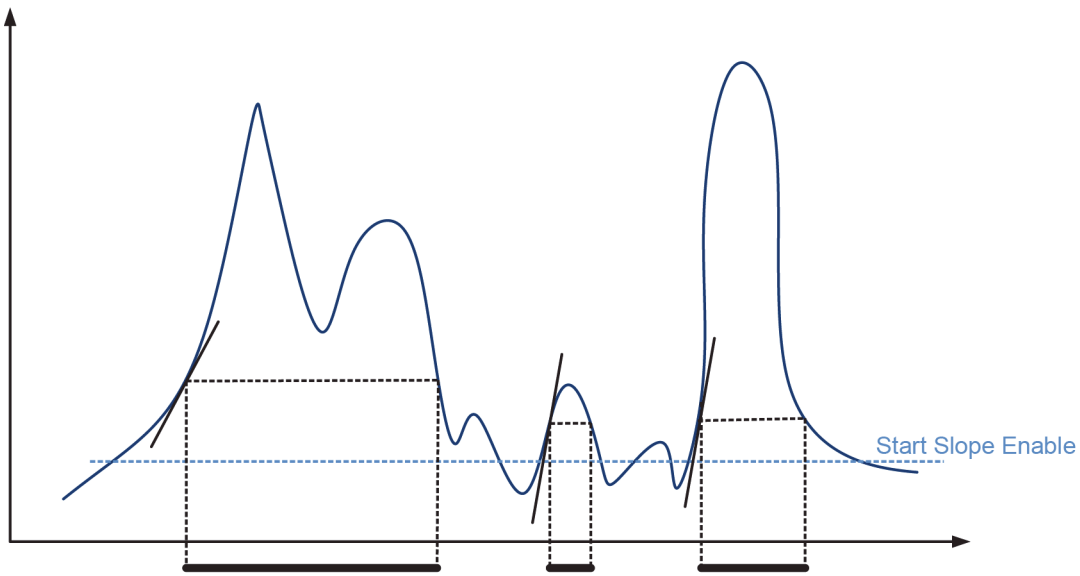
A.3 Collection and Fractionation Examples

A.3.1 Threshold Collection



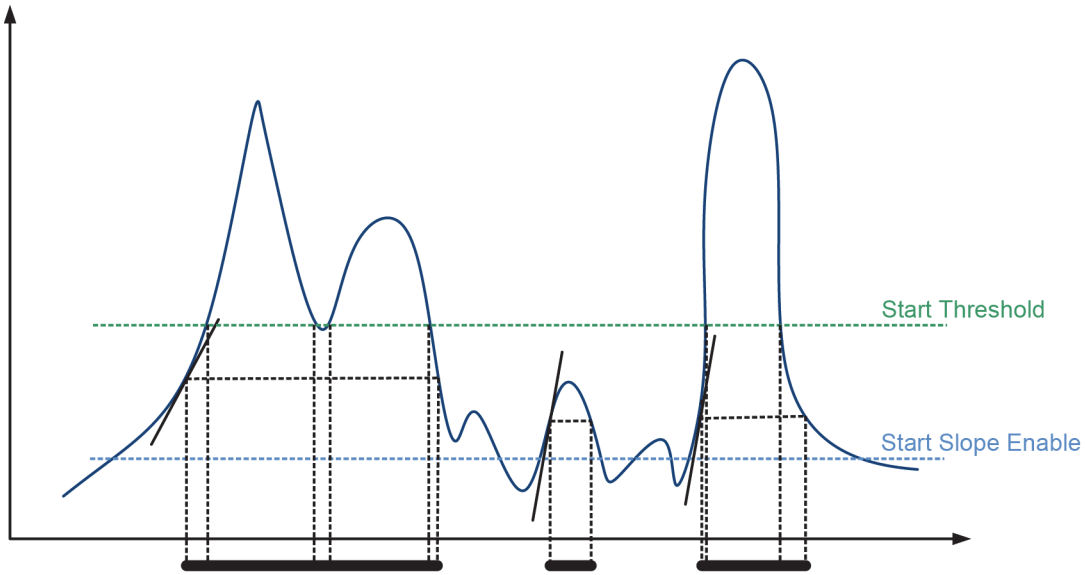
Collects when the absorption level is above the “Start Threshold” level.

