Biotage

SP System

HPFC System

Operator's Manual

P/N 09628, Version 2.0

May 12, 2005

Safety and Document Conventions

The equipment described in this manual should only be operated and maintained by trained individuals. Please read this manual carefully before working with the product. To guarantee safe and effective operation it is absolutely necessary to follow all of the instructions in this "Operator's 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. A NOTE gives information that is crucial to the operation being described. A WARNING indicates important information that will prevent harm to personnel or damage to the system.

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 product unless good laboratory practices (GLP) or manufacturer's guidelines are followed.

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User Documentation List

The following documents are provided on the "SP System User Documentation" CD (P/N 09624):
- "Unpacking and Installation", P/N 09625
- "Operator's Manual" (this publication), P/N 09628
- "Safety and Quick Start", P/N 09715

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Figure 1-1. SP System General Features

1.1.1   Basic System Components

The Biotage SP system is a liquid HPFC chromatography system, consisting of the following components:

- LCD Touch Screen
- FLASH Collector
- Pump Module
- Flow Path Module
- UV Detector

LCD Touch Screen

The Touch Screen is a solvent-resistant, color LCD screen with a resolution of 800 x 600 pixels (SVGA). It serves both as a display and as the system's input device via on-screen touch buttons. It displays operational, status, and purification data. The purification data can be displayed graphically and sent to a user-supplied printer.

Please observe the following Touch Screen operation rules:

- You may operate the Touch Screen with a finger or with a Touch Screen stylus. To avoid damaging the Touch Screen surface, do not use anything else.
- Avoid excessive pressure; a light touch is all that is required to activate the on-screen buttons and features.
- Avoid getting harsh chemicals on the Touch Screen. If this happens, wipe it clean immediately as described in "Clean the Exterior of the System" on page 4-8.

<p align="center">WARNING</p>

To avoid damaging the Touch Screen surface, never use sharp, hard, or abrasive tools such as ballpoint pens, pencils, or keys on the screen.

FLASH Collector

FLASH Collector features:

- Compression module platform.
- Collection arm.
- Collection vessel platform.
- PC-compatible computer, equipped with hard drive and communication ports.
- Collect valve.

Pump Module

The pump delivers flowrates of 1 to 100 mL/min (1 mL/min resolution). It contains gradient valves and is rated for continuous duty at pressures from 0 to 100 psi.

Flow Path Module

The Flow Path Module directs liquid flow through the system. It contains valves that control column inlet, outlet, bypass, and waste flow.

UV Detector

The UV Detector provides purification data to the FLASH Collector based on the UV absorbance rates of the solvents passing through its UV flow cell. It is available in two models:

- Standard model: variable wavelength (200-320 nm).
- Optional model: fixed wavelength (254 nm).

Wetted Materials

The wetted parts of the system contain 316 Stainless Steel, Chemraz, PEEK, PFE, Polyethylene, Polypropylene, PTFE, and Silica, which are not affected by common chromatographic solvents.

1.1.2 Programmed Functions

The basic SP instrument can be programmed to perform the following functions:

- Collect fractions into a wide variety of collection racks and vessels.
- Generate user-defined elution gradients.
- Store and implement a large number of independent chromatographic methods.
- Control and collect fractions based on UV absorbance.
- Produce detailed, printable purification reports.

1.1.3   User Documentation

The following documents are provided on the "SP System User Documentation" CD (P/N 09624):

- "Unpacking and Installation", P/N 09625.
- "Operator's Manual" (this publication), P/N 09628.
- "Safety and Quick Start", P/N 09715.

Online Help

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

⚠ : View the safety requirements for the SP system. You must observe all these safety requirements when installing and operating the SP system. Failure to use the equipment 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 or system mode).

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

▼/▲ : Scroll up or down 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.

1.1.4   Accessories

Compression Module and Cartridge

The FLASH Collector's compression module platform can hold the following Biotage FLASH compression modules:

- FLASH 12+
- FLASH 25+
- FLASH 40+
- FLASH 65i (requires Luer adapter conversion kit (P/N 08397) and must be mounted on a separate stand (P/N 09185) or a suitable stand near the right side of the instrument)
- FLASH 75 (requires an adapter conversion kit and must be mounted on a suitable stand near the right side of the instrument)

All Biotage disposable cartridges are made of virgin polyethylene and polypropylene, and they are available with silica (KP-SIL), C_{18} (KP-C18-HS), or NH (KP-NH) media.

Figure 1-2. FLASH+ Compression Modules, Cartridges, and Samplets

Injection Methods

Liquid samples can be injected through an optional three-way injection valve (P/N FIV-VLV-1000) or loaded onto a Samplet.

Solid samples, once dissolved in a good solvent, can be applied to a Samplet or pre-adsorbed onto silica, evaporated to dryness, and placed into a ZIF-SIM.

Biotage Samplets can be placed into a FLASH+ purification cartridge, and the ZIF-SIMs can be mounted in a slot on the FLASH Collector's compression module platform near the compression module. (All ZIF-SIMs include a tubing kit (P/N 01903).)

Figure 1-3. ZIF-SIM

FLASH Collection Racks and Vessels

The SP system supports a wide variety of collection racks and vessels. The supported vessel size range is from 9 mL test tubes to 240 mL bottles.

Rack Type	Description	Vessel Diameter x Length	Max mL/Fraction	No of Vessels
BLK-013	Aluminum	13 mm x 100 mm	9	128
HRAK-013	White	13 mm x 100 mm	9	120
BLK-016	Aluminum	16 mm x 100 mm	12	128
HRAK-016	White	16 mm x 100 mm	12	96
QRAK-016	White	16 mm x 150 mm	21	96
BLK-116	Aluminum	16 mm x 150 mm	21	96
HRAK-017	White	17 mm x 130 mm	18	48
BLK-018	Aluminum	18 mm x 150 mm	27	96
QRAK-018	White	18 mm x 150 mm	27	48
BLK-025	Aluminum	25 mm x 150 mm	51	48
QRAK-025	White	25 mm x 150 mm	51	48
HRAK-120	White	120 mL bottles	120	24
HRAK-240	White	240 mL bottles	240	12
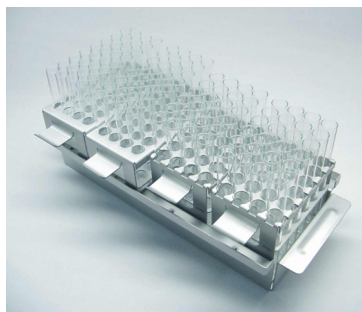
White Rack **Aluminum Rack** **Aluminum**
with Mixed Blocks **Iso-Block**

Figure 1-4. Typical Racks Used with the SP System

FLASH 65i Module Stand

The FLASH 65i module stand (P/N 09185) is used to mount the optional FLASH 65i compression module. The stand holds up to four FLASH 65i compression modules.

Compression Module Mounting Adapters

These reducing adapters allow the FLASH 12+ and FLASH 25+ compression modules to be mounted in the FLASH Collector mounting holes.

Figure 1-5. Compression Module Mounting Adapters

Order Accessories

To order accessories, please see the Biotage Product Catalog. The catalog can be downloaded at www.biotagedcg.com.

1.2 Software Description

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

- **Chemistry:** Set up, control, monitor and review a purification run.
- **System:** Change system settings, manage users, view the status of the Fraction Collector, Pump Module and UV Detector, and, if necessary, restore the system after e.g. overpressure or an arm movement failure.

The main menu also provides software version information through its **About** 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 system mode.
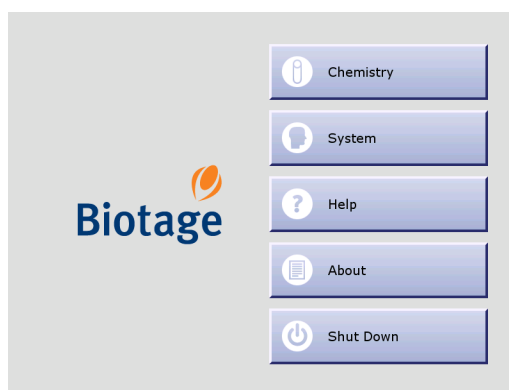
Figure 1-6. Main Menu

1.2.1 System Mode

In the system mode, the software is divided into eight (8) 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:

- **Cartridges:** Add, edit and delete 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.
- **Diagnostics:** View the status of the Fraction Collector, Pump Module and UV Detector, and, if necessary, restore the system after e.g. overpressure or an arm movement failure.
- **General:** Specify UV Detector model and turn on and off 1) monitoring of solvent and waste levels, 2) audible alarm (for rack change, leakage, high pressure, low solvent volume, full waste reservoir and misalignment of the Collection Arm), 3) mouse pointer, 4) auto print of reports, 5) line flush at end of runs, and 6) default allocation of the whole collection rack for each run (only for SP1 systems). When necessary, it is also possible to save the system log to a USB Flash drive.
- **Methods:** Export, import and delete methods. It is also possible to set a default method, i.e. a method that all new methods are based on, and delete unused projects.
- **Racks:** Change a rack's default maximum fraction volume and select the rack types that will appear in the rack selection list when setting up a method in the chemistry mode.
- **Results:** Delete result records.
- **Solvents:** Add, edit and delete 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.

Figure 1-7. System Mode

Buttons in the Right-hand Panel

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

- **Help:** View context-sensitive help.
- **Main Menu:** Go to the main menu to enter the chemistry mode (to set up, control, monitor and review a purification run), view software version information or shut down the system.

1.2.2 Chemistry Mode

In the chemistry mode, the software is divided into five (5) 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 methods. It is also here that you assign a method to the column mounted on the system and select the block(s), on the collection rack, to be used.
- **Columns:** View the status for a column loaded onto the system, start or queue up* an equilibration, request direct injection of a sample and start or queue up* a purification run. It is also possible to edit and remove a run.
- **Status:** Monitor and edit the purification run in progress, control the UV Detector lamp, and instruct the system to start collecting fractions (if you are running a purification run with manual collection) and to switch to a new collection vessel.
- **Results:** Search, view and print the results of your purification run. It is also possible to create a new method with the same purification parameters from a previous purification, and save a result record on a USB Flash drive as an HTML file.
- **Solvents:** Prime the solvent inlets, assign solvent types to the solvent inlets, and set the solvent and waste reservoirs' volumes.

* Equilibrations and purification runs can only be queued when using an SP4 system.

Status and Task Field

The system status can be "Idle", "Running", "Pausing", "Paused" or "Resuming". When the system status is "Running", the following tasks can be in progress:

- **Auto Ext.:** If remaining UV absorption is detected when the system nears the end of a purification run, the system enters the Auto-Extend mode. This extends the gradient purification stage of the run with 25% of the total gradient length.
- **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.
- **Equilibrate:** The system is running an equilibration.
- **Gradient:** The system is running a purification run.

- **Line Flush & Purge & Det. Flush:** Prior to finishing a purification run, i.e. after the gradient purification stage is completed, the system performs a Line Flush, a Purge (a system decompression process) and a UV Detector Flush.
- **Prime:** The system is priming the solvent inlet(s).
- **UV Zero:** The system is setting the UV zero level.
- **Warming Lamp:** The UV Detector lamp is being warmed up. This takes approximately 7.5 minutes.
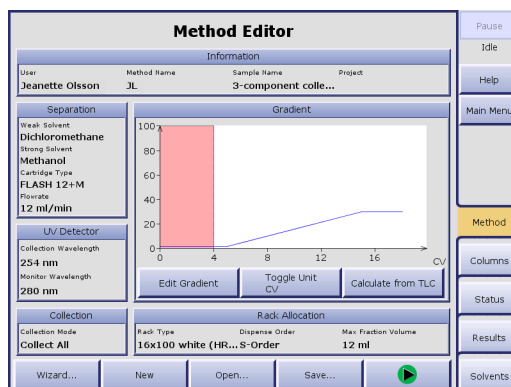
Figure 1-8. Chemistry Mode

Buttons in the Right-hand Panel

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

- **Pause/Resume:** To pause a purification run, press the Pause button. Once the current pump stroke finishes, 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.
- **Main Menu:** Go to the main menu to enter the system mode (to change system settings, manage users, view the status of the Fraction Collector, Pump Module and UV Detector, and, if necessary, restore the system after e.g. overpressure or an arm movement failure), view software version information or shut down the system.

NOTE
Only users with system owner privilege can log into the system mode.

1.2.3 Method Tab (Chemistry Mode)

At the **Method** tab, in the software's chemistry mode, you can create, open and edit methods. It is also here that you assign a method to the column mounted on the system and select the block(s), on the collection rack, to be used.

There are different ways of creating a new method:

- **Create a new method.** If the system has been configured to use a default method (see "Set a Default Method" on page 2-4), the default method is opened. After setting up the method, you can save it under a new name by entering a unique method name. The default method remains unaltered under its original name.

- **Base the new method on an existing method.** After editing, you can save it 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 preconfigured method with a default gradient.**
  The software comes with five preconfigured methods with only a defined gradient, see Table 3-1 on page 3-2. After completing the method, you can save it under a new name by entering a unique method name.
- **Base a new method on a previous run.** After editing the purification parameters, you can save it under a new name by entering a unique method name.

NOTE
You do not have to save your method to be able to run it.
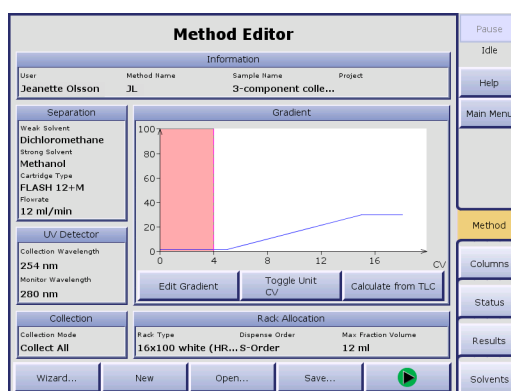
Figure 1-9. Method Tab

Parameters

A method is a specific group of purification parameters:

- **User:** Selecting your user name identifies you as the method's creator and, if you save the method and your user account is password-protected, it protects your method from being edited by another user. If you have not been assigned a user name, please contact your system supervisor.
- **Method Name:** The name of the method. It has to be unique for the user. If you are editing an existing method and save it under a new name, the original method remains unaltered under its original name. (If you do not wish to save the method, no method name has to be entered.)
- **Sample Name:** The name of the sample. A default sample name is assigned, but it can be changed. The name has to be unique for the user.
- **Project:** A method can be associated with a project. This parameter is optional.
- **Weak Solvent and Strong Solvent:** The weak and strong solvent to be used. They should be the two solvents determined by the TLC procedure.
- **Cartridge Type and Flowrate:** The cartridge type and flowrate to be used. The default flowrate for the selected cartridge type can be changed and set to a value between 1 to 100 mL/min, see Table 3-2 on page 3-5 for guidelines.
- **Gradient:** The purification gradient, which includes equilibration (optional) and gradient segment data. It is also possible to set an initial waste value, i.e. delay collection until a specified volume has been delivered to the waste reservoir.
- **Collection and Monitor Wavelength:** Only available for the Variable Wavelength UV Detector. The possibility to set these wavelengths can help optimize compound detection since a compound's absorption of UV light varies with the wavelength.

- **Collection Mode:** The collection mode to be used. See Table 3-3 on page 3-7 for help in choosing a collection mode.
- **Rack Type:** The rack type to be used. See Table 3-5 on page 3-9 for guidance.
- **Dispense Order:** The collection pattern, "S-Order" or "Z-Order", to be used.
- **Max Fraction Volume:** The vessels' fill capacity. The software prevents you from entering a value that will overflow the vessels.

Buttons

The following buttons are available at the **Method** tab:

- **New:** Create a new method. If the system has been configured to use a default method (see "Set a Default Method" on page 2-4), the default method is opened.
- **Open...:** Open an existing method or a preconfigured method with a default gradient. (The five preconfigured methods are available under the username "GLOBAL". To find out their TLC solvent ratio, see Table 3-1 on page 3-2.)
- **Wizard...:** Open the method wizard. The wizard will step by step guide you through the setup of a run.
- **Save...:** Save the current method to the system's hard drive. Saved methods can be recalled later by pressing the Open button.
- ▶**:** Open the Run Parameters dialog, where you can select collection block(s) to be used and, if you are using an SP4 system, the column to be used. When this is done, you can start or queue up (when using an SP4 system) the equilibration (if used) and the purification run at the Columns tab. (The Run Parameters dialog is not displayed if you are using an SP1 system and the Collect In All Blocks option is turned on.)
- **Edit Gradient:** Create or edit a gradient for the current method.
- **Toggle Unit:** Press this button repeatedly until the desired X-axis unit appears on this button. The choices are column volumes (CV), volume (mL), or minutes (min). The default setting is CV.
- **Calculate from TLC:** Enter the following parameters obtained from a TLC procedure: 1) R_f values for compound of interest and the closest eluting compounds on the leading and trailing sides of the compound of interest, and 2) solvent types and their percentages. The software uses these values to calculate a purification gradient and column/sample load recommendations.

1.2.4   Columns Tab (Chemistry Mode)

At the **Columns** tab, in the software's chemistry mode, you can:

- View information on a column loaded onto the system. Available information is: user, method name, sample name, status, allocated block(s), the estimated time when the system will have completed the task and the progress of a task in progress (in percentage).
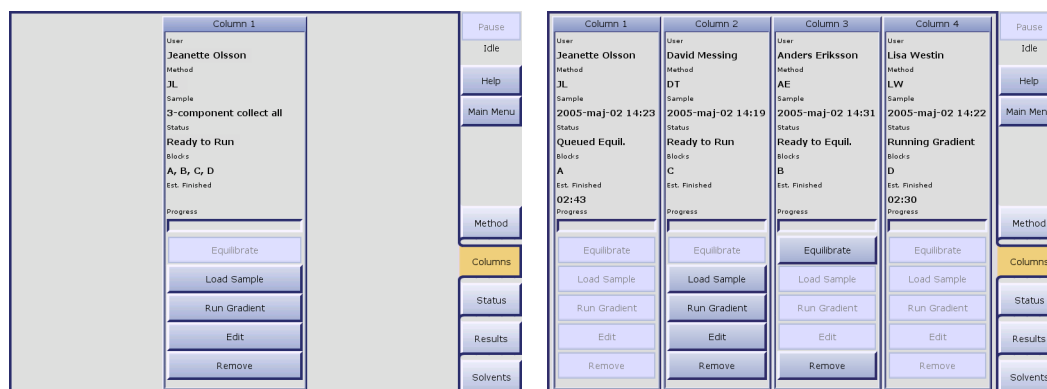
Figure 1-10. Columns Tab (SP1 and SP4)

- Start or queue up* an equilibration, request direct injection of a sample and start or queue up* a purification run.
- Edit and remove purification runs.

* Equilibrations and purification runs can only be queued when using an SP4 system.

Status

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

- **Ready to Equil.:** Press the Equilibration button to start or queue up (when using an SP4 system) the equilibration.
- **Queued Equil.:** The equilibration is queued and will be perform when the system has completed the task(s) in progress. This status only applies to SP4 systems.
- **Equilibrating:** The system is running the equilibration.
- **Valve Open:** The injection valve is open and it is now possible to inject the sample.
- **Ready to Run:** Press the Run Gradient button to run the gradient.
- **Queued Run:** The run is queued and will be performed when the system has completed the task(s) in progress. This status only applies to SP4 systems.
- **Running Gradient:** The system is running the gradient.
- **Finished:** The purification run is completed.
- **Editing:** The method is being edited.

NOTE

When using an SP4 system, queued equilibrations and gradient runs will be performed in the order they were queued up.

Buttons

The following buttons are available at the **Columns** tab:

- **Equilibrate:** Start or queue up (when using an SP4 system) the equilibration. This button is only enabled if an equilibration has been set up in the method.
- **Load Sample:** Request for loading of sample (direct injection). If an equilibration has been set up in the method, this button is enabled when the equilibration has been completed.
- **Run Gradient:** Start or queue up (when using an SP4 system) the purification run.
- **Edit:** Edit the method. It is not possible to edit a purification run during its equilibration.
- **Remove:** Remove the purification run. To remove a run during the gradient run, you have to press the End Run button at the Status tab.

NOTE

To be able to edit or remove a purification run in progress, you have to pause the system, i.e. press the Pause button in the right-hand panel. Once the current pump stroke finishes, the Collection Arm returns to its home position (the inner right corner) and the system is paused.

1.2.5 Status Tab (Chemistry Mode)

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

- Monitor the status and progress of a purification run in progress. While a purification is running, the graph displays the programmed gradient and a dynamic chromatogram.
- Control the UV Detector lamp.
- Instruct the system to start collecting fractions (if you have set up a purification run with manual collection) and to switch to a new collection vessel.

Rack/Information/>> field

By pressing the **Rack/Information/>>** field repeatedly, you can toggle between displaying a rack image, an information view and hiding the field (to expand the chromatogram).

If the rack image is displayed, fractions that are already collected can be located by matching their colors on the chromatogram with the vessel colors on the collection rack image. A fraction location feature is also available for the records that are accessible at the **Results** tab, see "Search for Records" on page 3-20.

