Grace Davison Discovery Sciences

Product Manual
Reveleris™ Flash Chromatography System

GRACE

NOTICES

This system is covered by a limited warranty. A copy of the warranty is included with this manual. The operator is required to perform routine maintenance as described herein on a periodic basis to keep the warranty in effect. For routine maintenance procedures, refer to Chapter 5.

All information in this manual is subject to change without notice and does not represent a commitment on the part of Grace Davison Discovery Sciences.

The system and various components in the system are the subject of pending US patents.

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Note: Changes or modifications not expressly approved by Grace Davison Discovery Sciences could void the user's authority to operate the equipment.

Grace Davison Discovery Sciences, Inc. is a product group of W.R. Grace & Co.-Conn. Alltech Associates is a wholly owned subsidiary of W.R. Grace & Co.-Conn.

Printed in the United States of America

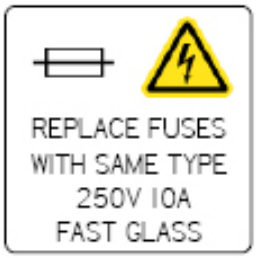
Labels on System

The following labels are affixed to the system:

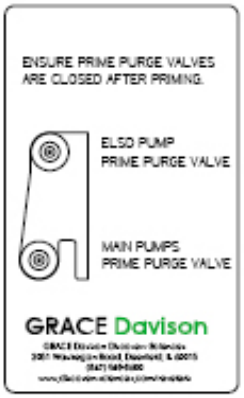
Serial Number Label

Safety Label for Lamp

Safety Label for Laser



Fuse Label



Purge Valve Label

Symbols used in this Manual

The Danger symbol indicates a hazardous situation that, if not avoided will result in death or serious injury.

The Warning symbol indicates a hazardous situation that, if not avoided could result in death or serious injury.

The Caution symbol with the safety alert symbol indicates a hazardous situation that, if not avoided could result in minor or moderate injury.

The Notice symbol is used to highlight information that will optimize the use and reliability of the system.

Important Safety Guidelines for the Reveleris™ Flash Chromatography System

Please read the following warnings and caution statements carefully before using the Reveleris™ Flash Chromatography System:

⚠WARNING This equipment must be used as specified by the manufacturer otherwise overall safety will be impaired

⚠WARNING If flammable solvents are to be used at any stage of the operation of the system, open flames must be prohibited in the facility.

⚠WARNING The operation of the instrument involves the use of potentially hazardous and/or poisonous solvents and the separation of potentially hazardous and/or poisonous samples. The operator should obtain an MSDS for all solvents and modifiers employed in the system.

⚠WARNING When poisonous or hazardous solvents or chemicals are used with the Reveleris™ instrument, it is possible that the concentration of the vapor may exceed the maximum exposure level recommended by OSHA Guide 1910.0000 (or other regulatory organizations). If the solvent vapor level may exceed this level, ensure that the system in placed in an appropriate fume hood. This protection should be in accordance with all applicable federal, state and local laws and regulations and in compliance with your organization's chemical safety program.

⚠WARNING Only use the power supply cord recommended by the manufacturer

⚠WARNING Make certain that the Reveleris™ system is powered down and the power cord from the unit is unplugged before the left side panel is removed.

⚠WARNING If the Reveleris™ system is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

⚠WARNING Any item that is connected to the Reveleris™ system via a data port must meet CE (or equivalent) certification requirements.

⚠WARNING The temperature of the laboratory should be maintained at least 25°C below the fire point of the solvents that are used for the separation. The quantity of flammable solvent that is kept in the immediate vicinity of the system should be minimized to prevent the spread of fire. All local and institutional regulations regarding the care and handling of flammable solvents as well as Good Laboratory Practies should be strictly obeyed.

⚠WARNING Do not use solvents with an autoignition temperature that is less than 25°C less than the highest ELSD temperature. The normal ELSD operating temperature is 30°C, but the thermofuses will open at 60°C, so solvents must have a minimum autoignition temperature of 85°C.