A.3.2 Slope Collection



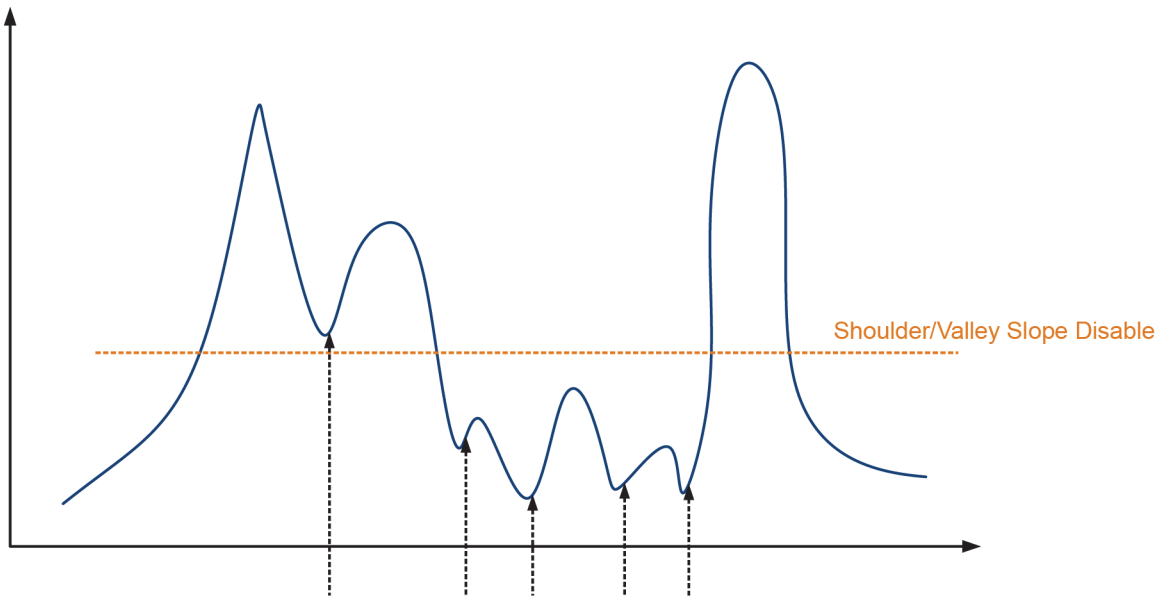
Starts collecting when the absorption level is above the “Start Slope Enable” level and the calculated slope is greater than the “Start Slope” setting. Stops collecting when the absorption level is below the level detected when the collection was started.

A.3.3 Slope Collection and Threshold Fractionation



Starts collecting when the absorption level is above the “Start Slope Enable” level and the calculated slope is greater than the “Start Slope” setting. Stops collecting when the absorption level is below the level detected when the collection was started. Fractionates when the “Start Threshold” level is reached.

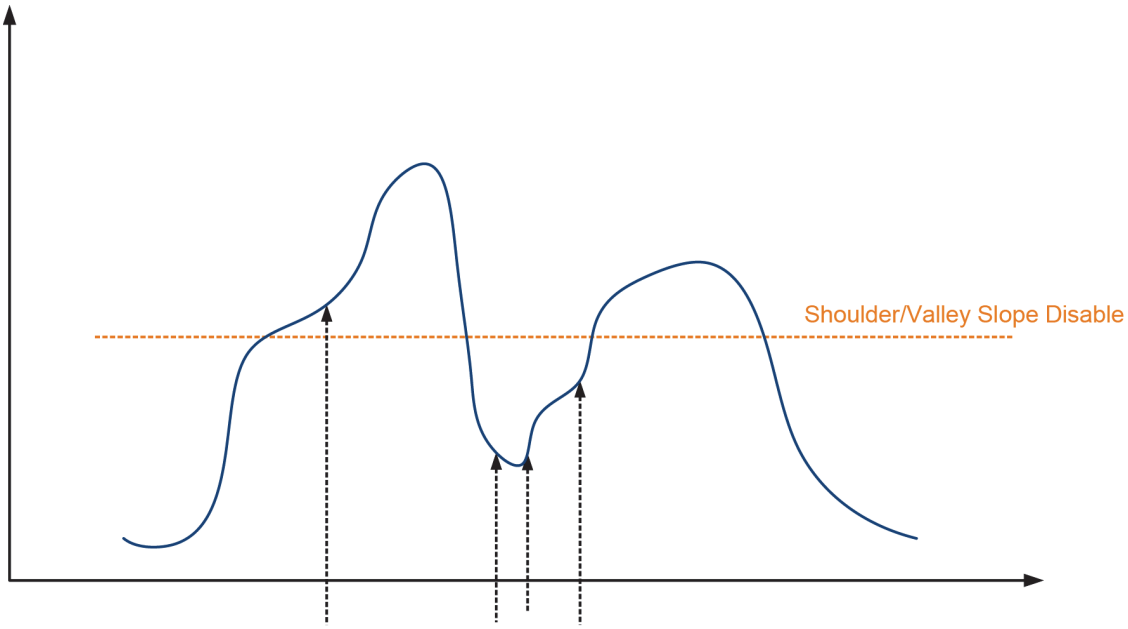
A.3.4 Valley Fractionation



No fractionation since the absorption level is above the “Shoulder/Valley Slope Disable” level.

Fractionates when a valley is detected.

A.3.5 Shoulder Slope Fractionation



A.4 Auto Extend of Gradient

When the system enters the Auto-Extend mode, the gradient is extended with an isocratic segment that is 25% of the total gradient length using the end solvent mix of the current gradient. The criteria for deciding if the gradient should be extended depend on the collection method and the parameters defined for the collection method:

Collection Method	Auto-Extend Criteria
Manual (Collect All)	If the current calculated slope* and/or absorption level is greater than the predefined system settings for auto extend, the gradient is extended.†
Threshold	If the current absorption level is greater than the threshold defined in the method and/or the current calculated slope* is greater than the predefined slope for auto extend†, the gradient is extended.
Slope	If the system is collecting, the current calculated slope* is greater than the slope defined in the method, and/or the current absorption level is above the predefined threshold for auto extend†, the gradient is extended.

* The Slope collection mode is only available with the UV1 and UV2 detection signals, and slope values will only activate auto extend if collection is based on UV1 and/or UV2 detection signals.

† Auto extend can be enabled or disabled in the system mode. The predefined system settings for auto extend are: Threshold = 100 mAU and Slope = 180 mAU/CV.

If the method in use is a combination of two or more of the methods above, the gradient is extended if one or more of the methods' collection criteria are met. If collecting on UV1 and UV2, either signal can auto extend the gradient. If collection is based on XIC or TIC, any signal can auto extend the method.

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