If the information view is displayed, you can see a color legend, the current % of strong solvent, defined wavelength(s) in nm, measured absorption in AU, defined threshold in AU and the estimated time when the system will have finished processing the purification run.

Figure 1-11. Status Tab

Buttons

The following buttons are available at the **Status** tab:

- **Scrolling:** View the present reading +/- 2 CV (Column Volumes) on the horizontal axis.
- **Show All:** View all readings on the horizontal axis.
- **Toggle Unit:** Toggle the horizontal axis unit between CV (Column Volumes), minutes and mL.
- **Zoom In:** Magnify (zoom) the area around the UV zero level.
- **Zoom Out:** Decrease the magnification.
- **Auto Scale:** Adjust the graph so that all readings on the vertical axis are presented.
- **UV Zero:** Used to set the current UV level to 0 (zero) AU.
- **Lamp On/Lamp Off:** Manually turning the lamp on and off.
- **Skip Warm Up:** Used to skip the UV Detector lamp warm-up period and begin the run. However, if the lamp has not reached operating temperature, purification results may not be optimal.
- **End Run:** End a purification during the gradient run. (The system has to be paused.)
- **Start Collecting:** Start collecting fractions. (Only if manual collection has been selected in the method.)
- **New Fraction:** Instruct the system to switch to a new collection vessel.
- **Edit:** Edit a purification in progress. (The system has to be paused.)

NOTE

To be able to edit or remove a purification run in progress, you have to pause the system, i.e. press the Pause button in the right-hand panel. It is not possible to edit a purification run during its equilibration.

1.2.6   Results Tab (Chemistry Mode)

Each purification run is stored as an individual record in the system's database. These purification records can be searched, viewed, printed and copied at the **Results** tab, in the software's chemistry mode.

Filter Parameters

All records that match the specified filter parameters are listed at the **Results** tab. If a record that you wish to view is not listed, change the filter parameters by pressing the **Result Filters** field. Possible filter parameters are 1) user name, 2) project name, 3) method name, and 4) date when the purification run was processed.

NOTE
If the system is restarted, only the records saved in the last 10 days are displayed until the filter parameters are changed.

Buttons

The following buttons are available at the **Results** tab:

*   **Show:** Open a result dialog with purification details, a chromatogram and a fraction locator for the selected record.
*   **Create:** Create a method with the same purification parameters as the ones used in the selected record.
*   ▲ / ▼ : Scroll the record list up and down.
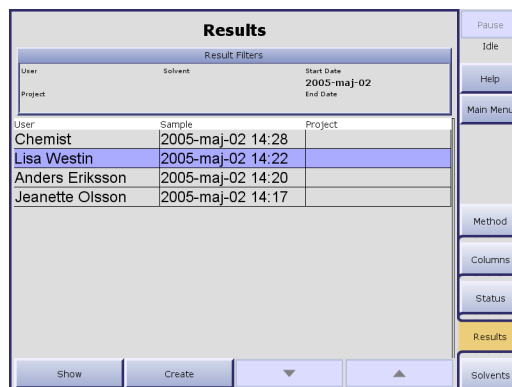
Figure 1-12. Results Tab

1.2.7   Solvents Tab (Chemistry Mode)

At the **Solvents** tab, in the software's chemistry mode, you can:

*   **Assign a solvent type to each of the four solvent inlets,** labeled A through D, on the Pump Module. 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. If you change the solvent type that is connected to a particular inlet, you must make the corresponding change in the software. To assign a solvent type, press the applicable Solvent Channel field. 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.
*   **Enter the capacities and current fluid levels for the waste and solvent reservoirs** each time you empty a waste reservoir or replenish a solvent. The system will issue a warning when it is time to replenish a solvent or empty a waste reservoir. To enter values,

press the applicable Channel field. (This feature is only enabled if the monitoring of solvent and waste are turned on, see "Turn On and Off Solvent and Waste Monitoring" on page 2-12.)

- **Prime the solvent inlets** to 1) remove any air bubbles from the pump and solvent inlets by flushing them with solvents, or 2) empty the solvent inlets of solvents used in the previous purification and fill them with new solvents. To prime, press the Prime button.
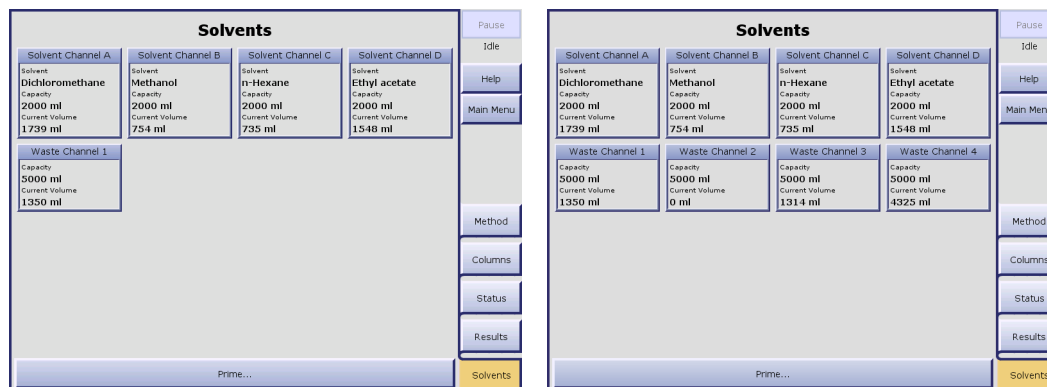
Figure 1-13. Solvents Tab (SP1 and SP4)

1.2.8 SP Remote Viewer

With the system connected to your network, users can access the SP Remote Viewer through a standard web browser.

The SP Remote Viewer quickly tells you:

- If the instrument is occupied.
- If the instrument is still processing or there is a problem that requires manual attention.
- The results of your purification runs.

To access the SP Remote Viewer, see page 3-22.
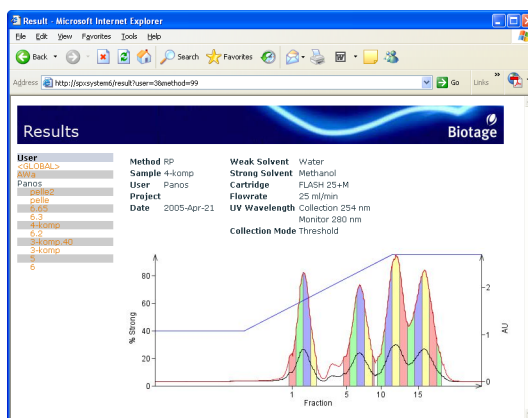
Figure 1-14. SP Remote Viewer

1.3 Specifications

1.3.1   General System Specifications

Input Voltage	
FLASH Collector	100-240 VAC, 50/60 Hz, 2.5 A; converted to 24 VDC internally
Flow Path Module	24 VDC, 2.0 A
Pump Module	24 VDC, 2.5 A
Fuses	
FLASH Collector input	T2.5 A/250 V, CSA certified, UL listed. Biotage P/N 09367 (2 required)
Internal	4.0 A/250 V (not user replaceable)
Overvoltage Category	
Category II. Complies with CSA C22.2 NO. 1010.1, and IEC 61010-1	
Agency Certification	
SP System	CE and cCSAus®
Fluid Path (Wetted) Materials	
316 Stainless Steel, Chemraz, PEEK, PFE, Polyethylene, Polypropylene, PTFE, and Silica	
Dimensions and Weights	
Dimensions (with Touch Screen in parked position)	Width: 19" (48 cm); Length: 23" (58 cm); Height: 24" (61 cm)
Weights	FLASH Collector: 33.4 lbs (15.2 kg)
	Pump Module: 7.9 lbs (3.6 kg)
	Flow Path Module for SP1: 7.6 lbs (3.4 kg)
	Flow Path Module for SP4: 14.5 lbs (6.6 kg)
Environmental	
Pollution Degree	2
Location	For indoor use only
Temperature	Temperature range is 15 to 30°C (59 to 86°F)
Humidity	Operating humidity range is 20 to 95% RH
Altitude	Maximum altitude for unpressurized solvent is 6,000 feet above sea level. For higher elevations, you may pressurize solvent to a maximum of 5 psi (0.35 bar).
Atmospheric Pressure	10 to 15.5 psi operating pressure (0.69 to 1.07 bar)
Interfaces	
Ethernet LAN, four USB ports, parallel port, mouse port, and CRT (VGA) port	

1.3.2   Operational Performance Specifications

FLASH Collector	
Fraction Volume	Programmable from 3-240 mL, depending on rack size
Fraction Number	128 max., 13x100 mm aluminum rack
Pump Module	
Flowrate	1 to 100 mL/min variable, in 1 mL/min increments
Solvent Delivery	3 mL/stroke
Pressure Resistance	100 psig maximum operating
Gradient Valve	
Gradient Precision	±2% Between 3% and 97% with any binary combination of A, B, C, and D
LCD Touch Screen	
Screen Overlay	Solvent stable
Input Power	12 VDC (from FLASH Collector)
Display Interface	VGA
Touch Screen Interface	USB 2.0
Screen	10.4" LCD; TFT Active Matrix; 800 x 600 resolution

1.3.3   UV Detector Specifications

Input Voltage	
Both Detector Models	110-120 VAC, 50/60 Hz
	220-240 VAC, 50 Hz (CE Certified)
Fuses (Main Power)	
Variable Wavelength Model	110-120 VAC: 2.0 A, 250 V, UL listed; Biotage (no P/N), Bussman P/N GDB-2A
	220-240 VAC: 1.0 A, 250 V, CSA UL listed; Biotage P/N 03120, Bussman P/N GDB-1A
Fixed Wavelength Model	110-120 VAC: 1.0 A, 250 V, UL listed; Biotage P/N 03120, Bussman P/N GDB-1A
	220-240 VAC: 0.5 A, 250 V, UL listed; Biotage (no P/N), Bussman P/N GDB-500ma
Wavelength	
Variable Wavelength Model	200 nm to 320 nm
Fixed Wavelength Model	254 nm
Agency Certification	
Both Detector Models	CE Certified
LED Indicator Lamps	
Both Detector Models	Power on and UV Lamp on
Amplitude Range	
Both Detector Models	0-2 AU (Absorbance Units); accuracy of ± 0.02 AU
UV Lamp	
Variable Wavelength Model	Deuterium Discharge
Fixed Wavelength Model	Low Pressure Mercury
Flow Cell Optical Path	
Variable Wavelength Model	0.3 mm
Fixed Wavelength Model	0.1 mm standard; 0.3 mm optional
Dimensions	
Variable Wavelength Model	15.59" (396 mm) L x 7.88" (200 mm) W x 7.07" (180 mm) H
Fixed Wavelength Model	9.62" (244 mm) L x 6.47" (164 mm) W x 7.34" (186 mm) H
Weight	
Variable Wavelength Model	26.4 pounds (12 kg)
Fixed Wavelength Model	13.2 pounds (6 kg)
Wetted Materials	
Variable Wavelength Model	SST, fused silica, PTFE, and PEEK
Fixed Wavelength Model	SST, Quartz, and PTFE

1.3.4   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 Material Safety Data Sheets (MSDS) and any other applicable regulations for the safe use, handling, transporting, storage, and disposal of these solvents.

Solvent	Abbreviation	Strength	Selectivity Class	UV Cutoff (nm)	Vapor Pressure (PSI)	Vapor Pressure (mm Hg)
1,2-Dichloroethane	DCE	0.49	5 (V)	228	1.2	62.0
1-Chlorobutane	1-ClBut	0.26	6 (VIa)	220	1.5	77.6
1-Chloropentane	1-ClPent	0.26	6 (VIa)	225	0.2	10.3
1-Chloropropane	1-ClProp	0.30	6 (VIa)	225	5.5	284.4
1-Propanol	PrOH	0.82	2 (II)	210	0.3	15.5
2-Chloropropane	2-ClProp	0.29	6 (VIa)	225	8.6	444.6
2-Propanol	IPA	0.82	2 (II)	210	0.6	31.0
Acetone	Acet	0.56	6 (VIa)	330	3.5	181.0
Acetonitrile	ACN or MeCN	0.65	6 (VIb)	190	1.4	72.4
Bromoethane	C2H5Br	0.37	6 (VIa)		7.3	377.4
Carbon tetrachloride	CCl4	0.18	0	265	1.8	93.1
Chloroform	CHCl3	0.40	8 (VIII)/9 (IX)	245	3.0	155.1
Cyclohexane	CyHex	0.04	0	210	1.5	77.6
Cyclopentane	CyPent	0.05	0	210	7.7	398.1
Decane	Dec	0.01	0	215	0.05	2.6
Dichloromethane (Methylene chloride)	DCM	0.42	5 (V)	235	6.6	341.3
Diethyl ether	Ether	0.38	1 (I)	218	8.4	434.4
Dimethyl sulfoxide	DMSO	0.62	3 (III)/6 (VI)	265	0.008	0.4
Dioxane	Diox	0.56	6 (VIa)	215	0.6	31.0
Ethanol	EtOH	0.88	2 (II)	210	0.8	41.4
Ethyl acetate	EtOAc	0.58	6 (VIa)	255	1.4	72.4
i-Propyl ether	i-PrEth	0.28	1 (I)	220	2.9	149.9
Iso-octane	IO	0.01	0	215	0.8	41.4
Methanol	MeOH	0.95	2 (II)	210	1.8	93.1
Methyl acetate	MeOAc	0.60	6 (VIa)	260	3.3	170.6
Methyl ethyl ketone	MEK	0.51	6 (VIa)	330	1.5	77.6
n-Butyl ether	BuEth	0.25	1 (I)	220	0.09	4.7
n-Heptane	Hept	0.01	0	210	0.8	41.4
n-Hexane	Hex	0.01	0	210	2.5	129.3
n-Pentane	Pent	0.01	0	210	8.1	418.9
t-Butyl methyl ether	MTBE	0.62	1 (I)	220	4.8	248.2
Tetrahydrofuran	THF	0.57	3 (III)	220	2.5	129.3
Toluene	Tol	0.29	7 (VII)	286	0.4	20.7
Water	H_2O	1.00	0	190	2.3	118.9

<table>
<tr><td>**Chapter**
2</td><td>*Administration*
(System Mode)</td></tr>
</table>

WARNING

Before performing any procedures in this chapter, please read and observe the safety requirements in the "Safety and Quick Start" document (P/N 09715). Failure to follow those requirements may result in personal injury and/or equipment damage.

2.1 Log into the System Mode

You must log into the system mode to be able to change system settings, manage users, view the status of the Fraction Collector, Pump Module and UV Detector, and, if necessary, restore the system after e.g. an arm movement or overpressure failure.

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 **Enter 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 1-6.

NOTE

The first time you log into the system mode, log in using the user account "GLOBAL" and the password "1234". Before you log out, it is strongly recommended that the password is changed. See "Administrate the User List" on page 2-7.
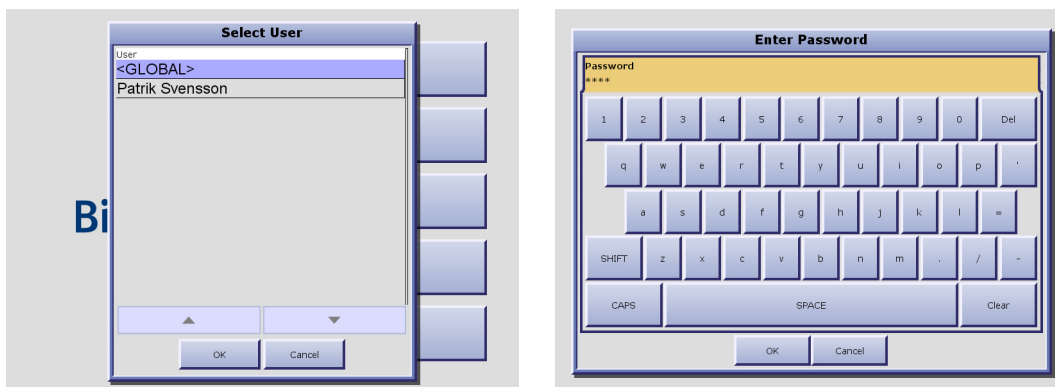

Figure 2-1. Log into the System Mode

2.2 Administrate the Cartridge List

The software comes with a preconfigured list of cartridges and their parameters. At the **Cartridges** tab, in the software's system 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.

The cartridge parameters are:

- The name of the cartridge type.
- The column volume in mL.
- The default flowrate in mL/min.
- The recommended maximum flowrate in mL/min.

NOTE
The preconfigured cartridges cannot be edited or deleted.
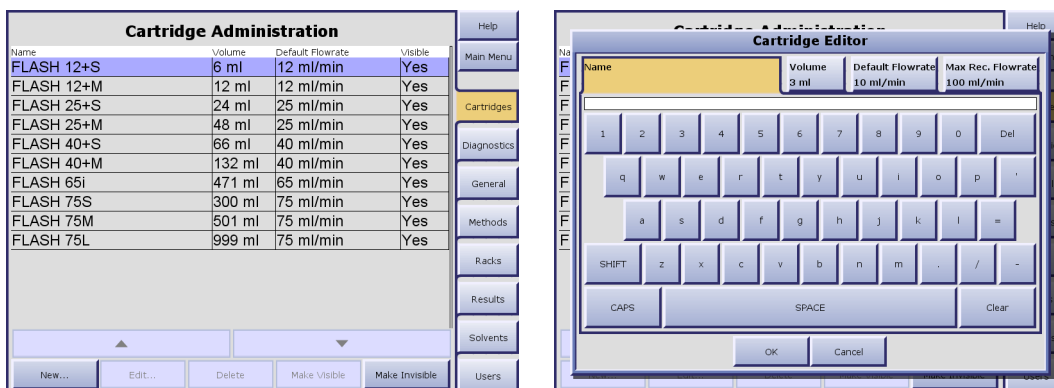
Figure 2-2. Cartridge Tab

2.2.1 Add a Cartridge Type

1. Select the **Cartridges** tab in the right-hand panel. All defined cartridge types are listed.
2. Press **New...**. The **Cartridge Editor** opens.
3. Enter the cartridge parameters.
4. To save the new cartridge type, press **OK**.

2.2.2 Delete an Unused Cartridge Type

1. Select the **Cartridges** tab in the right-hand panel. All defined cartridge types are listed.
2. Select the cartridge that you want to delete. To scroll the list down or up, press ▼ or ▲ .
3. Press **Delete**. The **Confirm Delete** dialog opens.
4. To confirm delete, press **Yes**.

2.2.3 Edit a Cartridge Type

1. Select the **Cartridges** tab in the right-hand panel. All defined cartridge types are listed.
2. Select the cartridge type that you want to edit. To scroll the list down or up, press ▼ or ▲ .
3. Press **Edit...**. The **Cartridge Editor** opens.
4. Edit the cartridge parameters.
5. To save the changes, press **OK**.

2.2.4 Select Cartridge Types to List

1. Select the **Cartridges** tab in the right-hand panel. All defined cartridge types are listed.
2. To make a cartridge type appear in the cartridge selection list when setting up a method, select it and press **Make Visible**. To scroll the list down or up, press ▼ or ▲ .
3. To make a cartridge type <u>not</u> available in the cartridge selection list when setting up a method, select it and press **Make Invisible**. To scroll the list down or up, press ▼ or ▲ .

2.3 Administrate the Method and Project Lists

At the **Methods** tab, in the software's system mode, you can export, import and delete methods. It is also possible to set a default method, i.e. a method that all new methods are based on in the chemistry mode, and delete unused projects.

NOTE
The preconfigured methods cannot be edited or deleted.
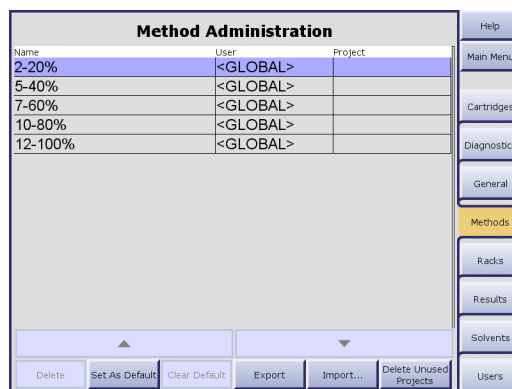
Figure 2-3. Methods Tab

2.3.1 Delete a Method

1. Select the **Methods** tab in the right-hand panel. All saved methods are listed.
2. Select the method that you want to delete. To scroll the list down or up, press ▼ or ▲ .
3. Press **Delete**. The **Confirm Delete** dialog opens.
4. To confirm delete, press **Yes**.

2.3.2 Delete Unused Projects

1. Select the **Methods** tab in the right-hand panel.
2. To delete all projects that do not contain any methods or result records, press **Delete Unused Projects**. The **Confirm Delete** dialog opens.
3. To confirm delete, press **Yes**.

2.3.3 Export a Method

1. Select the **Methods** tab in the right-hand panel. All saved methods are listed.
2. Connect a USB Flash drive to one of the SP system's USB ports. If two or more devices are connected, the first device to be connected will be used.
3. Select the method that you want to export. To scroll the list down or up, press ▼ or ▲ .
4. Press **Export**. The method is saved on the root of the USB Flash drive.