Table of Contents

1. INTRODUCTION

1.1 ABOUT THE REVELERIS™ SYSTEM

The Reveleris™ Flash Chromatography System is designed for the rapid purification of complex samples by liquid chromatography. A flash chromatographic separation is performed using a cartridge containing a stationary phase such as 40-63 µm silica and a mobile phase such as hexane: ethyl acetate (70:30) at a flow rate of 40 mL/min at a moderate pressure (typically less than 100 psi). Although the resolution of flash chromatography is less than that of analytical or preparative high performance liquid chromatography (HPLC), where 2-5 µm particles are employed, the technique is extremely useful as it provides the chromatographer with the ability to separate gram size samples in a short period.

In flash chromatography, as in analytical or preparative HPLC, the separation is a function of the relative interaction of the various compounds in the sample with the stationary phase and the mobile phase. Compounds with a stronger interaction with the stationary phase than with the mobile phase will be more strongly retained by the column and have a longer retention time (and vice versa). Typically, the chromatographer determines the general conditions for separation of the sample via thin layer chromatography (TLC). Although most flash chromatography separations are performed using a normal phase sorbent, a number of other stationary phases are available including C_{18}, Cyano, Diol and Amino. A detailed discussion of the nature of the separation and the benefit of each phase is presented in Section *7.5*.

The Reveleris™ Flash Chromatography system includes a UV detector that can monitor two wavelengths simultaneously as well as an evaporative light scattering detector (ELSD). The ELSD provides the ability to monitor all non-volatile compounds in the eluent and allows the user to monitor for compounds that do not absorb UV radiation. An additional benefit of the ELSD is the ability to employ mobile phases that absorb light at the same wavelength as the compound(s) of interest.

1.2 FEATURES OF THE REVELERIS™ FLASH CHROMATOGRAPHY SYSTEM

The Reveleris™ system includes a number of advanced features that are provided to enhance the utility of flash chromatography including:

- *Fraction collection can be based on any detector signal (UV1, UV2 or ELSD); this provides a superb control of the fractionation process and leads to a higher degree of purity of the collected compound(s).*
- *The fraction collector is configured with swappable fraction collection trays, extending collection capabilities and providing a more flexible system.*
- *Automatic sample loading can be performed on an unattended basis; you can set up a liquid or solid sample injection and walk away.*
- *The four solvent bottles with large capacity minimizes down time due to refilling mobile phases. Solvent reservoir tray is located on top of the system, thereby saving valuable laboratory space*
- *An exceedingly broad range of cartridges from Grace Davison Discovery Sciences and other manufacturers can be employed. If you have developed a method on a legacy system, it can be carried over to the Reveleris™ instrument with a minimum of effort.*

1.3 SYSTEM DESIGN

The Reveleris™ Flash Chromatography system is a computer controlled integrated system that includes the following:

- *Solvent Delivery System (Section 1.3.1)*
- *Sample Injection System (Section 1.3.2)*
- *Cartridge Assembly (Section 1.3.3)*
- *Detection System (Section 1.3.4)*
- *Fraction Collector (Section 1.3.5)*

A schematic diagram is presented in Figure 1-1.