2.3.4   Import Methods

NOTE

Methods to be imported must be saved on the root of a USB Flash drive.

If you import a method with a solvent, cartridge type, 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 type, etc., and will not consider their parameters.

1. Select the **Methods** tab in the right-hand panel. All saved methods are listed.
2. Connect the USB Flash drive to one of the SP system's USB ports. If two or more devices are connected, the first device to be connected will be used.
3. Press **Import...**. The **Import Methods** dialog opens.
4. To import one method, select it and press **Import Selected**. To scroll the list down or up, press ▼ or ▲.
5. To import all methods, press **Import All**.

### 2.3.5   Set a Default Method

To set a default method, i.e. a method that all new methods are based on:

1. Select the **Methods** tab in the right-hand panel. All saved methods are listed.
2. Select the method that you want to set as the default method. To scroll the list down or up, press ▼ or ▲.
3. Press **Set As Default**. The **Set As Default Method** dialog opens.
4. To confirm the default method, press **OK**.

If you do not want to use a default method, press **Clear Default**. (If no method has been set as the default method, this button is disabled.)

2.4   Administrate the Rack List

The software comes with a preconfigured list of racks and their parameters. At the **Racks** tab, in the system mode, you can change a rack's default maximum fraction volume and select the rack types that will appear in the rack selection list when setting up a method in the chemistry mode.

Figure 2-4. Racks Tab

2.4.1   Change a Rack's Default Max Fraction Volume

1. Select the **Racks** tab in the right-hand panel. The predefined rack types are listed.
2. Select the rack that you want to edit.
3. Press **Edit**. The **Rack Editor** opens.
4. Enter the default maximum fraction volume in mL.

2.4.2   Select Rack Types to List

1. Select the **Racks** tab in the right-hand panel. The predefined rack types are listed.
2. To make a rack type appear in the rack selection list when setting up a method, select it and press **Make Visible**.
3. To make a rack type <u>not</u> available in the rack selection list when setting up a method, select it and press **Make Invisible**.

2.5   Administrate the Result List

Each purification run is stored as an individual record in the system's database. At the **Results** tab, in the software's system mode, you can delete result records.

1. Select the **Results** tab in the right-hand panel.
2. All records that fit the specified filter parameters are listed at the **Results** tab. If a record that you wish to delete is not listed, change the filter parameters:
   a. Press the **Result Filters** field. The **Filter Parameters Editor** opens.
   b. Specify the filter parameters. (If you want to list all result records, press **Clear All**.)
   c. To search, press **OK**. If there are records matching your filter parameters, they are listed in chronological order at the **Results** tab.
3. To delete a single record, select it and press **Delete**. To scroll the list down or up, press ▼ or ▲ .
4. To delete all the listed records, i.e. delete all the records that fit the parameters of the result filter, press **Delete All**.
5. To confirm delete, press **Yes** in the **Confirm Delete** dialog.

Figure 2-5. Results Tab

2.6   Administrate the Solvent List

The software comes with a preconfigured list of solvents and their parameters. At the **Solvents** tab, in the software's system 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.
- The minimum time, in seconds, for the pump to be refilled.

NOTE

The preconfigured solvents cannot be edited or deleted.

2.6.1 Add a Solvent

1. Select the **Solvents** tab in the right-hand panel. All defined solvents are listed.
2. Press **New...**. The **Solvent Editor** opens.
3. Enter the solvent parameters.
4. To save the new solvent, press **OK**.

Figure 2-6. Solvents Tab

2.6.2 Delete an Unused Solvent

1. Select the **Solvents** tab in the right-hand panel. All defined solvents are listed.
2. To delete an unused solvent, select it and press **Delete**. To scroll the list down or up, press ▼ or ▲ .
3. To delete all unused solvents, i.e. delete solvents that are not used by any of the methods stored on the system or assigned to a solvent inlet, press **Delete Unused**.
4. To confirm delete, press **Yes** in the **Confirm Delete** dialog.

2.6.3 Edit a Solvent

1. Select the **Solvents** tab in the right-hand panel. All defined solvents are listed.
2. Select the solvent that you want to edit. To scroll the list down or up, press ▼ or ▲ .
3. Press **Edit...**. The **Solvent Editor** opens.
4. Edit the solvent parameters.
5. To save the changes, press **OK**.

2.6.4 Select Solvents to List

1. Select the **Solvents** tab in the right-hand panel. All defined solvents are listed.
2. To make a solvent appear in the solvent selection list when setting up a method, select it and press **Make Visible**. To scroll the list down or up, press ▼ or ▲ .
3. To make a solvent not available in the solvent selection list when setting up a method, select it and press **Make Invisible**. To scroll the list down or up, press ▼ or ▲ .

2.7 Administrate the User List

At the **Users** tab, in the software's system 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 when entering the system mode.

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 system mode (the user can change system settings, manage users, view the status of the Fraction Collector, Pump Module and UV Detector, and, if necessary, restore the system after e.g. an arm movement or overpressure failure).
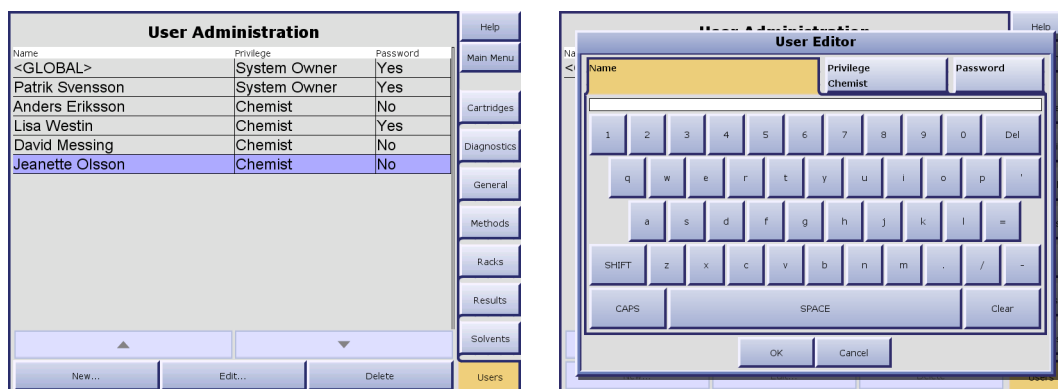
Figure 2-7. Users Tab

2.7.1 Add a User Account

1. Select the **Users** tab in the right-hand panel. All defined users are listed.
2. Press **New...**. The **User Editor** opens.
3. Enter the user parameters.
4. To save the new user, press **OK**.

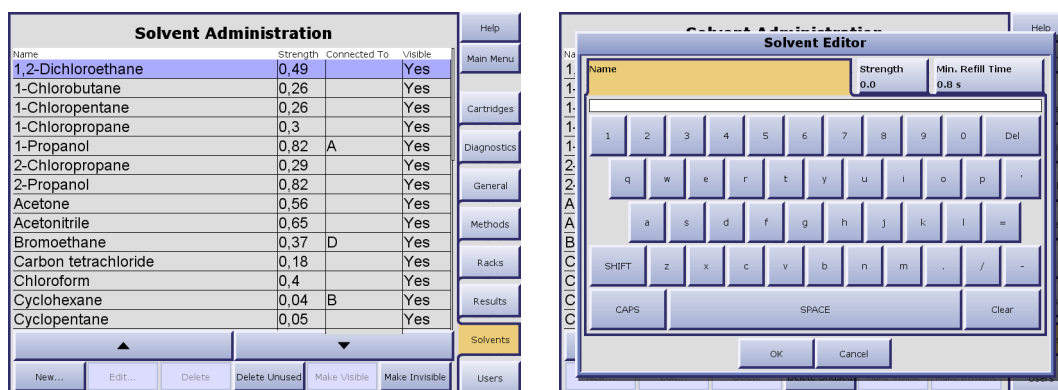## 2.7.2 Change the Password or Privilege

1. Select the **Users** tab in the right-hand panel. All defined users are listed.
2. Select the user account that you want to edit. To scroll the list down or up, press ▼ or ▲ .
3. Press **Edit...**. The **User Editor** opens.
4. Edit the user parameters. If you change the user name, all methods and result records associated with the user will be updated.
5. To save the changes, press **OK**.

2.7.3 Delete a User Account

1. Select the **Users** tab in the right-hand panel. All defined users are listed.
2. Select the user account that you want to delete. To scroll the list down or up, press ▼ or ▲ .
3. Press **Delete**. The **Confirm Delete** dialog opens.
4. To confirm delete, press **Yes**.

2.8 Export the System Logs

The system logs can be saved on a USB Flash drive connected to one of the SP system's USB ports.

1. Select the **General** tab in the right-hand panel.
2. Connect a USB Flash drive to one of the SP system's USB ports. If two or more devices are connected, the first device to be connected will be used.
3. Press **Export Logs**. The system logs are saved as a zip file (logs.zip) with log files that can be viewed in Notepad.

2.9 Set Up a Printer

2.9.1 Install a Plug and Play Printer

To use this procedure, you must have a Windows XP compatible printer. If Windows XP is not able to automatically detect and install your printer, see "Install a Non-Plug and Play Printer" on page 2-9.

1. If you are not at the main menu, press **Main Menu** in the right-hand panel and then press **Shut Down**.
2. When the SP software has shut down, connect a Windows XP compatible mouse either to one of the SP system's USB ports, or to the SP system's mouse port.
3. Turn the printer on. Windows XP will recognize it as a new piece of hardware and will install it automatically.
4. Select **Printers and Faxes** from the Windows **Start** menu. The **Printers and Faxes** dialog opens.
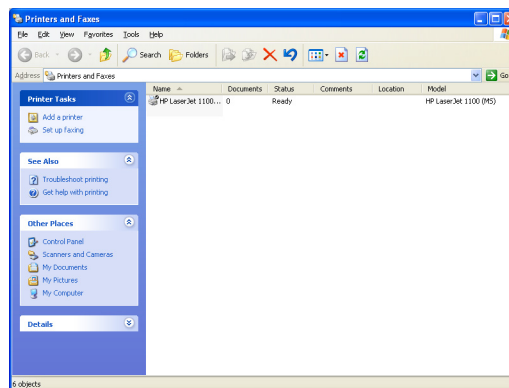
Figure 2-8. Printers and Faxes Dialog

5. If the printer was installed correctly, its name will appear in the printer list. If this is the first printer installed on this system, it is automatically designated as the default printer and will be used by the SP system. If more than one printer is installed, you may change the default printer as described in "Set the Default Printer" on page 2-9.
6. Select **Close** from the **File** menu.

WARNING

To avoid possible injury and equipment damage, keep hands away from the collection rack area while the homing routine runs in step 7 below.

7. Double-click the SPX icon on the desktop to start up the SP software. The Collection Arm moves through its homing routine and the system boots to the system's main menu.
8. Disconnect the mouse.

2.9.2   Install a Non-Plug and Play Printer

Use this procedure if Windows XP cannot automatically identify and install your printer.

1. If you are not at the main menu, press **Main Menu** in the right-hand panel and then press **Shut Down**.
2. When the SP software has shut down, connect a Windows XP compatible mouse either to one of the SP system's USB ports, or to the SP system's mouse port.
3. Connect your printer either to one of the SP system's USB ports, or to the SP system's parallel port.
4. Turn the printer on.
5. Select **Printers and Faxes** from the Windows **Start** menu. The **Printers and Faxes** dialog opens.
6. Click **Add a Printer**. The **Add Printer Wizard** opens.
7. Follow the instructions in the wizard to install your printer. The wizard will prompt you to supply a driver for your printer. Here are some recommended ways to install your printer driver files:
   - If the driver file is on a CD-ROM disk, connect a Windows XP compatible external CD-ROM drive to one of the SP system's USB ports and insert the disk.
   - If the driver file is on a floppy disk, connect a Windows XP compatible external floppy disk drive to one of the SP system's USB ports and insert the disk.
   - Copy your driver file to a USB Flash drive and plug it into one of the SP system's USB ports.
8. Navigate to the connected drive in the wizard's browse dialog and select your driver file.
9. Once the printer is installed, its name will appear in the printer list. If this is the first printer installed on this system, it will be designated as the default printer and will be used by the SP system. If more than one printer is installed, you may change the default printer as described in "Set the Default Printer" on page 2-9.
10. Select **Close** from the **File** menu.

WARNING

To avoid possible injury and equipment damage, keep hands away from the collection rack area while the homing routine runs in step 11 below.

11. Double-click the SPX icon on the desktop to start up the SP software. The Collection Arm moves through its homing routine and the system boots to the system's main menu.
12. Disconnect the mouse.

2.9.3   Set the Default Printer

If you have more than one printer installed, the SP system uses the one designated as the default printer. The software automatically designates the first printer installed as the default printer. This procedure describes how to change the default printer assignment.

1. If you are not at the main menu, press **Main Menu** in the right-hand panel and then press **Shut Down**.
2. When the SP software has shut down, connect a Windows XP compatible mouse either to one of the SP system's USB ports, or to the SP system's mouse port.
3. Select **Printers and Faxes** from the Windows **Start** menu. The **Printers and Faxes** dialog opens.
4. In the list of printers, select the printer you wish to designate as the default.
5. Select **Set as Default Printer** from the **File** menu. The selected printer now has a small check mark next to its icon.
6. Select **Close** from the **File** menu.

WARNING

To avoid possible injury and equipment damage, keep hands away from the collection rack area while the homing routine runs in step 7 below.

7. Double-click the SPX icon on the desktop to start up the SP software. The Collection Arm moves through its homing routine and the system boots to the system's main menu.
8. Disconnect the mouse.

2.10 Set the Date and Time

The date and time settings are used to "time stamp" the result records. Setting the date and time correctly ensures an accurate date and time stamp for your records and can help you search for specific records.

1. If you are not at the main menu, press **Main Menu** in the right-hand panel and then press **Shut Down**.
2. When the SP software has shut down, connect a Windows XP compatible mouse either to one of the SP system's USB ports, or to the SP system's mouse port.
3. Select **Control Panel** from the Windows **Start** menu. The **Control Panel** dialog opens.
4. Click **Date, Time, Language, and Regional Options**.
5. Click **Date and Time**. The **Date and Time Properties** dialog opens.
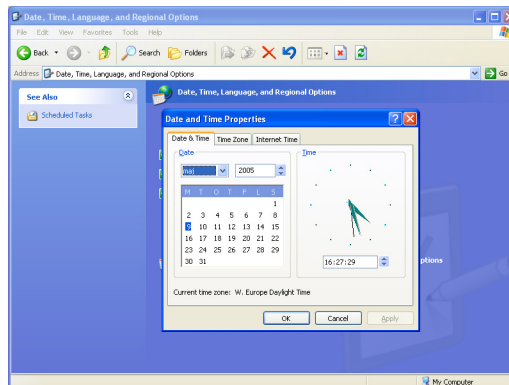
Figure 2-9. Date and Time Properties Dialog

6. Click the current date on the calendar. If you need to change the month and/or year, use the drop-down lists at the top of the calendar.
7. Click the up or down arrow buttons to change the hour, minutes, seconds.
8. To apply your settings and close the dialog, click **OK**.

WARNING

To avoid possible injury and equipment damage, keep hands away from the collection rack area while the homing routine runs in step 9 below.

9. Double-click the SPX icon on the desktop to start up the SP software. The Collection Arm moves through its homing routine and the system boots to the system's main menu.
10. Disconnect the mouse.

2.11 Specify the UV Detector Model

1. Select the **General** tab in the right-hand panel.
2. Press **Change** in the **UV Detector Type** field repeatedly until the correct detector model is shown.

Figure 2-10. General Tab (SP1 and SP4)

2.12 Turn On and Off Audible Alarm

If the Audible Alarm option is turned on, an audible warning will sound in the following situations: 1) rack change, 2) leakage (if using the optional Leak Detector), 3) high pressure, 4) low solvent volume (if the Solvent Monitoring option is turned on), 5) full waste reservoir (if the Waste Monitoring option is turned on), and 6) misalignment of the Collection Arm.

1. Select the **General** tab in the right-hand panel.
2. To turn on or off the audible alarm, press **ON** or **OFF** in the **Audible Alarm** field.

2.13 Turn On and Off Auto Print of Reports

If the Auto Print option is turned on, a report will be printed to the default printer after a purification run is completed. To install a printer, see "Set Up a Printer" on page 2-8.
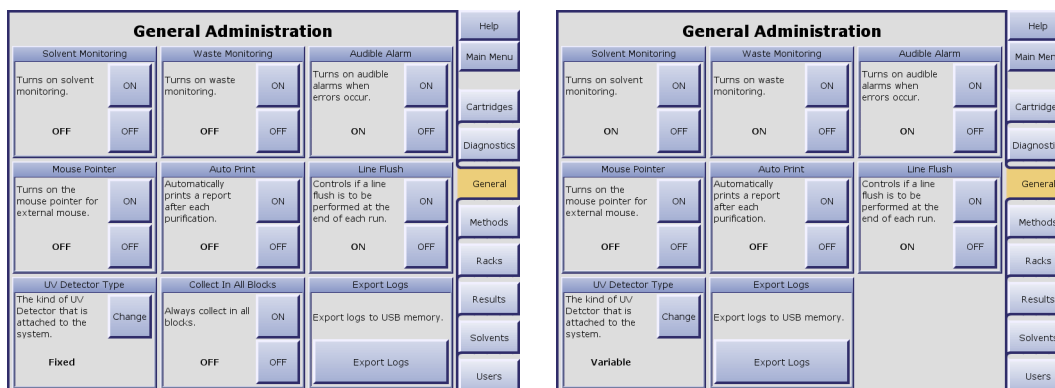
1. Select the **General** tab in the right-hand panel.
2. To turn on or off the auto print of reports, press **ON** or **OFF** in the **Auto Print** field.

2.14 Turn On and Off Default Rack Allocation

If you turn on the Collect In All Blocks option, the whole collection rack will by default be allocated for each run. This option is only available if you are using an SP1 system.

1. Select the **General** tab in the right-hand panel.
2. To turn on or off the default allocation of the whole collection rack for each run, press **ON** or **OFF** in the **Collect In All Blocks** field.

2.15 Turn On and Off Line Flush at the End of Runs

If the Line Flush option is turned on, the system will flush the inlet line and the column with 9 mL of the weakest solvent before finishing the purification run. (The liquid is dispensed to the waste.)

1. Select the **General** tab in the right-hand panel.
2. To turn on or off the line flush at the end of runs, press **ON** or **OFF** in the **Line Flush** field.

2.16 Turn On and Off Mouse Pointer

When connecting a Windows XP compatible mouse to one of the SP system's USB ports, or to the SP system's mouse port, you need to turn on the mouse pointer.

1. Select the **General** tab in the right-hand panel.
2. To turn on or off the mouse pointer, press **ON** or **OFF** in the **Mouse Pointer** field.

2.17 Turn On and Off Solvent and Waste Monitoring

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

1. Select the **General** tab in the right-hand panel.
2. To track solvent and/or waste levels:
    a. Press **ON** in the **Solvent Monitoring** and/or **Waste Monitoring** field.
    b. If you want an audible alarm to sound when a warning is issued, make sure the Audible Alarm option is turned on. (To turn on the audible alarm option, press **ON** in the **Audible Alarm** field.)
    c. Enter capacities and current fluid levels for the solvent and/or waste reservoirs, see "Set the Reservoir Volumes" on page 3-23. (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 have to be entered each time you empty a waste reservoir or replenish a solvent.)
3. To turn off solvent and/or waste monitoring, press **OFF** in the **Solvent Monitoring** and/or **Waste Monitoring** field.

2.18 View the System Status and Restore the System

At the **Diagnostics** tab (in the software's system mode) you can view the status of the Fraction Collector, Pump Module and UV Detector, and, if necessary, restore the system after e.g. overpressure or an arm movement failure.

2.18.1 View the System Status

1. Select the **Diagnostics** tab in the right-hand panel.
2. If the UV Detector's lamp is <u>not</u> on (Lamp On/Off = OFF), press **Lamp On**.
3. Check that the Reference and Sample Intensity is 50% or higher.
4. Check that there is <u>no</u> "Fault" status.

Figure 2-11. Diagnostics Tab

2.18.2 Restore the System

Intensity Below 50%

If the Reference or Sample Intensity is below 50%, the lamp might need to be replaced. Please contact your local Biotage 1-Point Support Team. (If the UV Detector lamp is not turned on, the intensity is 0%.)

"Fault" Status

WARNING

To avoid possible injury and equipment damage, keep hands away from the collection rack area while the homing routine runs in step 2, 4 and 5 below.

1. Ensure all liquid lines and electrical cables between the system modules are connected correctly and securely. For details, see the "Unpacking and Installation" document (P/N 09625).
2. If there is a Fraction Collector error, press **Initialize Arm**.
3. If there is a Pump Module error, press **Initialize Pump**.
4. If there is a Flow Path Module error, or the action in step 2 or 3 failed, press **Reset All and Clear**.

WARNING

Failure to perform an orderly system shutdown may result in user data corruption.

5. If the action in step 4 failed, restart the system in the following way:
   a. Press **Main Menu** in the right-hand panel.
   b. Press **Shut Down**. The Windows desktop appears.
   c. Press **Start** on the Windows task bar. The **Start** menu appears.
   d. Press **Turn Off Computer...**. The **Turn off computer** dialog opens.
   e. Press **Turn Off**.
6. Wait until the Touch Screen goes blank and then turn off the FLASH Collector and the UV Detector. The power switches are located at the rear of the units.
7. Start up the system, i.e. turn on the FLASH Collector and the UV Detector, and then press the System Start-up push button on the front of the FLASH Collector.
8. If the error remains after restart of the system, please contact your local Biotage 1-Point Support Team.

Chapter	*Operation*
3	*(Chemistry Mode)*

WARNING

Before performing any procedures in this chapter, please read and observe the safety requirements in the "Safety and Quick Start" document (P/N 09715). Failure to follow those requirements may result in personal injury and/or equipment damage.

WARNING

To avoid arcing or other electrical hazards, and/or data loss, do not connect or disconnect electrical cords or cables (including mains, 12 VDC and 24 VDC) while the system is turned on.

3.1 Start Up the System

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

1. Ensure that all liquid lines and electrical cables between the system modules are connected correctly and securely. For details, see the "Unpacking and Installation" document (P/N 09625).
2. Plug in the FLASH Collector power cord and turn on the FLASH Collector. The power switch is located at the rear of the unit.
3. Plug in the UV Detector power cord and turn on the UV Detector. The power switch is located at the rear of the unit.

WARNING

To avoid possible injury and equipment damage, keep your hands out of range of the Collection Arm while the homing routine runs in step 4 below.

4. Press the System start-up push button on the front of the FLASH Collector. The Collection Arm moves through its homing routine and the system boots to the system's main menu.
5. The first time the system is started, specify the UV Detector model (see page 2-11).
6. To be able to set up, control, monitor and review a purification run, press **Chemistry**. The system is opened in the chemistry mode.
7. To be able to change system settings, manage users, view the status of the Fraction Collector, Pump Module and UV Detector, and, if necessary, restore the system after e.g. overpressure or an arm movement failure, see "Log into the System Mode" on page 2-1.

NOTE

Only users with system owner privilege can log into the system mode.

For more information about the software modes, see "Software Description" on page 1-6.

3.2 Shut Down the System

<div align="center">

WARNING

</div>

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

Use the following procedure to perform an orderly system shutdown:

1. Ensure that there is no purification run in progress; otherwise, it will be aborted.
2. If you are not at the main menu, press **Main Menu** in the right-hand panel.
3. Press **Shut Down**. The Windows desktop appears.
4. Press **Start** on the Windows task bar. The **Start** menu appears.
5. Press **Turn Off Computer...**. The **Turn off computer** dialog opens.
6. Press **Turn Off**.
7. If you wish to remove power from the system, wait until the Touch Screen goes blank and then turn off the FLASH Collector and the UV Detector. The power switches are located at the rear of the units.
8. If desired, unplug the two power cords from their power outlets.

3.3 Create, Open and Edit Methods

There are different ways of creating a new method:

- **Create a new method.** If the system has been configured to use a default method (see "Set a Default Method" on page 2-4), the default method is opened. After setting up the method, you can save it under a new name by entering a unique method name. The default method remains unaltered under its original name.
- **Base the new method on an existing method.** After editing, you can save it 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 preconfigured method with a default gradient.**
  The software comes with five preconfigured methods with only a defined gradient, see Table 3-1. After completing the method, you can save it under a new name by entering a unique method name.
- **Base a new method on a previous run.** After editing the purification parameters, you can save it under a new name by entering a unique method name.

<div align="center">

Table 3-1: Default Gradient Selection Table

TLC Solvent Ratio (weak:strong)	Method Name*
1:1	12-100
4:1	5-40
9:1	2-20
3:2	10-80
2:3	7-60

</div>

* The method names indicate the strong solvent gradient profile from lowest % to highest %. Gradients occur over 13 CV (Column Volumes).

3.3.1 Create or Open a Method

NOTE

When creating a method for the first time, you can choose to use the method wizard, see "Create a Method Using the Method Wizard" on page 3-12.

1. To create a new method, select the **Method** tab in the right-hand panel and press **New**. If the system has been configured to use a default method (see "Set a Default Method" on page 2-4), the default method is opened at the Method tab.
2. To base a new method on the method for the previous run:
   a. Select the **Method** tab in the right-hand panel and press **Open...**.
   b. In the **Open Method** dialog, press **Previous Run**. The method is opened at the **Method** tab.
3. To change an existing method, or base a new method on an existing method or on a preconfigured method with a default gradient:
   a. Select the **Method** tab in the right-hand panel and press **Open...**.
   b. In the **Open Method** dialog, select user. All methods for that user are listed. (To select one of the five preconfigured methods with a defined gradient, select the user "GLOBAL". To find out their TLC solvent ratio, see Table 3-1 on page 3-2.)
   c. Select a method and press **OK**.
4. To base a new method on a performed purification run, see "View, Print and Copy Record Details" on page 3-20.
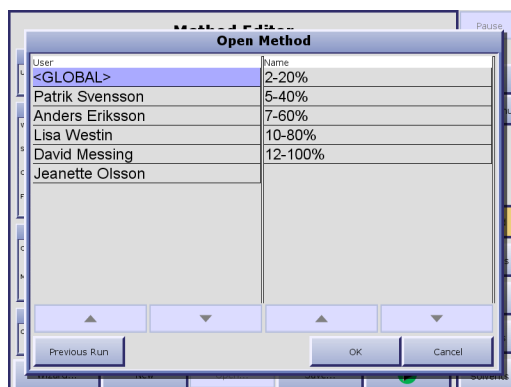
Figure 3-1. Open Method Dialog

Enter Method Information

1. Press the **Information** field. The **Information Editor** opens.
2. At the **User** tab, select your user name. If you have not been assigned a user name, please contact your system supervisor.
3. At the **Method Name** tab, enter the name of the method. If you are editing an existing method and save it under a new name, the original method remains unaltered under its original name. (If you do not wish to save the method, no method name has to be entered.)
4. If you want to change the default sample name, change it at the **Sample Name** tab.
5. At the **Project** tab, select the project that you want the method to be associated with. (This is optional.) To add, delete and rename projects, see page 3-10.
6. To enter a method comment, press **Next** and enter the comment.
7. You can now either continue setting up the method by pressing **Next** (and then continue with step 2 in "Select Solvent and Cartridge"), or save the entered information and close the **Information Editor** by pressing **OK**.

Select Solvent and Cartridge

1. Press the **Separation** field. The **Separation Editor** opens.
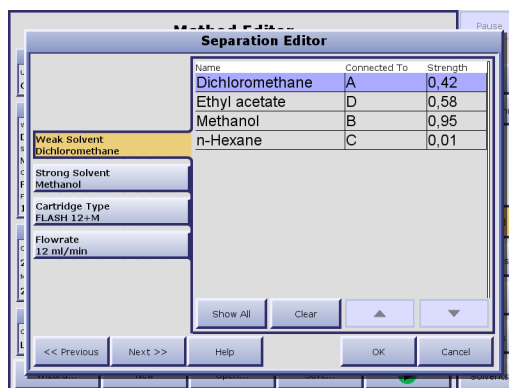
Figure 3-2. Separation Editor

2. At the **Weak Solvent** and **Strong Solvent** tabs, select two solvents for this method. (They should be the two solvents determined by the TLC procedure.) To show all solvents, press **Show All**. To only show the solvents mounted on the system, press **Hide Unconnected**. Note that you do not select solvent percentages in the **Separation Editor**. Solvent percentages are assigned using the **Gradient Editor**.

3. At the **Cartridge Type** tab, select the cartridge type for this method.

4. If you do not want to use the default flowrate for the cartridge type, change it at the **Flowrate** tab. (The range is 1 to 100 mL/min, see Table 3-2 for guidelines.)

NOTE

If you use the collection mode Low Slope or Medium Slope, set the flowrate to a maximum of 60 mL/min.

5. You can now either continue setting up the method by pressing **Next** (and then continue with step 3 in "Create Gradient"), or save the entered information and close the **Separation Editor** by pressing **OK**.

Create Gradient

1. If you want to calculate the gradient from TLC data, see page 3-11.

2. Press the **Gradient** field. The **Gradient Editor** opens.

Figure 3-3. Gradient Editor

3. The Y-axis indicates percent of the strong solvent, and the X-axis refers to solvent volume in mL or CV (Column Volumes), or time in minutes. CV is the default unit. To change the X-axis unit from CV (Column Volumes) to volume (mL) or minutes (min), press the **Toggle Unit** repeatedly until the desired X-axis unit appears on the button.

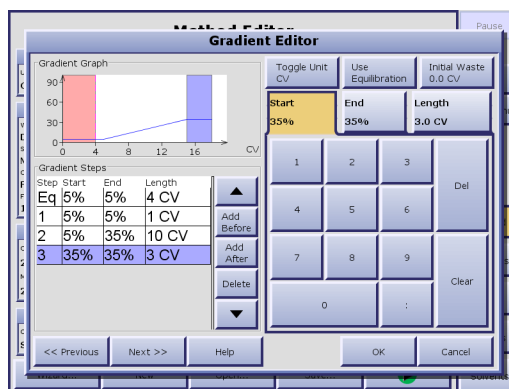4. If you wish to delay collection until a specified volume has been delivered to the waste, set the Initial Waste:
   a. Press **Initial Waste**.
   b. Enter the value for Initial Waste (in 3 mL increments).
   c. To save the value, press **OK**.

5. If you wish to equilibrate:
   a. Press **Use Equilibration**.
   b. At the **Start** tab, enter the percent of the strong solvent to be used when starting the equilibration.
   c. At the **End** tab, enter the percent of the strong solvent to be used when ending the equilibration.
   d. At the **Length** tab, enter the equilibration volume, based on the guidelines in Table 3-2.

Table 3-2: Equilibration Volume and Flowrate Table

Column	*Column Volume (mL)	**Recommended Equilibration Volume (mL)	Recommended Flowrates (mL/min)
FLASH 12+S	6	18	12
FLASH 12+M	12	36	12
FLASH 25+S	24	72	25
FLASH 25+M	48	144	25
FLASH 40+S	66	198	40
FLASH 40+M	132	396	40
FLASH 65i	470	1410	65***
FLASH 75S	300	900	75***
FLASH 75M	500	1500	75***
FLASH 75L	1000	3000	75***

* Volume of dry column.
** Three times the column volume.
*** If you use the collection mode Low Slope or Medium Slope, set the flowrate to a maximum of 60 mL/min.

6. To add gradient steps:
   a. Press **Add After** or **Add Before** depending on whether you want to add the step before or after the selected step.
   b. At the **Start** tab, enter the percent of the strong solvent to be used when starting the gradient step.
   c. At the **End** tab, enter the percent of the strong solvent to be used when ending the gradient step.
   d. At the **Length** tab, enter the length of the step in CV (Column Volumes) or minutes.
   e. To add more steps, repeat step a through step d.

7. To delete a gradient step, select it using the ▼ and ▲ buttons and then press **Delete**.

8. When you have finished setting up the gradient, you can either continue setting up the method by pressing **Next** (and then continue with step 2 in "Select Variable Wavelengths"), or save the entered information and close the **Gradient Editor** by pressing **OK**.

Select Variable Wavelengths

If you have the Variable Wavelength UV Detector:

1. Press the **UV Detector** field. The **UV Detector Editor** opens.
2. At the **Collection Wavelength** tab, enter the value for the collection wavelength that you wish to use with this method. (The range is 200-320 nm.)
3. At **Monitor Wavelength** tab, enter the value for the monitor wavelength that you wish to use with this method. (The range is 200-320 nm.)
4. You can now either continue setting up the method by pressing **Next** (and then continue with step 2 in "Select Collection Mode"), or save the entered information and close the **UV Detector Editor** by pressing **OK**.

The possibility to set these wavelengths can help optimize compound detection since a compound's absorption of UV light varies with the wavelength.

Select Collection Mode

1. Press the **Collection** field. The **Collection Editor** opens.

Figure 3-4. Collection Editor

2. At the **Collection Mode** tab, select the collection mode. Refer to Table 3-3 on page 3-7 for help in choosing a collection mode.
3. If you selected the Threshold mode, enter a UV Collect Threshold AU value (in mAU) at the **Start Threshold** tab.
4. If you selected the Slope mode enter the following parameters (please refer to Table 3-4 on page 3-7 for parameter details):
   a. A UV Collect Threshold value (in mAU) at the **Start Threshold** tab.
   b. Enter a Shoulder Slope value (in mAU/sec) at the **Shoulder Slope** tab.
   c. Enter a Shoulder Slope Disable value (in mAU) at the **Shoulder Slope Disable** tab.
   d. Enter a Start Slope value (mAU/sec) at the **Start Slope** tab.
   e. Enter a Start Slope Enable value (in mAU) at the **Start Slope Enable** tab.
5. You can now either continue setting up the method by pressing **Next** (and then continue with step 2 in "Select Rack Type and Dispense Order"), or save the entered information and close the **Collection Editor** by pressing **OK**.

Table 3-3: Collection Modes

Mode	Operation	Used
Collect All	UV absorbance of sample is monitored, but collection is based on the gradient's total volume and the max fraction volume for the vessel type.	Where UV absorbance is recorded but is not the primary consideration for fractionation.
Threshold	Collection occurs only if AU level is above a user-defined UV absorbance threshold point. Portions of the sample with absorption lower than the AU threshold point are dispensed to the waste.	Where UV absorbance (AU value) is the primary consideration for fractionation.
Slope	This customizable collection mode allows you to set values for the five collection parameters shown in Table 3-4.	Refer to "Slope Detection Collection" on page A-1 for details.
Low Slope	Collection is based on the slope of UV peaks. This mode is pre-programmed to collect samples with low slopes and/or poor UV absorbance. Fixed settings: • Start Threshold: 2000 mAU • Shoulder Slope: 1 mAU/sec • Shoulder Slope Disable: 2000 mAU • Start Slope: 3 mAU/sec • Start Slope Enable: 50 mAU	Where low absorbance is expected. This mode gives higher yield and lower purity than Medium Slope collection.
Medium Slope	Collection is based on the slope of UV peaks. This mode is pre-programmed to collect samples with medium slopes. Fixed settings: • Start Threshold: 2000 mAU • Shoulder Slope: 1 mAU/sec • Shoulder Slope Disable: 2000 mAU • Start Slope: 8 mAU/sec • Start Slope Enable: 100 mAU	Where higher absorbance is expected. This mode gives lower yield and higher purity than Low Slope collection.
Manual	You can at any time instruct the system to start and stop collecting fractions. The system will fill collection vessels according to the defined max fraction volume. You can at any time switch to a new collection vessel. See "Control a Manual Collection" on page 3-18.	

Table 3-4: Collection Mode Parameters

Parameter	Description
Start Threshold	The Start Threshold parameter is used to collect samples with an absorbance exceeding the set absorbance threshold in Absorbance Units (AU).
Shoulder Slope	Shoulder peaks are secondary peaks connected to a main peak. A shoulder peak generally has a lower amplitude than the main peak it is connected to, and is considered to be an "unresolved" peak because it is not separated from the main peak. The start or end of a shoulder peak usually indicates that a different compound is being sensed. Use of the Shoulder Slope parameter allows sensing this new compound so that it can be separated from the previous compound in the collection vessels. Two criteria must be met in order for a shoulder to be detected by the SP system: 1. The slope must change and its absolute value drop below the Shoulder Slope value. 2. The slope must then increase to twice (2x) the Shoulder Slope value.
Shoulder Slope Disable	The Shoulder Slope Disable is a level above which all peak collection criteria are based upon fraction volume, disregarding slope and slope change values. This value is typically set high (>1500 mAU) to prevent false fractionation due to noise as the UV Detector approaches UV absorption maximum.

Table 3-4: Collection Mode Parameters

Parameter	Description
Start Slope	The Start Slope parameter is used to set a minimum slope (rate of AU change) trigger value. While the measured AU slope value (ΔAU/Δtime) is equal to or above this value, sample collection based on slope may occur, depending on the setting of the Start Slope Enable and Shoulder Slope Disable parameters. While the AU slope is below this value, collection based on slope may not occur.
Start Slope Enable	The Start Slope Enable is an absorbance level used to enable slope collection. Only peaks that are above the Start Slope Enable value and that meet the Start Slope criteria will be collected. Peaks below the Start Slope Enable value will be dispensed to the waste. For example, if a sample meets the Start Slope criterion, but the slope is below the Start Slope Enable threshold it will not be collected except when the Start Threshold value is lower than the Start Slope Enable value.

NOTE
If you use the collection mode Low Slope or Medium Slope, set the flowrate to a maximum of 60 mL/min.

Select Rack Type and Dispense Order

1. Press the **Rack Allocation** field. The **Allocation Editor** opens.
2. At the **Rack Type** tab, select the rack type to be used. See Table 3-5 for guidance.
3. At the **Dispense Order** tab, select "S-Order" or "Z-Order" collection pattern.
4. If you do not want to use the default max fraction volume for the vessel type, change it at the **Max Fraction Volume** tab. The software prevents you from entering a value that will overflow the vessels.
5. To save the entered information, press **OK**. The **Allocation Editor** closes.
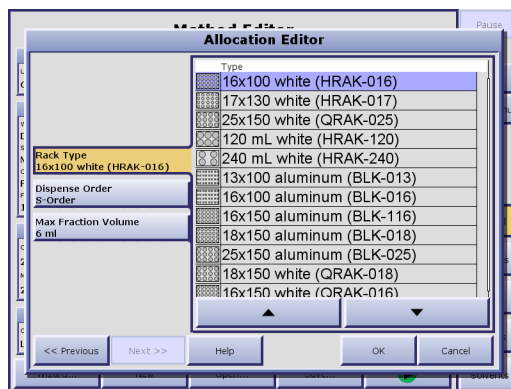

Figure 3-5. Allocation Editor

Table 3-5: Rack Selection Table

Rack Type	No. of Blocks	Definition	Vessel D x L, mL	No of Vessels	Vol. (mL)	12+S (6)	12+M (12)	25+S (24)	25+M (48)	40+S (66)	40+M (132)	75S (300)	65i (470)	75M (500)	75L (1000)
						FLASH Cartridge Type (CV in mL)									
BLK-013	4	Aluminum	13 x 100 mm, 9 mL	32 x 4	288 x 4				2	3					
HRAK-013	4	White	13 x 100 mm, 9 mL	30 x 4	270 x 4			2	3	3					
BLK-016	4	Aluminum	16 x 100 mm, 12 mL	32 x 4	384 x 4				2	3					
HRAK-016	4	White	16 x 100 mm, 12 mL	24 x 4	288 x 4				2	3					
BLK-116	4	Aluminum	16 x 150 mm, 21 mL	24 x 4	504 x 4				2	2	4	8			
QRAK-016	4	White	16 x 150 mm, 21 mL	24 x 4	360 x 4				2	3					
HRAK-017	4	White	17 x 125 mm, 18 mL	20 x 4	360 x 4				2	3	3	6			
BLK-018	4	Aluminum	18 x 150 mm, 27 mL	24 x 4	648 x 4				2	2	3				
QRAK-018	4	White	18 x 150 mm, 27 mL	12 x 4	324 x 4					2	3				
BLK-025	4	Aluminum	25 x 150 mm, 51 mL	12 x 4	612 x 4					3	3				
QRAK-025	4	White	25 x 150 mm, 51 mL	12 x 4	612 x 4					2	3	6			
HRAK-120	4	White	120 mL	6 x 4	720 x 4					2	3	5	8	9	17
HRAK-240	3	White	240 mL	4 x 3	960 x 3						2	4	6	7	13

Requires 1 block of this type, based on 12 CV gradient in "Collect All" mode

Requires multiple blocks of this type, based on 12 CV gradient in "Collect All" mode. (See the number of blocks in the table.)

Rack change required during the run, based on 12 CV gradient in "Collect All" mode. (See the number of blocks in the table.)

Not recommended.
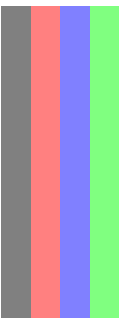
Save and/or Run the Method

1. If you want to save the method and reuse it in the future, press **Save...**. The **Create Method?** dialog opens.
2. To confirm save, press **Yes**. If your user account is password-protected, the **Enter Password** dialog opens. Enter your password using the keypad, and then press **OK**.
3. To run a (saved or unsaved) method, see "Prepare and Run a Purification" on page 3-13.

NOTE

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

3.3.2   Add, Delete and Rename Projects

A method can be associated with a project. To add, delete or rename a project, follow the instructions below.

NOTE

To be able to delete a project, you have to delete all methods and result records in the project (see page 2-3 and page 2-5).