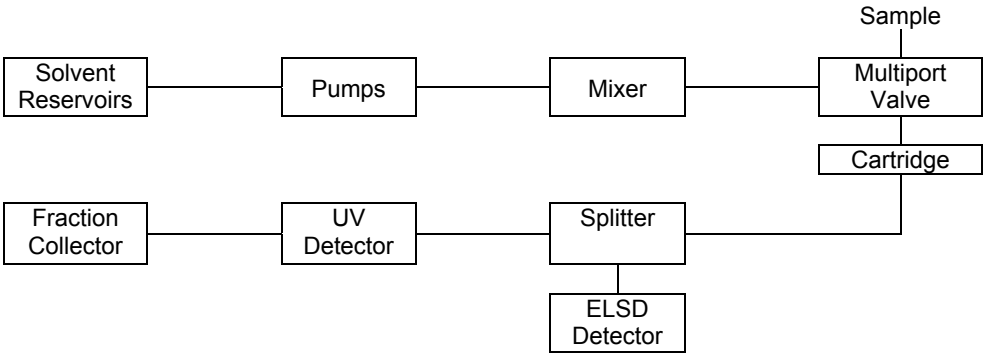


Figure 1-1: Schematic Design of Reveleris™ Flash Chromatography System

1.3.1 SOLVENT DELIVERY SYSTEM

A detailed diagram of the solvent delivery system is presented in Figure 1-3. The Reveleris™ instrument includes four solvent reservoirs with filter fittings that deliver mobile phase to the pumps. A fluid level sensor is included in each reservoir to detect when the solvent in a reservoir has been depleted and pause the separation. The pumps can deliver an isocratic mobile phase or a binary gradient mobile phase at a flow rate of 4-200 mL/min with a maximum pressure of 200 psi. A passive high-pressure mixer is included after the pumps to ensure that the gradient is thoroughly mixed and the pressurized mobile phase is delivered to the multi-port valve. In addition, a prime purge valve is included in the solvent delivery system to allow for priming and rapid delivery of solvent as required (e.g. to remove all air when starting up).

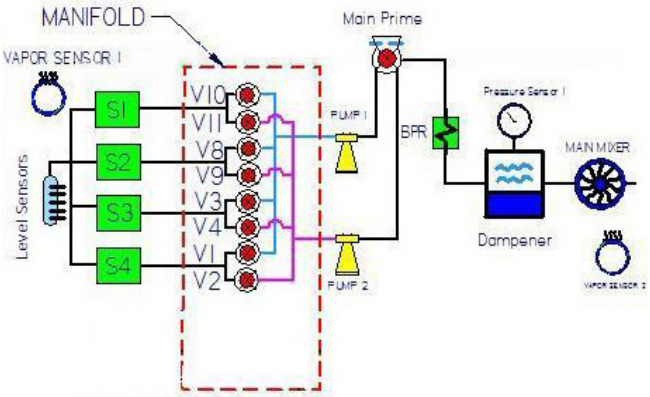


Figure 1-3: Solvent Delivery System (S=Solvent Bottle, V=Valve, BPR=Back Pressure Regulator)

The solvent management system allows the user to control the delivery of solvents. This system is used to generate the desired gradient. Level sensors are included to monitor solvent levels. If the solvent level is low, the sensor will pause the run and inform the user of the low solvent level or the system can be set up to automatically withdraw solvent from another reservoir.

⚠CAUTION The waste level and vapor sensors are safety devices designed to detect/prevent solvent spills and fire hazards. Do not disable or bypass these sensors.

In addition, the system will monitor the waste container to prevent over filling. The run will be paused if the sensor detects that the waste container is full. Sensors to monitor the vapor levels in and around the instrument are provided to protect against solvent leaks (the system is stopped when leak is detected).

1.3.2 SAMPLE INJECTION SYSTEM

The sample injection system (Figure 1-4) includes an interface to allow dry or liquid sample loading of the cartridge.

For samples that are readily soluble in the mobile phase, the sample is dissolved in the appropriate solvent and is injected on top of the column using a syringe (10 mL to 140 mL) while the flow is off. After the injection, the flow of mobile phase is used to transport the sample through the cartridge.

For samples that are <u>not</u> readily soluble in the mobile phase, the sample is adsorbed onto a solid phase (e.g. silica or alumina). The adsorbent with the sample is then placed in a cartridge (solid sample loader (5-70 g capacity)) in series before the column. The mobile phase flow begins and the sample is desorbed from the loader to the top of the cartridge.

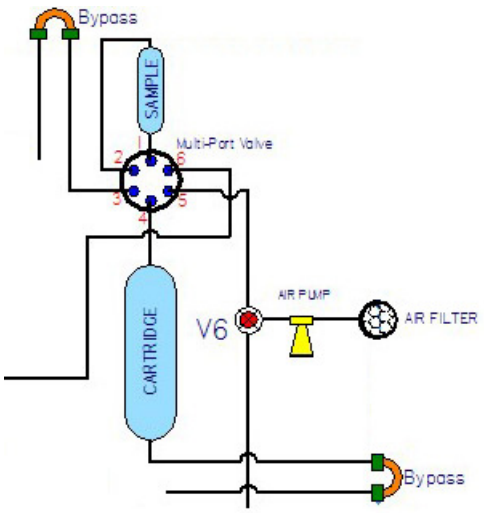


Figure 1-4: Sample Injection System and Cartridge

The multi-port valve is employed in a number of activities including equilibrating the column, delivering solvent for the separation, providing pre- and/or post- injection flush operations and air purging the column. Additionally, a sample bypass connector is included for flushing the system. The air pump indicated in Figure 1-4 can be used to purge solvent from the cartridge between separations or before discarding the column.