1. Select the **Method** tab in the right-hand panel.
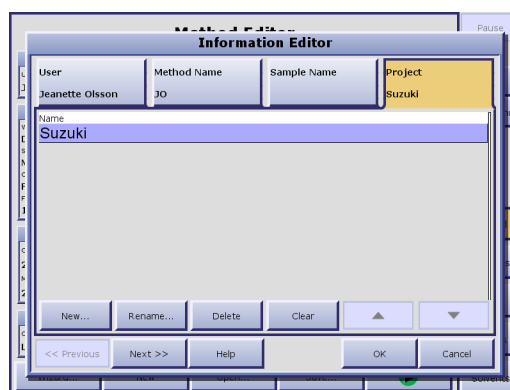2. Press the **Information** field. The **Information Editor** opens.

Figure 3-6. Information Editor

3. Select the **Project** tab.
4. To add a new project:
    a. Press **New...**. The **Project Editor** opens.
    b. Enter the name of the project.
    c. To save the new project, press **OK**.
5. To rename a project:
    a. Select the project that you want to rename. To scroll the list down or up, press ▼ or ▲.
    b. Press **Rename...**. The **Project Editor** opens.
    c. Enter the new project name.
    d. To save the new name, press **OK**.
6. To delete a project:
    a. Select the project that you want to delete. To scroll the list down or up, press ▼ or ▲.
    b. Press **Delete**. The **Confirm Delete** dialog opens.
    c. To confirm delete, press **Yes**.
7. To close the **Information Editor**, press **OK**.

3.3.3   Calculate Gradient from TLC Data

The software's **TLC Plate Measurements Editor** is used to enter the following parameters obtained from a TLC procedure:
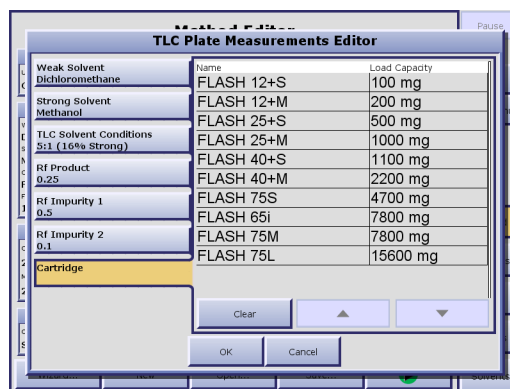
- R_f values for compound of interest and the closest eluting compounds on the leading and trailing sides of the compound of interest.
- Solvents and their percentages.

The software uses these values to calculate a purification gradient and column/sample load recommendations.

NOTE
The values you enter into the TLC Plate Measurements Editor must be those obtained from the TLC procedure.

1. Select the **Method** tab in the right-hand panel.
2. Press **Calculate from TLC**. The **TLC Plate Measurements Editor** opens.

Figure 3-7. TLC Plate Measurements Editor

3. At the **Weak Solvent** and **Strong Solvent** tabs, select two solvents for this method. They must be the same two solvents as the ones used in the TLC procedure.
4. At the **TLC Solvent Conditions** tab, enter either the percent of strong solvent or the solvent ratio (i.e. weak solvent:strong solvent).
5. At the **Rf Product** tab, enter the TLC's R_f value for the compound of interest.
6. At the **Rf Impurity 1** tab, enter the TLC's R_f value for Impurity 1.
7. At the **Rf Impurity 2** tab, enter the TLC's R_f value for Impurity 2.
8. At the **Cartridge** tab, select a column/load size combination.
9. To save the data, press **OK**. The **TLC Plate Measurements Editor** closes and the software calculates the purification gradient based on the TLC data and displays it at the **Method** tab.

3.3.4 Create a Method Using the Method Wizard

When creating a method for the first time, you can choose to use the method wizard. The wizard will step by step guide you through the setup of a run.

1. Using standard TLC procedures, determine the appropriate solvents and composition for the purification.
2. Prepare the instrument for the run as described on page 3-13.
3. Select the **Method** tab in the right-hand panel.
4. Press **Wizard...**. The method wizard opens.
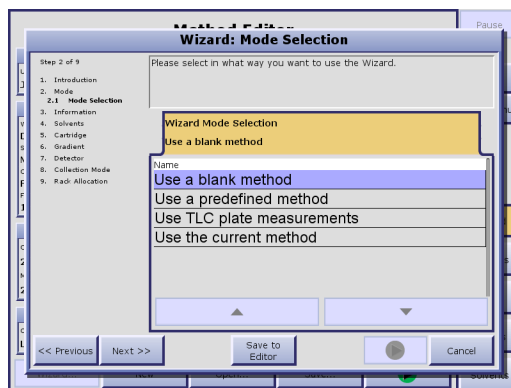
Figure 3-8. Method Wizard

5. Read and follow the instructions that appear in the wizard. (For help to select block type, see Table 3-5 on page 3-9.)
6. If you want to save the method and reuse it in the future:
   a. Press **Save to Editor**.
   b. Press **Save...**. The **Create Method?** dialog opens.
   c. To confirm save, press **Yes**. If your user account is password-protected, the **Enter Password** dialog opens. Enter your password using the keypad, and then press **OK**.

7. To run a purification run using the method, press the ▶ button.
8. Proceed with step 5 in "Run a Purification" on page 3-13.

3.4 Prepare and Run a Purification

3.4.1 Prepare the Instrument for a Run

1. On the Touch Screen, select the **Columns** tab in the right-hand panel and check that the column is free/empty, i.e. **Status** = " " or "Finished".

2. Install the compression module, cartridge and collection rack that you want to use. For connection drawings, see the "Unpacking and Installation" document (P/N 09625).

3. Ensure that a sufficient quantity of the correct solvent is present in each solvent reservoir and that the waste reservoir has sufficient capacity for this run.

4. On the Touch Screen, select the **Solvents** tab and ensure that the solvents are assigned accurately to the solvent inlets. If the monitoring of solvent and waste levels is turned on, check that the capacity and current volume values are correct. To make changes, see "Assign Solvent Types to the Solvent Inlets" on page 3-23 and "Set the Reservoir Volumes" on page 3-23.

5. If you need to prime the system due to change of solvent type or air bubbles, see page 3-24.

6. To turn on the UV Detector lamp in advance, select the **Status** tab and press **Lamp On**.

7. Ensure that connections are correct and secure.

<div align="center">

NOTE
</div>

It is recommended that you first create the method to be used before setting up to run a purification, see page 3-2.

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

3.4.2 Run a Purification

<div align="center">

WARNING
</div>

If you at any time during a purification run need to pause the system, press the Pause button in the right-hand panel. Once the current pump stroke finishes, the Collection Arm returns to its home position (the inner right corner) and the system is paused. To resume operation, press the Resume button.

1. Prepare the instrument as described in "Prepare the Instrument for a Run" above.

2. Select the **Method** tab in the right-hand panel

3. Open or create a new method, see "Create, Open and Edit Methods" on page 3-2.

4. To run a purification run using the displayed method, press the ▶ button. The **Run Parameters** dialog opens.

5. In the **Run Parameters** dialog, select/press the collection block(s), and, if using an SP4 system, the column to be used. "Check mark" = the block is selected (see Figure 3-9). If you want to use the whole rack, press **All Blocks**. (If the **Run Parameters** dialog is not displayed, your system has been configured to allocate the whole rack for each run.)

6. To proceed, press **OK**. The **Columns** tab is selected.

7. To start or queue up (when using an SP4 system) an equilibration step, press **Equilibrate** at the **Columns** tab (see Figure 3-11). Before the equilibration is started, the system empties the solvent inlets of solvents used in the previous purification and fills them with new solvents.

8. Prepare the sample for injection. See Table 3-6 and Table 3-7 on page 3-15 for FLASH cartridge loading capacity.
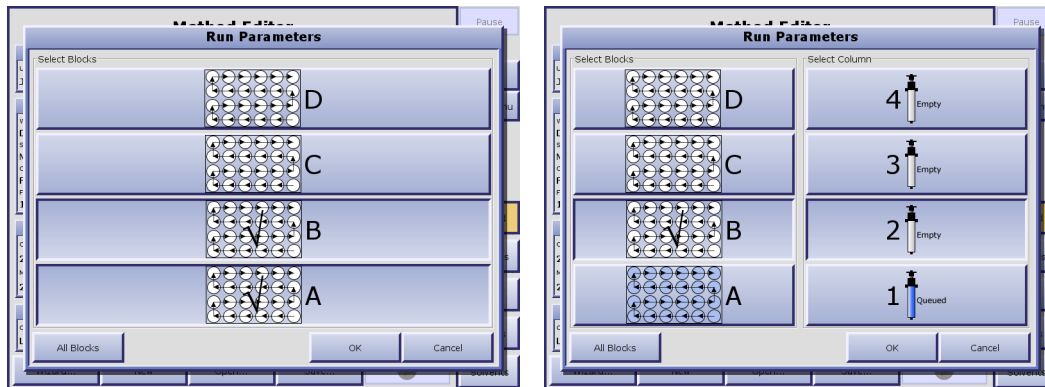
Figure 3-9. Run Parameters Dialog (SP1 and SP4)

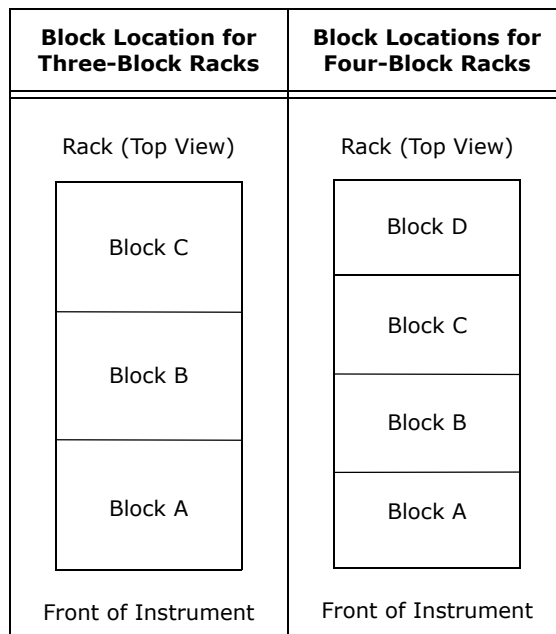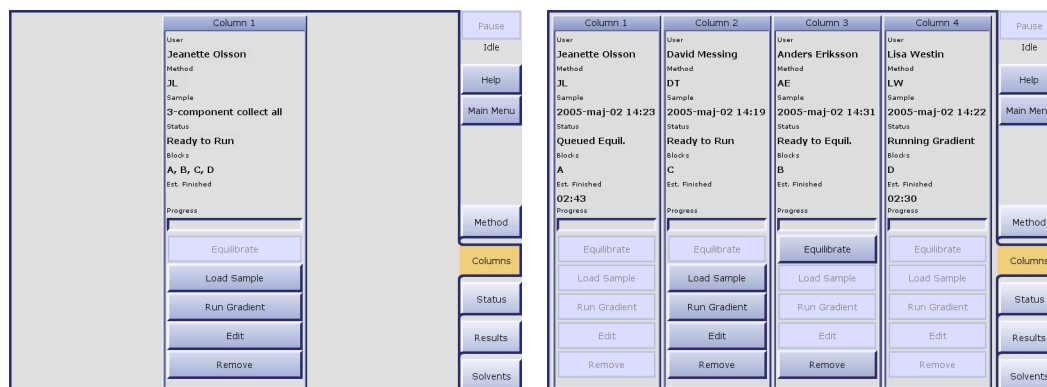| Block Location for Three-Block Racks	Block Locations for Four-Block Racks
Rack (Top View)	Rack (Top View)
Block C	Block D
Block B	Block C
	Block B
Block A	Block A
Front of Instrument	Front of Instrument

Figure 3-10. Block Locations

Figure 3-11. Columns Tab (SP1 and SP4)

Table 3-6: Cartridge Loading Capacity for Gradient Elution

FLASH Cartridge	Column Volume (mL)	Silica Weight (g)	ΔCV 0.1-0.4 (mg)	ΔCV 0.5-0.9 (mg)	ΔCV 1.0-1.4 (mg)	ΔCV 1.5-1.9 (mg)	ΔCV 2.0-2.4 (mg)	ΔCV 2.5-3.0 (mg)	ΔCV 3.1-4.0 (mg)	ΔCV >4.0 (mg)
12+S	6	5	13	25	50	75	100	125	150	200
12+M	12	9	25	50	100	150	200	250	300	400
25+S	24	20	50	100	200	300	400	500	600	800
25+M	48	39	100	200	400	600	800	1000	1200	1600
40+S	66	50	138	275	550	825	1100	1375	1650	2200
40+M	132	100	275	550	1100	1650	2200	2750	3300	4400
65i	470	350	975	1950	3900	5850	7800	9750	11700	15600
75+S	300	200	588	1175	2350	3525	4700	5875	7050	9400
75+M	500	400	975	7800	15600	23400	31200	39000	46800	62400
75+L	1000	800	1950	3900	7800	11700	15600	19500	23400	31200

Table 3-7: Cartridge Loading Capacity for Isocratic Elution

FLASH Cartridge	Column Volume (mL)	Silica Weight (g)	Difficult ΔCV = 1 (mg)	Typical ΔCV = 2 (mg)	Easy ΔCV = 6 (mg)
12+S	6	4.5	4-20	20-100	100-200
12+M	12	9	8-40	40-200	200-400
25+S	24	20	15-80	80-400	400-800
25+M	48	40	30-160	160-800	800-1600
40+S	66	50	40-200	200-1000	1000-2000
40+M	132	100	80-400	400-2000	2000-5000
65i	470	350	300-1600	1600-8000	8000-20000
75+S	300	200	160-800	800-4000	4000-10000
75+M	500	400	400-2000	2000-10000	10000-20000
75+L	1000	800	800-4000	4000-20000	20000-40000

9. When the **Load Sample** button at the **Columns** tab is enabled, introduce the sample onto the cartridge using a Samplet, a ZIF-SIM, or direct injection. To open the injection valve:
   a. Press **Load Sample**. The **Load Sample** dialog opens.
   b. Introduce the sample onto the cartridge through the injection valve. (The loading state will time-out after approximately 15 minutes and the **Load Sample** dialog is then closed.)
   c. When you have finished loading the sample, press **OK** in the **Load Sample** dialog.

10. To start or queue up (when using an SP4 system) the purification run, press **Run Gradient**. Before the gradient is started, the system empties the solvent inlets of solvents and fills them with new solvents. Note that the run is not started until the UV lamp is sufficiently warmed up.

NOTE

When using an SP4 system, queued equilibrations and gradient runs will be performed in the order they were queued up.

When your purification run is completed, make sure to remove the cartridge and place the compression module head in a slot on the FLASH Collector's compression module platform.

Load New Blocks During the Run

If more fractions are to be collected than can fit in the allocated block(s), the system pauses, the Collection Arm returns to the home position (the inner right corner), and the **Load New Block(s)** dialog opens. Replace the block(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 block that you selected. For example, if you selected blocks B and C (see Figure 3-10 on page 3-14), the collection is resumed in block B in vessel 1.

Auto-Extend Mode

If remaining UV absorption is detected when the system nears the end of a purification run, the system enters the Auto-Extend mode. This extends the gradient purification stage of the run with 25% of the total gradient length.

Line Flush, Decompression and Detector Flush

Prior to finishing a purification run, i.e. after the gradient purification stage is completed, the system performs a line flush (if the Line Flush option is turned on), system decompression, and a detector flush. To turn on and off the Line Flush option, see "Turn On and Off Line Flush at the End of Runs" on page 2-11.

3.5 Monitor and Control a Purification Run

3.5.1 Monitor the Purification in Progress

Select the **Status** tab in the right-hand panel. While a purification is running, the graph displays the programmed gradient and a dynamic chromatogram.

Graph View

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

- **Scrolling:** View the present reading +/- 2 CV (Column Volumes) on the horizontal axis.
- **Show All:** View all readings on the horizontal axis.
- **Toggle Unit:** Toggle the horizontal axis unit between CV (Column Volumes), minutes and mL.
- **Zoom In:** Magnify (zoom) the area around the UV zero level.
- **Zoom Out:** Decrease the magnification.
- **Auto Scale:** Adjust the graph so that all readings on the vertical axis are presented.
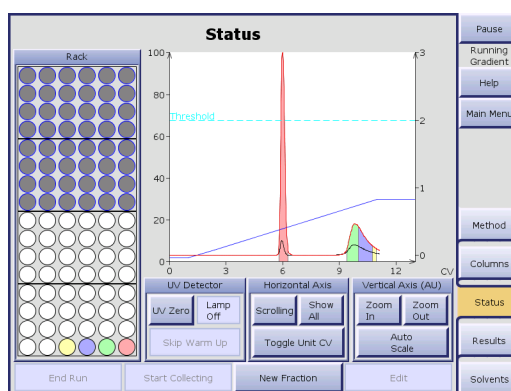

Figure 3-12. Status Tab

Fraction Location and View Information

By pressing the **Rack/Information/>>** field repeatedly, you can toggle between displaying a rack image, an information view and hiding the field (to expand the chromatogram).

If the rack image is displayed, fractions that are already collected can be located by matching their colors on the chromatogram with the vessel colors on the collection rack image. A fraction location feature is also available for the records that are accessible at the **Results** tab, see "Search for Records" on page 3-20.

If the information view is displayed, you can see a color legend, the current % of strong solvent, defined wavelength(s) in nm, measured absorption in AU, defined threshold in AU and the estimated time when the system will have finished processing the purification run.

3.5.2 Control the UV Detector Lamp

The UV Detector uses an ultraviolet (UV) lamp in its detection process. The lamp must be warmed up in order for the signal to be accurate and stable.

<div align="center">

NOTE

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

</div>

The UV Detector lamp is turned on via two methods:

1. **Automatically.** If the UV Detector lamp is not on when you press the **Equilibration** or **Run Gradient** (if equilibration is not used) button at the **Columns** tab, the software sends a command to the UV Detector to turn the lamp on. However, before starting the run, the software waits (approximately 7.5 minutes) for the lamp to warm up.

2. **Manually.** You can turn the UV Detector lamp on manually. By doing this ahead of time, you can avoid the delay that is required if the software turns the lamp on at the start of a run.

To prolong lamp life, the UV Detector will turn the lamp off automatically if it the unit is idle (no purification is started) for approximately two hours.

Manually Turn the UV Lamp On

You can turn the UV Detector lamp on manually by pressing **Lamp On** at the **Status** tab.

Bypass the Automatic UV Lamp Warm-up Period

You can bypass the automatic UV Detector lamp warm-up period by pressing **Skip Warm Up** at the **Status** tab. However, if the lamp has not reached operating temperature, purification results may not be optimal.

3.5.3   Control a Manual Collection

If you have set up a purification run with manual collection, you can at any time instruct the system to start collecting fractions by pressing **Start Collecting** at the **Status** tab. 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**. To stop collecting fractions, press **Stop Collecting**.

3.5.4   Edit and Manually Extend a Purification Run

If remaining UV absorption is detected when the system nears the end of a purification run, the system enters the Auto-Extend mode. This extends the gradient purification stage of the run with 25% of the total gradient length.

To edit and manually extend a purification run:

1. If the purification run is in progress or queued*, pause the system by pressing **Pause** in the right-hand panel. Once the current pump stroke finishes, the Collection Arm returns to its home position (the inner right corner) and the system is paused.

2. To edit the purification run, press **Edit** at the **Columns** tab. The **Gradient Editor** opens.

3. Edit the gradient parameters.

4. To edit more parameters, press **Next**.

5. To save the changes, press **OK**.

6. If the system is paused, resume the operation by pressing **Resume** in the right-hand panel.

7. If the system was not paused, load the sample and/or start or queue up* the run by pressing **Load Sample** and/or **Run Gradient**.

   * Purification runs can only be queued when using an SP4 system.

NOTE

It is not possible to add or edit the initial waste and an equilibration step, and to edit a purification run during its equilibration.

When using an SP4 system, queued equilibrations and gradient runs will be performed in the order they were queued up.

3.5.5   Pause, Resume or End a Purification Run

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).

Pause and Resume a Purification Run

1. To pause a purification in progress, press **Pause** in the right-hand panel. Once the current pump stroke finishes, the Collection Arm returns to its home position (the inner right corner) and the system is paused.

2. To resume the operation, press **Resume** in the right-hand panel.

End and Remove a Purification Run

1. If the purification run is in progress, press **Pause** in the right-hand panel. Once the current pump stroke finishes, the Collection Arm returns to its home position (the inner right corner) and the system is paused.

2. If the purification run is not started or if it was paused during the equilibration, select the **Columns** tab in the right-hand panel and press **Remove**.

3. If the purification run was paused during the gradient run, select the **Status** tab in the right-hand panel and press **End Run**. A line flush (if the Line Flush option is turned on), system decompression and detector flush are performed. To turn on and off the Line Flush option, see "Turn On and Off Line Flush at the End of Runs" on page 2-11.

3.6 Unload a Purification Run

1. To check if a purification run is completed, select the **Columns** tab in the right-hand panel and check that the column's status is "Finished".

2. When the purification run is completed, unload the block(s) or rack. The allocated block(s) for the run is/are listed underneath the **Blocks** heading at the **Columns** tab.

3. Remove the cartridge and place the compression module head in a slot on the FLASH Collector's compression module platform.

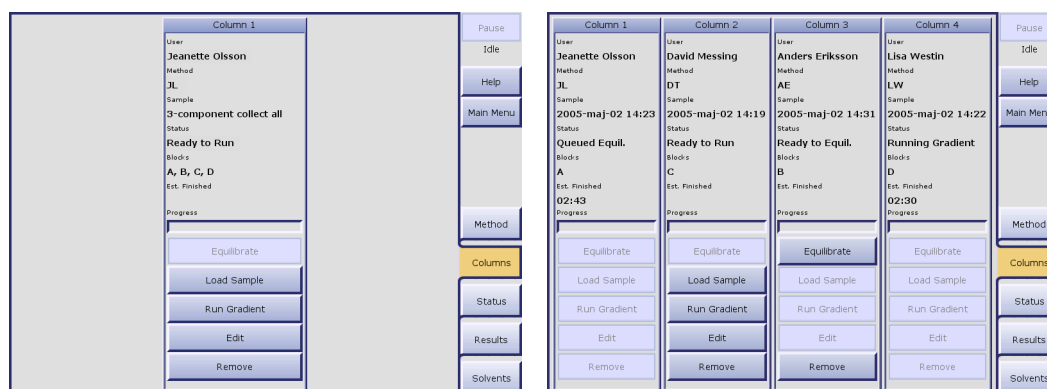4. To clear the column position in the software, press **Remove** at the **Columns** tab.

Figure 3-13. Columns Tab (SP1 and SP4)

3.7 Access Result Records and Locate Fractions

3.7.1 Search for Records

1. Select the **Results** tab in the right-hand panel.
2. Press the **Result Filters** field. The **Filter Parameters Editor** opens.
3. Specify the filter parameters. (If you want to list all result records, press **Clear All**.)
4. To search, press **OK**. If there are records matching your filter parameters, they are listed in chronological order at the **Results** tab. If more records are found than can be displayed at one time, use the ▲ and ▼ buttons to scroll through the records.
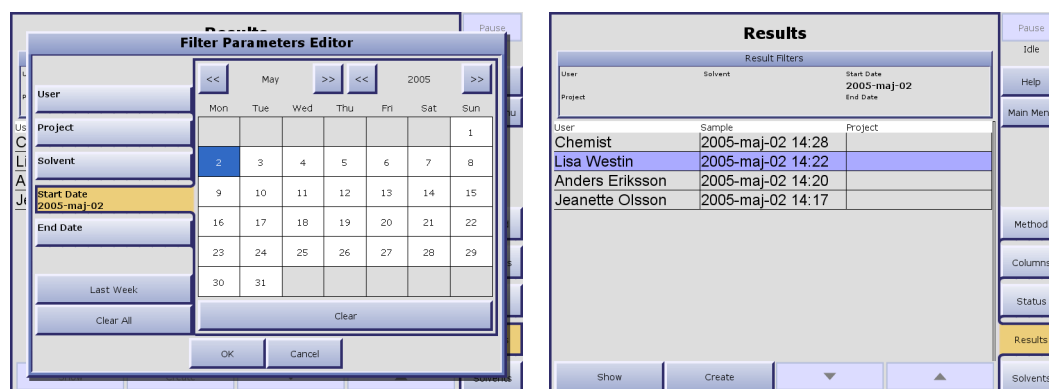

Figure 3-14. Results Tab

3.7.2 View, Print and Copy Record Details

1. Select the **Results** tab in the right-hand panel.
2. Select the record that you want to view, print and/or copy. If the record is not listed, see "Search for Records" above.
3. Press **Show**. A result dialog with purification details and a chromatogram for the selected record opens. The colors used in the chromatogram are:
   - Blue = The gradient.
   - Red = The collection wavelength.
   - Black = The monitor wavelength. (If using the Variable Wavelength UV Detector.)
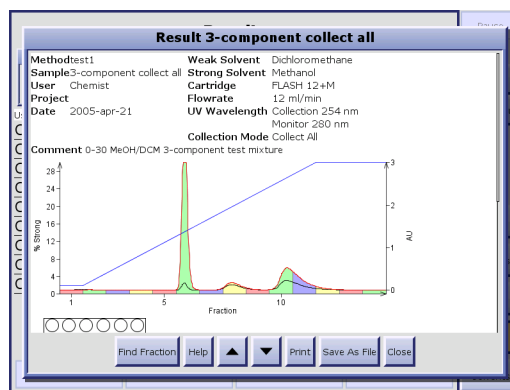

Figure 3-15. Result Dialog

4. To print the record, press **Print**. The report is sent to the system's default printer (i.e. a printer must be installed on your system, see "Set Up a Printer" on page 2-8).

5. To save the result record on a USB Flash drive as an HTML file:
   a. Connect a USB Flash drive to one of the SP system's USB ports. (If two or more devices are connected, the first device to be connected will be used.)
   b. Press **Save As File**. The record is saved on the drive (in a "spx 2.0 results" folder) as an HTML file, which can be viewed with a web browser such as Microsoft Internet Explorer.
6. To create a method with the same purification parameters as the ones used in the selected record, press **Create**. The method is opened at the **Method** tab and can be edited and saved, see "Create, Open and Edit Methods" on page 3-2.

3.7.3 Locate Fractions

1. Select the **Results** tab in the right-hand panel.
2. Select the record that you want to search for fractions. If the record that you wish to search is not listed, see "Search for Records" on page 3-20.
3. Press **Show**. A result dialog opens.
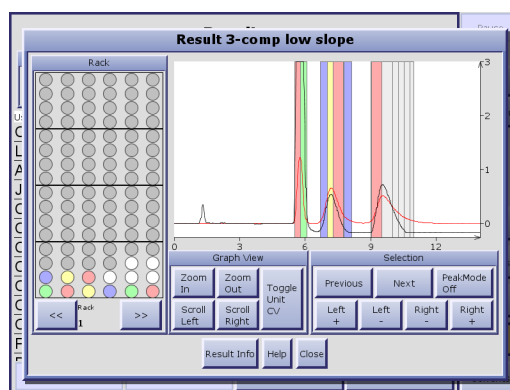4. Press **Find Fraction**. A rack image appears next to the chromatogram.

Figure 3-16. Find Fraction Dialog

5. To select a single fraction, either select it in the chromatogram or use the **Previous** and **Next** buttons. The corresponding collection vessel on the rack image is marked. It is also possible to select a collection vessel in the rack image to view the fraction in the chromatogram. (If several racks have been used, you can toggle the viewed rack by pressing the **<<** and **>>** buttons.)
6. To select a peak, press **PeakMode On** and select a fraction in the peak. (The **PeakMode** button is disabled when the collection mode Collect All or Manual has been used).
7. To select several fractions, select one of the fractions and then expand the selection by using the **Left +** and **Right +** buttons. The corresponding collection vessels on the rack image are marked. To reduce a selection, use the **Left -** and **Right -** buttons.
8. To change the graph view, use the **Zoom In**, **Zoom Out**, **Scroll Left** and **Scroll Right** buttons. For example, if the chromatogram displays many fractions close together, you can magnify (zoom in) an area of interest. You can also toggle the horizontal axis (the x-axis) unit between CV (Column Volumes), minutes and mL by repeatedly pressing **Toggle Unit**.

Color Legend

The colors used in the rack image are:
- Grey = The vessel has not been used for the viewed purification run.
- White = The vessel contains a fraction.
- Red, green, blue or yellow = The vessel is selected. (The vessel color corresponds with the fraction color in the chromatogram.)

3.8 View System Status and Results from Your Office

With the system connected to your network, users can access the SP Remote Viewer through a standard web browser.

The SP Remote Viewer quickly tells you:

- If the instrument is occupied.
- If the instrument is still processing or there is a problem that requires manual attention.
- The results of your purification runs.

To access the SP Remote Viewer:

1. Enter the URL *http://MACHINENAME* in a web browser (where MACHINENAME is the network identity of the system, or an IP-address).
2. Press **Enter** and the SP Remote Viewer is loaded. The page is automatically updated every five seconds.

NOTE

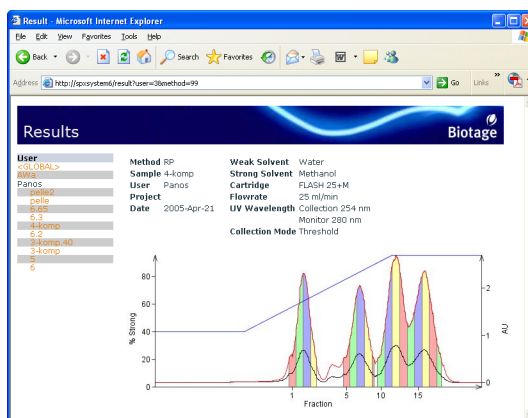**If you need help accessing the Remote Viewer, contact your IT administrator.**

Figure 3-17. SP Remote Viewer

3.9 Liquid Handling

3.9.1 Assign Solvent Types 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 solvents used in the method.

1. Select the **Solvents** tab in the right-hand panel.
2. Press one of the four **Solvent Channel** fields. The **Solvent Channel** dialog opens.
3. At the **Solvent** tab, select the solvent that you wish to assign to that inlet. The software comes with a preconfigured list of solvents and their parameters. You may add other solvents to this list, and edit user defined solvents:
    a. To add a solvent, press **New...**.
    b. To edit a solvent, select the solvent that you want to edit and press **Edit...**. To scroll the list down or up, press ▼ or ▲.
    c. In the **Solvent Editor**, enter/edit the solvent parameters. The solvent parameters are 1) the solvent name, 2) the solvent strength value - a value between 0 and 1, and 3) the minimum time, in seconds, for the pump to be refilled.
    d. To save, press **OK**.
4. If the **Capacity** tab is available, enter the capacity of the solvent reservoir.*
5. If the **Current Volume** tab is available, enter the reservoir's current solvent level.*
6. To save, press **OK**.
7. Repeat step 2 through 6 for the other three inlets.

   * These values are used by the system to track reservoir capacity. To enable this feature, see "Turn On and Off Solvent and Waste Monitoring" on page 2-12.
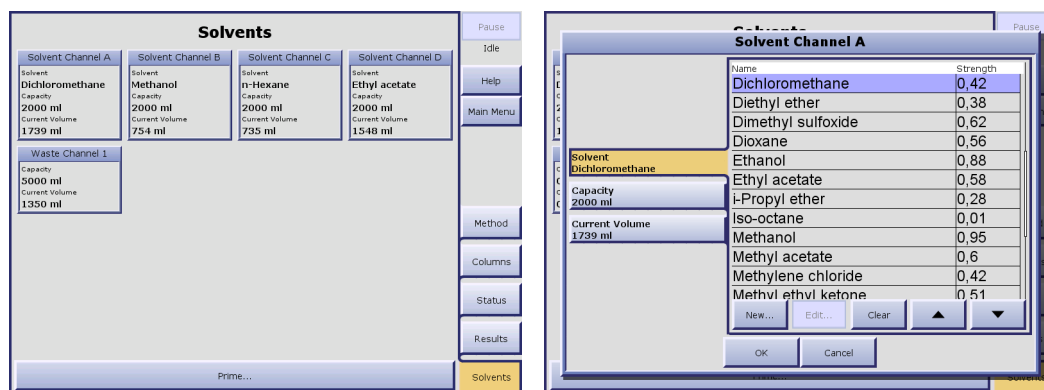

Figure 3-18. Solvents Tab (SP1 System Shown)

3.9.2 Set the Reservoir Volumes

If the monitoring of solvent and/or waste levels is turned on (see "Turn On and Off Solvent and Waste Monitoring" on page 2-12), 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 solvent.

1. Select the **Solvents** tab in the right-hand panel.
2. Press the applicable **Channel** field. The **Channel** dialog opens.
3. At the **Capacity** tab, enter the capacity of the reservoir.
4. At the **Current Volume** tab, enter the reservoir's current level
5. To save, press **OK**.
6. To enter values for another reservoir, repeat step 2 through 5.

3.9.3   Prime the System

Before you start a purification run on your SP 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.

<div align="center">

WARNING

Never prime the system without a column mounted on the system.

</div>

To prime the system:

1. Make sure that the column (compression module and cartridge), and solvent and waste reservoirs that you want to use are mounted on the system.
2. Ensure that a sufficient quantity of the correct solvent type is present in each solvent reservoir and that the waste reservoir has sufficient capacity for the prime.
3. Select the **Solvents** tab in the right-hand panel.
4. Make sure that the solvents are assigned accurately to the solvent inlets. If the capacity and current volume values are available for the waste and/or solvent reservoirs (i.e. the monitoring of levels is turned on), check that values are correct. To make changes, see "Assign Solvent Types to the Solvent Inlets" on page 3-23 and "Set the Reservoir Volumes" on page 3-23.
5. Press **Prime...**. The **Prime Parameters** dialog opens.
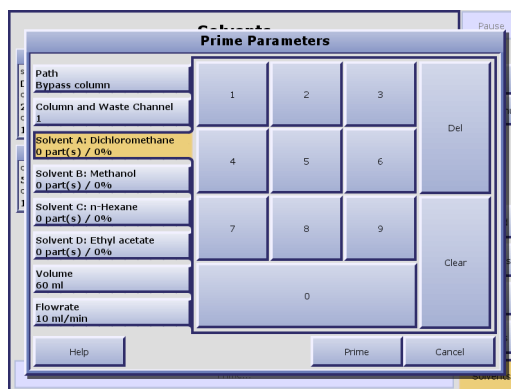

Figure 3-19. Prime Parameters Dialog (SP4 System Shown)

6. If using an SP4 system, select the column and waste reservoir to be used at the **Column and Waste Channel** tab.
7. At the **Path** tab, select the prime path. To only prime the inlets for the used solvents and not the column, press **Bypass column**.
8. Enter how many parts of each solvent mounted on the system that shall be used in the prime. (If several solvents are listed and you only want to use one solvent, enter "1" for the solvent that you wish to use and "0" for the other solvents.)
9. At the **Volume** tab, enter the total prime volume.
10. At the **Flowrate** tab, enter the flowrate.
11. To start priming, press **Prime**.

Chapter

4

Maintenance

WARNING

Before working on the Biotage SP system, read and observe the safety requirements in the "Safety and Quick Start" document (P/N 09715). Failure to follow the safety requirements may result in personal injury and/or equipment damage. If the equipment has been damaged and does not function properly, shut down the system safely and contact Biotage immediately.

4.1 Contact the Biotage 1-Point Support Team

If your system has been damaged and does not function properly, shut down the system safely and contact your local Biotage 1-Point Support Team:

E-Mail: 1-pointsupport@biotage.com (US) or 1-pointsupport@eu.biotage.com

Telephone: see www.biotagedcg.com

4.2 Accessories and Spare Parts

To order accessories and spare parts, please see the Biotage Product Catalog. The catalog can be downloaded at www.biotagedcg.com.

4.3 FLASH Collector

4.3.1 Collection Arm Guide bar

It is recommended that the guide bar be cleaned and lubricated every 100 hours of operation or if the Collection Arm shows signs of gradual misalignment during normal operation.

1. Shut down the system as described in "Shut Down the System" on page 3-2.
2. Turn the FLASH Collector off and unplug the power cord from the back of the unit.
3. Wipe the guide bar clean with a clean, lint-free cloth.
4. Apply a small amount of oil-free fluoropolymer lubricant (P/N 08440) to a clean, lint-free cloth and wipe onto the guide bar.

Collection Arm Guide Bar

Figure 4-1. Collection Arm Guide Bar

4.4 UV Detector

The UV Detector has few requirements for maintenance. However, it is necessary to keep it clean and protect it from dust and chemical spills. Particular attention should be paid to preventing the UV cell from leaking. Absolute cleanliness of all optical components is necessary for correct operation. Avoid contamination of the optical components, particularly the photodiode window, cell windows, beam splitter, and lamp.

WARNING

UV light is harmful to human eyes. It is imperative that the UV Detector be operated with its covers properly attached to prevent injury from UV light.

NOTE

If the UV lamp fails to ignite after four or more tries of turning the lamp on, contact your local Biotage 1-Point Support Team.

4.4.1 Clean the Flow Cell

A dirty cell has decreased transmissivity, which causes increased noise level and difficulties to perform UV zero.

- For **minor contamination** of the cell windows, clean by flushing with a series of miscible solvents through it. Select the solvents based on the contamination. It is possible to use both organic and inorganic solvents and diluted solutions of acids (e.g. H2SO4 or HNO3 diluted with distilled water in a ratio of 1:20 to 1:10), unless they attack stainless steel or silica (sapphire) windows.
- For **major contamination**, cleaning must be performed at the factory. Please contact the Biotage 1-Point Support Team for return instructions.

To clean the UV Detector flow cell:

1. If using the Variable Wavelength UV Detector:
   While placing one hand under the flow cell (to prevent it from falling), pull outward on the retaining latch to unlock the flow cell, and then carefully remove the flow cell from the unit:

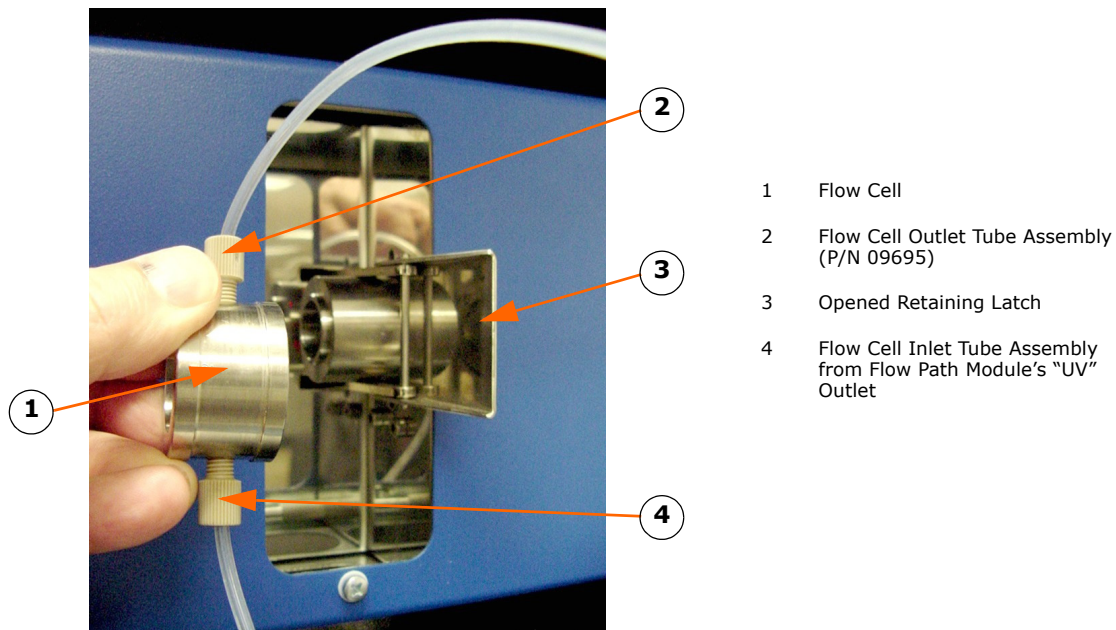|	
1	Flow Cell
2	Flow Cell Outlet Tube Assembly (P/N 09695)
3	Opened Retaining Latch
4	Flow Cell Inlet Tube Assembly from Flow Path Module's "UV" Outlet

Figure 4-2. Cleaning the Variable Wavelength UV Detector Flow Cell

2. If using the Fixed Wavelength UV Detector:
    While placing one hand under the flow cell (to prevent it from falling), loosen the retaining latch screw, unhook the retaining latch and then carefully remove the flow cell from the unit:
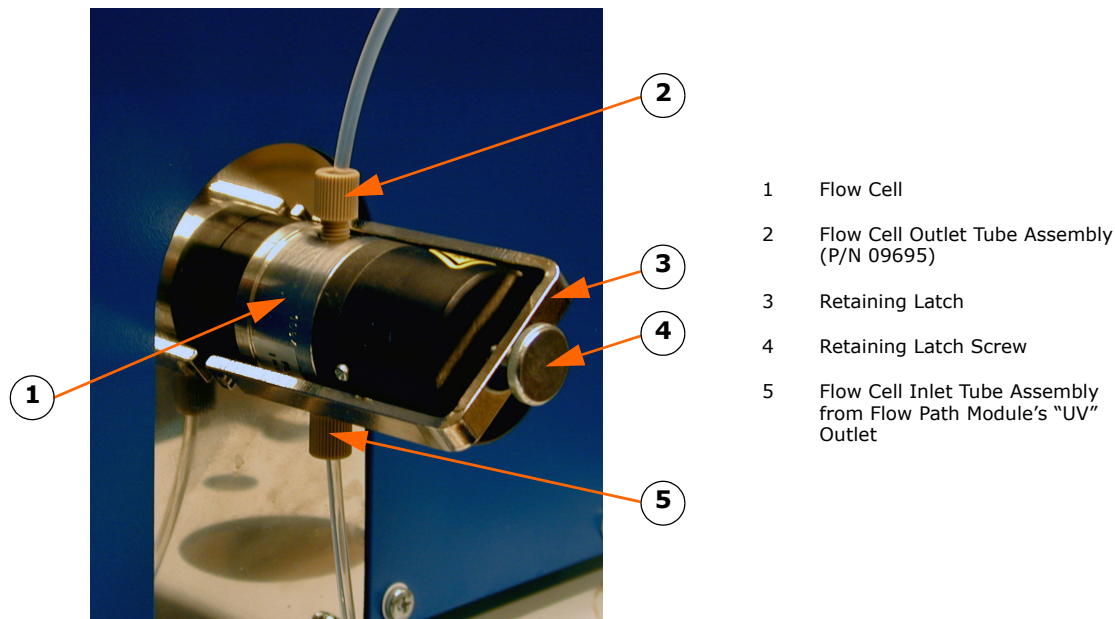

1	Flow Cell
2	Flow Cell Outlet Tube Assembly (P/N 09695)
3	Retaining Latch
4	Retaining Latch Screw
5	Flow Cell Inlet Tube Assembly from Flow Path Module's "UV" Outlet

Figure 4-3. Cleaning the Fixed Wavelength UV Detector Flow Cell

3. Disconnect the tube assemblies from the flow cell.
4. Flush the flow cell with a series of miscible solvents. 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 or silica (sapphire) windows.
5. Reconnect the tube assemblies to the flow cell.
6. Carefully replace the flow cell and the retaining latch.

NOTE

If using the Variable Wavelength UV Detector, note that the retaining latch should close with little effort. If the retaining latch is difficult to close, the flow cell is probably misaligned.

4.4.2   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 3-24), 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 but also in other parts of the system. A typical case is a change from certain buffers to methanol-containing mobile phases. For example, to change from an organic mobile phase to aqueous solutions, it is necessary to flush the whole system with acetone or isopropanol.

4.4.3 UV Detector Cooling Fan(s)

Prolonged UV Detector operation without proper cooling can damage the unit. Visually check the operation of the fan(s) regularly. If a cooling fan fails, turn off the UV Detector as soon as possible to avoid damage, and then contact your local Biotage 1-Point Support Team for instructions.
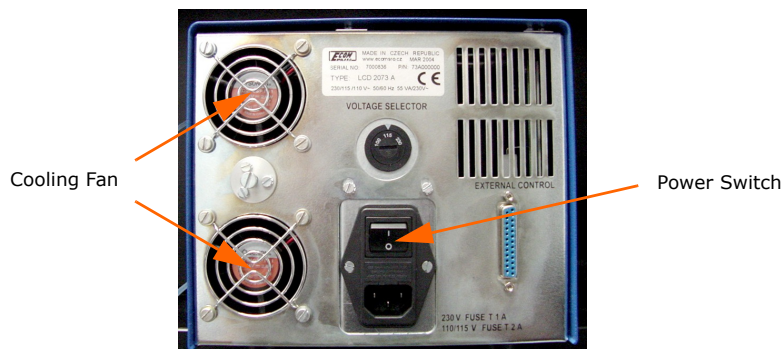

Cooling Fan

Power Switch

Figure 4-4. UV Detector Cooling Fans (Variable Wavelength Model Shown)

4.5 Pump Module

4.5.1 Flush the Pump Check Valve

The pump check valve (P/N 05516) should be flushed every 3 months or 50 hours of operation, whichever comes first. Flush with 100 mL of methanol and then with 100 mL of a 50% methanol/ethyl acetate mixture.

Use the following procedure to flush the pump check valve:

1. Prepare 100 mL of methanol and 100 mL of a 50% methanol/ethyl acetate mixture.
2. Place the solvent inlet A line into the methanol. (Solvent inlet letters are marked at the rear of the Pump Module.)
3. Ensure that a suitable waste reservoir is in place to receive the flushing solvent.
4. At the **Solvents** tab, press **Prime...**. The **Prime Parameters** dialog opens.
5. If using an SP4 system, select the column and waste reservoir to be used at the **Column and Waste Channel** tab.
6. At the **Path** tab, press **Bypass column**.
7. Enter "1" at the **Solvent A** tab and "0" for any other solvent that is listed (solvent B, C and/or D). If no solvent is assigned to solvent inlet A, see "Assign Solvent Types to the Solvent Inlets" on page 3-23.
8. At the **Volume** tab, set the prime volume to 100 mL, i.e. enter "100".
9. At the **Flowrate** tab, set the flowrate to 50 mL/min, i.e. enter "50".
10. To start flushing the pump check valve, press **Prime**. The pump will stop after 100 mL has been pumped.
11. When the system has stopped flushing, remove the solvent A inlet tubing from the methanol and place it in the methanol/ethyl acetate mixture (prepared in step 1).
12. Repeat step 3 through step 10 for the methanol/ethyl acetate mixture.
13. When the system has stopped flushing, place the inlet A tubing into its configured solvent.
14. Repeat step 3 through step 10 to flush the system using the configured inlet A solvent.

4.6 Solvent Inlet Filters

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.

The Biotage filter part number is (P/N 09376 – quantity of one), and one filter is installed on the solvent end of each of the four solvent inlet lines.

NOTE
Only finger-tighten the solvent inlet filter connections.
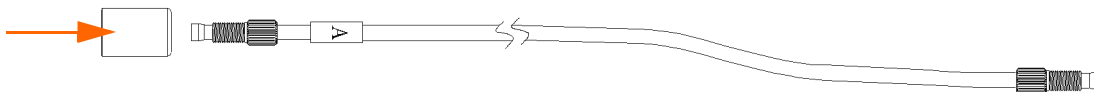

Figure 4-5. Solvent Inlet Filter

4.7 Leaks

If leaks are observed, pause the system (if the system is processing a run) and check for loose fittings or damaged tubing. Use caution when tightening fittings to prevent stripped threads or crushed ferrules. Replace damaged tubing.

4.7.1 Assembling Tubes Correctly

It is important to understand how to install the two different types of nuts and ferrules used on the SP system: 1) compression nut and double ferrule, and 2) flangeless nut and single ferrule.

Compression Nut and Double Ferrule

A compression nut and ferrule, as shown in Figure 4-6, is used on tubing for the injection valve and compression modules. Please note the presence of a main ferrule and a backing ferrule. Proper sealing will not occur unless both ferrules are oriented as shown below, with the tapered portion of the ferrules facing **away** from the nut.

Note that the tubing should extend 3 mm (1/8") past the end of the main ferrule and the backing ferrule should be against the back side of the main ferrule. They are shown separated in Figure 4-6 in order to show both ferrules clearly.
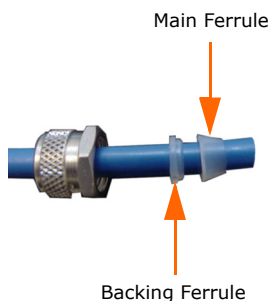

Figure 4-6. Compression Nut and Double Ferrule

Flangeless Nut and Single Ferrule

A flangeless nut and ferrule, as shown in Figure 4-7, is used on tubing for the Pump Module, UV Detector, and UV collect valve. Please notice that the ferrule is a single component. Proper sealing will not occur unless it is oriented as shown in Figure 4-7, with the tapered portion of the ferrule facing **toward** the nut. The ferrule should be placed near the end of the tubing as shown below.

Figure 4-7. Flangeless Nut and Single Ferrule

4.8 Replace Fuses

4.8.1 Fuse Specifications

Refer to "Specifications" on page 1-15 for fuse sizes and types.

4.8.2 UV Detector AC Input Fuse Replacement Procedure

1. Turn the UV Detector off and unplug the power cord from the back of the unit.
2. Loosen the hinged fuse holder cover by carefully prying under the bottom notch of the cover with a small standard (flat blade) screwdriver. See Figure 4-8:

Figure 4-8. Loosening the Hinged Fuse Holder Cover

3. Once the hinged fuse holder cover is loose, use your fingers to carefully pull it outward until it stops. See Figure 4-9:
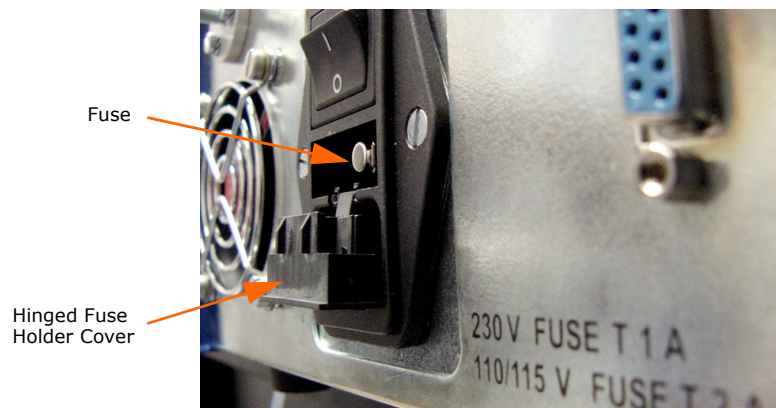

Figure 4-9. Hinged Fuse Holder Cover in the Open Position

4. Remove the fuse from the fuse holder.
5. Place a good fuse of the correct type and rate (see the **WARNING** note below) into the fuse holder and carefully close the hinged fuse holder cover.

WARNING

Fire hazard; always replace with the same type and rate fuse only. Do not remove equipment covers. There are no other user-replaceable parts inside the unit. If the equipment is damaged or does not function properly, contact your local Biotage 1-Point Support Team for repair information.

4.8.3 FLASH Collector Fuse Replacement Procedure

1. Shut down the system as described in "Shut Down the System" on page 3-2.

2. Turn the FLASH Collector off and unplug the power cord from the back of the unit. (You will not be able to open the hinged fuse holder cover if the power cord is plugged into the FLASH Collector.)

3. Loosen the hinged fuse holder cover by carefully prying in one of the notches on the right end of the cover with a small standard (flat blade) screwdriver. See Figure 4-10:
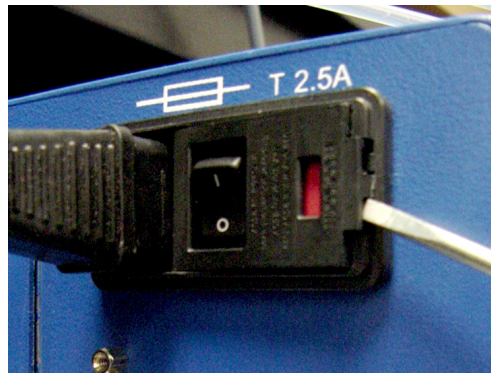

Figure 4-10. Loosening the Hinged Fuse Holder Cover

4. Open the hinged fuse holder cover and then loosen the red slide-out fuse holder by carefully prying in the notch on the right side of the holder with a small standard (flat blade) screwdriver. See Figure 4-11:
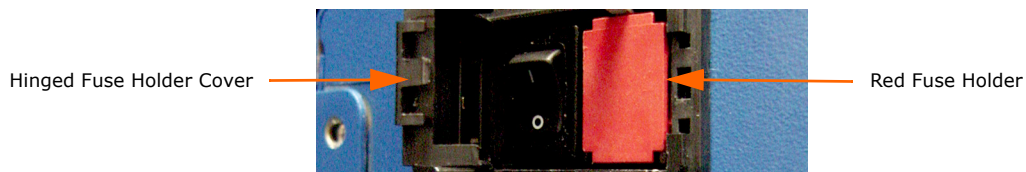

Hinged Fuse Holder Cover

Red Fuse Holder

Figure 4-11. Loosening the Red Fuse Holder

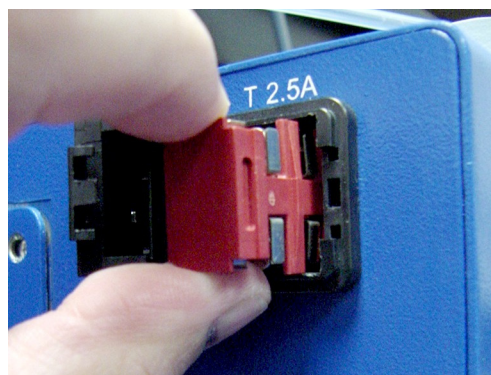5. Grasp the red fuse holder with your fingers and pull it out of the unit. See Figure 4-12:

Figure 4-12. Removing the Red Fuse Holder

6. The two fuses are clipped into the red fuse holder. Replace them with good fuses of the same type and rate (see the **WARNING** note below).

<div align="center">

WARNING
</div>

Fire hazard; always replace with the same type and rate fuse only. Do not remove equipment covers. The FLASH Collector uses double-pole fusing (both sides of the line are fused). There are no other user-replaceable parts inside the unit. If the equipment is damaged or does not function properly, contact the Biotage 1-Point Support Team for repair information.

7. Put the red fuse holder back in place and carefully close the hinged fuse holder cover.

4.9 Clean the Exterior of the System

Should it become necessary to clean the outside of the instrument and/or the Touch Screen:

1. Shut down the system as described in "Shut Down the System" on page 3-2.
2. Disconnect the two power cords from their power outlets.
3. To clean the Touch Screen, see the Touch Screen supplier recommendations in section 4.9.1.
4. To clean the exterior of the instrument, clean the surfaces 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.

<div align="center">

NOTE
</div>

Avoid harsh cleaners and chemicals, and getting moisture inside the unit.

4.9.1 Touch Screen Recommendations

Regular cleaning, if performed properly, extends the Touch Screen life and protects it from contaminants that cause unnecessary wear. The Touch Screen supplier recommends the following:

*   Keep all abrasive materials away from the Touch Screen.
*   Always turn the unit off before cleaning the screen.
*   The preferred way to clean the screen is by wiping with a clean, non-abrasive, **dry** cloth. If this does not clean the screen properly, then you may apply a small amount of **non-ammonia** based window cleaner to a clean, non-abrasive cloth and then wipe the screen with the damp cloth. The cleaning liquid must be applied to the cloth only.

<div align="center">

WARNING
</div>

Use only non-ammonia based cleaner and do not apply the liquid directly to the screen as this could introduce liquid into the Touch Screen electronics section, which could damage electronic components.

<table>
<tr><td>Chapter
5</td><td># Troubleshooting</td></tr>
</table>

5.1 Contact the Biotage 1-Point Support Team

If your problem persists, or for assistance at any time during troubleshooting, contact your local Biotage 1-Point Support Team:

E-Mail: 1-pointsupport@biotage.com (US) or 1-pointsupport@eu.biotage.com

Telephone: see www.biotagedcg.com

5.2 Accessories and Spare Parts

To order accessories and spare parts, please see the Biotage Product Catalog. The catalog can be downloaded at www.biotagedcg.com.

5.3 Compression Module-Related Problems

Problem	Possible Causes	Solution
1. Solvent leaks from the compression module.	a. Tube fittings are not tight and leaks occur at the inlet or outlet of the compression module.	Check tube fittings and tighten. See "Assembling Tubes Correctly" on page 4-5.
	b. Tube fittings or tube assembly defective.	Replace tube fittings or tube assembly. See "Assembling Tubes Correctly" on page 4-5.
	c. The compression module ZIF head was not seated properly.	Loosen and re-tighten the ZIF head to 1 to 1.5 turns past finger-tight.

5.4 Gradient Problems

Problem	Solution
1. Low composition gradient is not correct.	When generating solvent mixtures where the composition contains less than 10% of one solvent, it is recommended that you premix the solvent using the final % strong solvent desired in the weak solvent and use this as the solvent B. Program the gradient from 0 – 100% B using the pre-mixed B solvent. See "Create, Open and Edit Methods" on page 3-2.

5.5 FLASH Collector-Related Problems

Problem	Possible Causes	Solution
1. The FLASH Collector is inoperable.	a. No electrical power to the FLASH Collector.	1. Check that the power cord is plugged in. 2. Check the fuses. See "Replace Fuses" on page 4-6. See the **FUSE REPLACEMENT WARNING** note below this table.
	b. The Touch Screen is off.	1. Press the System Start-Up push button, on the front of the FLASH Collector, to power up the unit. 2. Check that the 12 VDC power cable is connected between the FLASH Collector and the screen. To plug in the cable: a) Shut down the system, i.e. perform step 1 through step 7 on page 3-2. b) Plug in the power cable. c) Start up the system, see page 3-1.
2. The Collection Arm does not position correctly over each collection vessel.	a. Misaligned block(s) and/or rack.	Ensure that the blocks and/or rack is/are aligned correctly.
	b. Wrong rack selected in the software.	Check the rack configuration for the current method. See "Create, Open and Edit Methods" on page 3-2.
	c. The Collection Arm is obstructed.	1. Remove any obstacles that obstruct or restrict arm movement. 2. Log into the system mode (see page 2-1) and press **Initialize Arm** at the **Diagnostics** tab. Note that only users with system owner privilege can log into the system mode.
	d. Improper calibration of the Collection Arm.	Please contact your local Biotage 1-Point Support Team.
3. "xxx" alarm message appears on the screen.		See "Alarm Messages" on page 5-6 for recommended corrective action.

FUSE REPLACEMENT WARNING

Fire hazard; replace with the same type and rate fuses only. See "Specifications" on page 1-15 for fuse specifications. The FLASH Collector uses double-pole fusing (both sides of the line are fused). There are no other user-replaceable parts inside this unit. If the equipment is damaged or does not function properly, contact Biotage for repair information.

5.6 Pump-Related Problems

Problem	Possible Causes	Solution
1. Air bubbles moving through the compression module outlet tubing in a steady stream during solvent delivery. Note that saturation of a cartridge takes approximately 3 Column Volumes.	a. Little or no solvent in the lines.	1. Replenish the solvent reservoir(s). 2. Ensure that the solvent inlet lines are submerged in solvent. See "Prime the System" on page 3-24.
	b. Loose compression module inlet or outlet fittings.	Check fittings, loosen and retighten. See "Assembling Tubes Correctly" on page 4-5.
	c. The solvent inlet or outlet tubing is not seated correctly.	1. Check solvent tubing connections at the rear of the Pump Module. Unscrew one nut at a time and ensure that the end of the tubing is flush with the reverse ferrule. The nuts should be finger-tight. See "Assembling Tubes Correctly" on page 4-5. 2. See Problem 4, which discusses low pump delivery volume.
	d. One or more solvent inlet filters are plugged.	Replace the solvent inlet filters (P/N 09376). See "Solvent Inlet Filters" on page 4-4. Re-circulating the solvent is not recommended. Particulate-free solvent is required.
2. Air is drawn into a FLASH Collector dispensing line during the refill stroke (for a distance greater than 15 centimeters/6 inches).	The pump check valve is not functioning properly.	Remove any particles in the check valve by flushing it. See "Flush the Pump Check Valve" on page 4-4.
3. Air is observed in a pump inlet line after each stroke.	a. One or more solvent lines are not submerged in solvent.	Ensure that all lines are submerged. See "Prime the System" on page 3-24.
	b. The pump check valve is not functioning properly.	Remove any particles in the check valve by flushing it. See "Flush the Pump Check Valve" on page 4-4.
	c. One or more pump inlet feed lines are loose.	Tighten nuts at the rear of the Pump Module.
	d. Solvent inlet filters need to be cleaned or replaced.	Clean or replace the solvent inlet filters (P/N 09376). See "Solvent Inlet Filters" on page 4-4.
4. Pump delivery volume is very low or inconsistent (20-50 percent low).	a. The pump check valve is not functioning properly.	1. Flush the pump check valve. See "Flush the Pump Check Valve" on page 4-4. 2. Check for leaks in the tubing or fittings. See "Leaks" on page 4-5. 3. If the problem persists, the pump piston seal or the check valve seal may be worn. Please contact your local Biotage 1-Point Support Team.
	b. The pump check valve is not functioning properly due to particulates in the solvent.	1. Remove any particles in the check valve by flushing it. See "Flush the Pump Check Valve" on page 4-4. 2. If the problem persists, please contact your local Biotage 1-Point Support Team.

Problem	Possible Causes	Solution
	c. Solvent vaporization during refill stroke.	1. Elevate the solvent reservoir(s) 30 centimeters or more (12 or more inches) above pump head. 2. Clean or replace the solvent inlet filters (P/N 09376). See "Solvent Inlet Filters" on page 4-4.
5. The pump does not deliver solvent.	a. No or insufficient solvent in the reservoir(s).	Replenish the solvent reservoir(s).
	b. The pump check valve is plugged.	Remove any particles in the check valve by flushing it. See "Flush the Pump Check Valve" on page 4-4.
	c. Blockage in solvent line(s).	1. Check the solvent inlet filters. See "Solvent Inlet Filters" on page 4-4. 2. Flush the entire system with methanol or isopropanol at a low flowrate. See "Prime the System" on page 3-24.
	d. Loose pump feed line(s).	Tighten the lines.

Table 5-1: Solvent Pumping Guide (Vapor Pressure at 20°C)

Solvent	PSI (20°C)	mm Hg
Water*	0.3	15.5
Toluene*	0.4	20.7
Isopropanol*	0.6	31.0
Ethanol*	0.8	41.4
Heptane*	0.8	41.4
Isooctane*	0.8	41.4
Acetonitrile*	1.4	72.4
Ethyl acetate*	1.4	72.4
Methanol*	1.8	93.1
Hexane*	2.5	129.3
Tetrahydrofuran*	2.5	129.3
Chloroform*	3.0	155.1
Acetone*	3.5	181.0
Dichloromethane**	6.6	341.3
Pentane***	8.1	418.9
Diethyl ether***	8.4	434.4
* Easily pumped solvents under normal conditions.		
** Reservoir elevation suggested, not required, under normal conditions.		
*** Reservoir elevation is strongly recommended. Maximum flowrate is 50 mL/min.		

NOTE

If using highly volatile (i.e. high vapor pressure) solvents such as pentane, reservoir elevation is strongly recommended. It is also highly advisable to limit the flowrate to a maximum of 50 mL/min and mount the solvent on solvent channel A or B.

5.7 Touch Screen

5.7.1   Calibrate the Touch Screen

If the Touch Screen needs to be recalibrated, use the following procedure:

1. Connect a Windows XP compatible mouse either to one of the SP system's USB ports, or to the SP system's mouse port.

2. If you are not at the main menu, press **Main Menu** in the right-hand panel and then press **Shut Down**.

3. When the SP software has shut down, double-click the Elo icon ( ) in the task bar. The **Elo Touchscreen Properties** dialog opens.

4. Click the button. Read and follow the instructions that appear on the screen.

WARNING

To avoid possible injury and equipment damage, keep hands away from the collection rack area while the homing routine runs in step 5 below.

5. When you are done, double-click the SPX icon on the desktop to start up the SP software. The Collection Arm moves through its homing routine and the system boots to the system's main menu.

6. Disconnect the mouse.

5.8 Alarm Messages

If an alarm cannot be cleared and operation resumed, contact you local 1-Point Support Team.

Alarm Message Title	Possible Causes	Solution
Arm Movement Failure	The system failed to move the Collection Arm.	1. Ensure that there is no obstacle blocking the Collection Arm. 2. To retry performing the task, press **Retry** in the alarm dialog.
Empty Waste Container	One or more waste reservoirs are full.* Note that this alarm will only occur if the monitoring of waste level(s) is turned on (see page 2-12). * SP4 systems have four waste reservoirs.	1. Press **OK** in the alarm dialog. 2. Empty or replace the waste reservoir(s). 3. Select the **Solvents** tab and enter the new reservoir volume(s). See "Set the Reservoir Volumes" on page 3-23. 4. To resume the run, press **Resume** in the right-hand panel.
Incorrect UV Detector Type Detected	a. The UV Detector Model is not specified.	1. Press **OK** in the alarm dialog. 2. Log into the system mode, see page 2-1. Note that only users with system owner privilege can log into the system mode. 3. Select the **General** tab in the right-hand panel. 4. Press **Change** in the **UV Detector Type** field repeatedly until the correct detector model is shown.
	b. The UV Detector is not turned on.	1. Press **OK** in the alarm dialog. 2. To plug in the UV Detector power cord: a) Shut down the system, i.e. perform step 1 through step 7 on page 3-2. b) Plug in the power cord. c) Start up the system, see page 3-1. 3. Turn the UV Detector on. (The power switch is located at the rear of the unit.)
	c. The UV Detector signal cable is defective or disconnected.	1. Press **OK** in the alarm dialog. 2. Shut down the system and unplug the two power cords from their power outlets, see page 3-2. 3. Plug in the cable or try a new cable (P/N 09324). (The UV Detector's **EXTERNAL CONTROL** port shall be connected to the FLASH Collector's **J1** port.) 4. Start up the system, see page 3-1.
	d. The UV lamp is defective.	The UV lamp must be replaced. Contact your local Biotage 1-Point Support Team.
Leak Detected	The Leak Detector (optional) reported a leak.	1. Check for leaks in the tubing or fittings. See "Leaks" on page 4-5. 2. To resume the run, press **Resume** in the alarm dialog.

Alarm Message Title	Possible Causes	Solution
Load New Block(s)	More fractions are to be collected than can fit in the allocated block(s).	1. Replace the block(s) according to the alarm dialog. 2. To resume the run, press **Resume** in the alarm dialog. The collection is resumed in the first vessel in the lowest lettered block that you selected. For example, if you selected blocks B and C (see Figure 3-10 on page 3-14), the collection is resumed in block B in vessel 1.
Overpressure Detected	a. Blockage in the column, tubing, or flow cell from precipitations or kinked tubing.	1. For an overpressure alarm due to kinked tubing: a) Press **Purge** in the alarm dialog. b) When the purge is done, straighten or replace the kinked tubing. For more information, see "Assembling Tubes Correctly" on page 4-5. c) To resume the run, press **Resume** in the alarm dialog. 2. For an overpressure alarm due to sample precipitation, see "Overpressure Alarm Procedure" on page 5-8. (Note that there may be precipitation in areas not visible.)
	b. The flowrate is too high for the type of cartridge.	1. Press **Purge** in the alarm dialog. 2. Press **Ignore** in the alarm dialog. The system is now paused. 3. If the overpressure occurred during a prime: a) Press **End Prime** in the **Prime** dialog. b) Start a new prime with a lower flowrate. 4. If the overpressure occurred during an equilibration: a) Remove the run by pressing **Remove** at the **Columns** tab. b) Open the method at the **Methods** tab. c) Lower the flowrate. d) Restart the run. 5. If the overpressure occurred during a gradient run: a) Edit the run by pressing **Edit** at the **Columns** tab. The **Gradient Editor** opens. b) Press **Next**. c) Lower the flowrate and press **OK**. d) To resume the run, press **Resume** in the right-hand panel.
Refill Solvent Container	One or more solvent reservoirs need to be refilled. Note that this alarm will only occur if the monitoring of solvent levels is turned on (see page 2-12).	1. Press **OK** in the alarm dialog. 2. Replenish the solvent reservoir(s). 3. Select the **Solvents** tab and enter the new reservoir volume(s). See "Set the Reservoir Volumes" on page 3-23. 4. To resume the run, press **Resume** in the right-hand panel.
Startup Problem Detected	A module (FLASH Collector, Pump Module, Flow Path Module) has failed its built-in test.	1. Press **OK** in the alarm dialog. 2. Log into the system mode, see page 2-1. Note that only users with system owner privilege can log into the system mode. 3. Correct the detected problem. See "View the System Status and Restore the System" on page 2-12.

5.8.1 Overpressure Alarm Procedure

For an overpressure alarm due to sample precipitation (note that there may be precipitation in areas not visible), use the following procedure:

1. Press **Purge** in the alarm dialog.
2. When the purge is done, press **Shut Down** in the alarm dialog.
3. When the SP software has shut down, visually inspect all tubing for precipitation. If found, remove and clean precipitate from tubing.
4. If you need to clean the flow cell, see "Clean the Flow Cell" on page 4-2.

WARNING

To avoid possible injury and equipment damage, keep hands away from the collection rack area while the homing routine runs in step 5 below.

5. Start up the SP software by double-clicking the SPX icon on the desktop. The Collection Arm moves through its homing routine and the system boots to the system's main menu.
6. Press **Chemistry**.
7. At the **Solvents** tab, press **Prime...**. The **Prime Parameters** dialog opens.
8. If using an SP4 system, select the column and waste reservoir to be used at the **Column and Waste Channel** tab.
9. Put the waste line in a clean reservoir of appropriate size to collect the effluent.
10. At the **Path** tab, press **Bypass column**.
11. Enter "1" for the strongest solvent, and "0" (zero) for all the other solvents. (To assign a solvent to a solvent inlet, see page 3-23.)
12. At the **Volume** tab, set the prime volume to 99 mL, i.e. enter "99".
13. At the **Flowrate** tab, set the flowrate to 5 mL/min, i.e. enter "5".
14. Ensure that a sufficient quantity of the solvent to be used is present in the solvent reservoir.
15. To start flushing, press **Prime**.
16. If it is necessary to flush the cartridge, repeat step 7 through step 15 with the following changes:
    - In step 10, press **Through column**.
    - In step 12, set the prime volume to twice (2x) the Column Volume (the value depends on the cartridge size used).
17. Once the system and/or cartridge are/is cleaned, it is recommended that a new method is created for the sample.

Chapter A

Slope Detection Collection

In addition to collecting fractions by volume and time, the SP system has a customizable UV peak collection feature. This feature allows the operator even greater flexibility for challenging purifications. The SP system can detect changes in slope, including shoulders and valleys, and fractionate using the detected changes.

This appendix discusses the application and use of the Slope feature and how it can be used in a chromatographic method.

Changes in UV absorbance produce peaks and occasional valleys on the chromatogram. Using the SP system's slope detection parameters, peaks can be collected or sent to the waste, depending on the settings chosen. A typical chromatogram containing UV absorbance changes is shown in Figure A-1. Slope is calculated by the SP system as follows:

$$\text{Slope} = \frac{\text{AU}_{\text{Current}} - \text{AU}_{\text{Previous}}}{\Delta t}$$

Where:

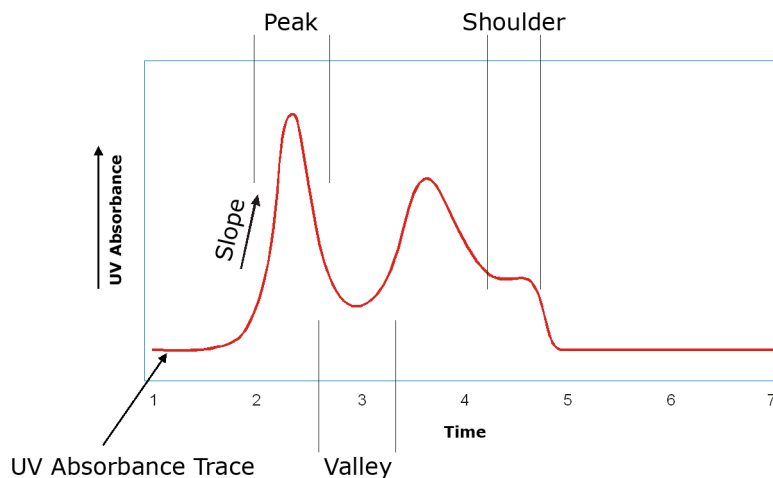|	
$\text{AU}_{\text{Current}} =$	Current value of UV absorbance as indicated by the Detector.
$\text{AU}_{\text{Previous}} =$	Previous value of UV absorbance as indicated by the Detector.
$\Delta t =$	Elapsed time from $\text{AU}_{\text{Current}}$ to $\text{AU}_{\text{Previous}}$.

Figure A-1. UV Absorbance Chromatogram Slopes

The SP system can be programmed to collect by UV threshold and to recognize peak, valley and shoulder slopes (see Figure A-1). The collection mode Slope is used to manually set the slope, threshold, and other conditions for optimizing a fractionation as described below:

Start Threshold (mAU): The Start Threshold setting is used to collect samples with an absorbance exceeding the set absorbance threshold in milli-Absorbance Units (mAU).

Shoulder Slope (mAU/sec): A shoulder is a peak partially resolved from the main peak and may be located on either the increasing or decreasing side of a peak. The value of the Shoulder Slope setting is normally set to a significantly lower value than the main slope (the Start Slope value). Two criteria must be met in order for a shoulder to be detected by the SP system:

1. The slope must change and its absolute value drop below the Shoulder Slope value.
2. The slope must then increase to twice (2x) the Shoulder Slope value.

Shoulder Slope Disable (mAU): The Shoulder Slope Disable is a level above which all peak collection criteria are based upon fraction volume, disregarding slope and slope change values. This value is typically set high (>1500 mAU) to prevent false fractionation due to noise as the UV Detector approaches UV absorption maximum.

Start Slope (mAU/sec): The rate of change of the UV absorbance per second.

Start Slope Enable (mAU): The Start Slope Enable setting is an absorbance level used to enable slope collection. Only peaks that are above the Start Slope Enable setting and that meet the Start Slope criteria will be collected. Peaks below the Start Slope Enable setting will be dispensed to the waste. For example, if a sample meets the Start Slope criterion, but the slope is below the Start Slope Enable threshold it will not be collected except when the Start Threshold value is lower than the Start Slope Enable setting.

Types of UV absorbance chromatogram slopes and SP system settings used to collect the slopes are described in the following sections.

A.1 Threshold Collection

In threshold collection, the sample is collected when the absorbance exceeds the preset UV threshold (the Start Threshold setting). In the example shown in the following figure, portions of peaks A and B are collected at and above the threshold. To collect more of each peak, a lower Start Threshold setting is required.
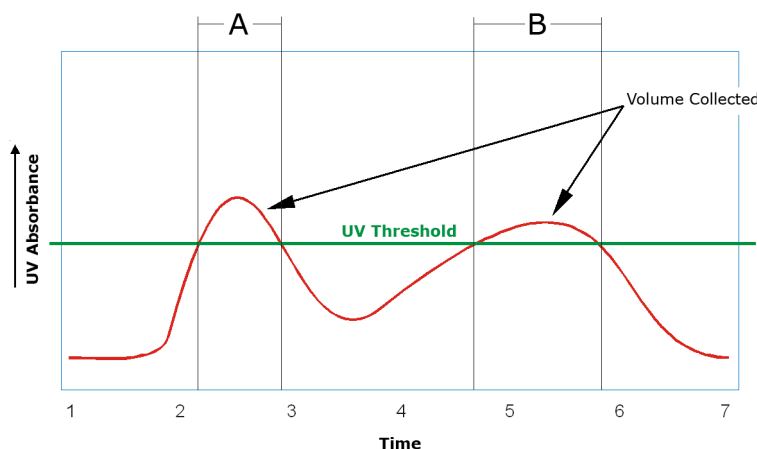

Figure A-2. Threshold Only Collection

A.2 Slope Collection

A.2.1   Medium and Low Slope Collection

The SP system includes two pre-programmed slope collection modes. The first setting, Medium Slope, is used for collecting fractions of relatively steep slope. In the example in the following figure, peak A (relatively high slope change) is collected, whereas peak B (lower slope change) is sent to the waste.
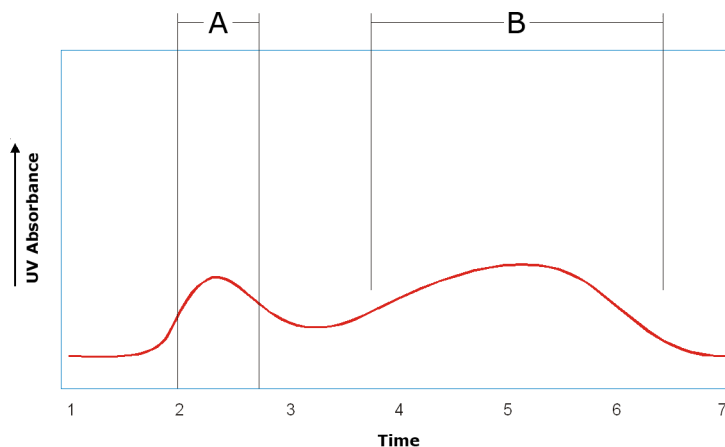

Figure A-3. Medium Slope Collection

The second pre-programmed setting is Low Slope. The example in the next figure shows fractionation of a lower slope value peak. The Low Slope setting enables the collection of both peak A and B.
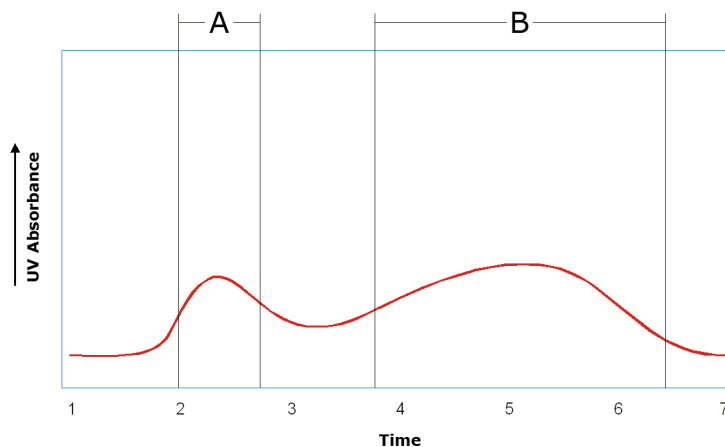

Figure A-4. Low Slope Collection

Table A-1: Low and Medium Slope Preset Default Values
(The default values are used to collect by slope and threshold.)

Parameter	Low Slope	Medium Slope
Start Threshold (mAU)	2000	2000
Shoulder Slope (mAU/sec)	1	1
Shoulder Slope Disable (mAU)	2000	2000
Start Slope (mAU/sec)	3	8
Start Slope Enable (mAU)	50	100

A.2.2   Start Slope Enable and Shoulder Slope Disable

Start Slope Enable and Shoulder Slope Disable thresholds allow even more specific fractionation. In the example shown in the next figure, fractionation based on slope occurs between the upper and lower limits set by the Start Slope Enable and Shoulder Slope Disable levels. Peak collection does not occur below Start Slope Enable. Peak collection above Shoulder Slope Disable occurs based on volume until the Shoulder Slope Disable level (threshold) is crossed again.
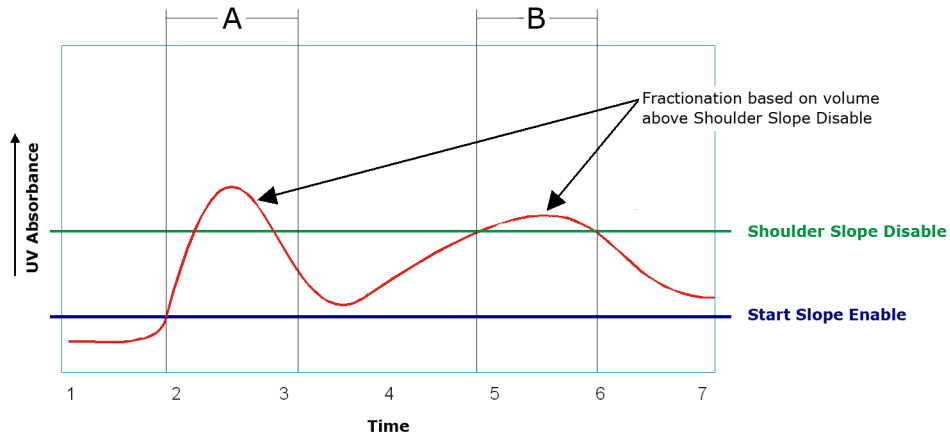

Figure A-5. Start Slope Enable and Shoulder Slope Disable

A.3 Shoulder Collection

A shoulder is a partially resolved peak of lower-magnitude than the main peak. The SP system has the ability to collect and fractionate shoulder peaks. The example shown in the following figure shows an example of a main peak (Peak A) and shoulder (Peak B). Peak A exceeds the Start Slope value and is collected. Peak B is not collected because a high value for Shoulder Slope has been used (>17 mAU/sec).
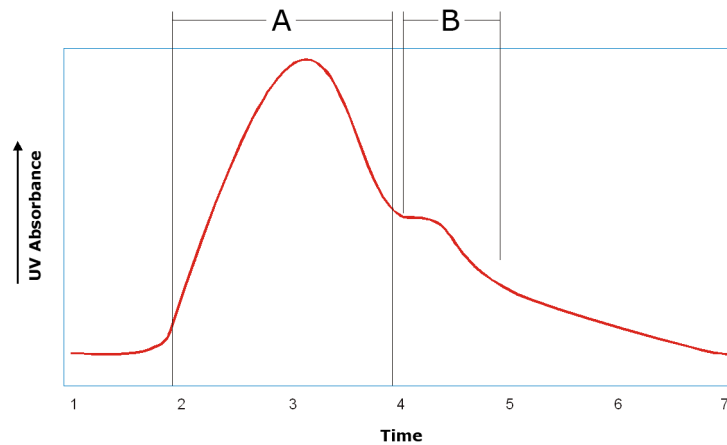

Figure A-6. Shoulder Slope Collection Off

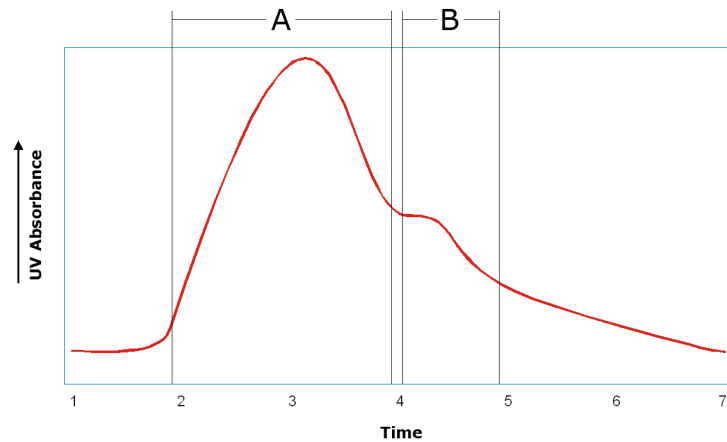The following figure shows the same slope where peak B is collected because a lower value has been used for Shoulder Slope (10 mAU/sec).

Figure A-7. Shoulder Slope Collection On