1.3.3 CARTRIDGE ASSEMBLY

The cartridge contains the stationary phase that performs the separation of the compounds in the sample. The Reveleris™ cartridge assembly accepts a broad range of columns from Grace Davison Discovery Sciences and other suppliers ranging in capacity from 4 to 330 g (Section 7.3.3). A leak proof universal mounting for rapid, installation of the cartridge is included and the type of column that is installed is determined by an RFID (radio frequency identification) system. This system can be overridden by the operator to permit the use of columns that do not include the RFID identification.

NOTICE If the RFID system is overridden, the maximum operating pressure is 45 psi.

⚠CAUTION A column bypass and a sample bypass are provided for system flushing and an air pump is provided to purge the solvent from the cartridge after each run and prepare for the next separation.

1.3.4 DETECTION SYSTEM

Two detectors are incorporated into the system, a UV detector and an Evaporative Light Scattering detector (ELSD). The user can select the signal from either or both detectors to trigger fraction collection or diversion of the eluent to waste.

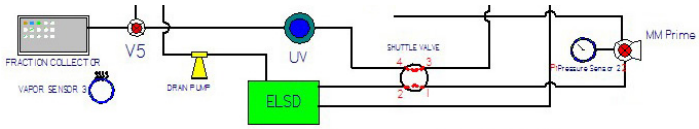


Figure 1-5: Detection/Fraction Collection System

The UV detector provides variable wavelength detection between 200 and 360 nm and allows for simultaneous monitoring of two wavelengths for triggering of the fraction collector. The ELSD is a mass sensitive detector that evaporates the mobile phase and monitors the particulate matter that passes through a photometric detector. A major benefit of this detector is that all non-volatile compounds can be detected, regardless of their spectroscopic properties. In addition, the ELSD detector allows for the use of mobile phases that absorb light at the same wavelength as the compound of interest (e.g. acetone).

1.3.5 FRACTION COLLECTOR

The eluent will be diverted to the fraction collector if the signal from any detector meets the user selected criteria. The fraction collector can employ multi-position racks for 12, 13, 16, 18, and 25 mm tubes as well as 480 mL bottles. The capacity of the racks is indicated in Table 1-1. Each vial rack has an RFID (radio frequency identification) key and a sensor is used to identify the vial size, number of tubes, tube position, and maximum acceptable vial volume. The sensor detects if the rack is installed properly or out of place (misaligned). Once the vial rack and rack holder combo are placed into the system, this information will be used to auto-populate the appropriate method fields (system default or user saved values will be used).

TABLE 1-1: RACK CAPACITY

Size of tube	Number of Tubes
12 mm OD x 75 mm H	252 (2 racks)
13 mm OD x 100 mm H	224 (2 racks)
16 mm OD x 125 mm H	168 (2 racks)
16 mm OD x 150 mm H	168 (2 racks)
18 mm OD x 150 mm H	120 (2 racks)
25 mm OD x 150 mm H	72 (2 racks)
480 mL French Square Bottles	9 (1 rack)

1.4 SYSTEM CONTROL AND DATA STORAGE

The primary user interface is a color touch screen panel with a stylus that can be used to control all aspects of the system. Four USB connections are provided to allow the use of external devices such as printers, a mouse, a keyboard, data storage devices, COM ports, memory downloads as well as the uploading software upgrades and transfer of application data. In addition, an Ethernet connection is provided to allow the control of the system via a personal computer or network. The Ethernet connection will allow for file transfer, data storage, error notification, software upgrades, access to service control features, data logs, service logs, and all other service modes via an external personal computer or external web browser by up to 10 remote computers. Where an Ethernet line is impractical to use, the system can be interfaced via a wireless adapter.

The Reveleris™ automatically saves data about each run and saves methods, which can be readily recalled. The user will be warned when the memory is nearly full so that data and/or methods can be transferred to a USB Flash drive or a mapped network hard drive.

RFID technology is employed to automatically identify the cartridge (size, media) and the racks for the fraction collector. The information retrieved by RFID automatically populates the recommended method to be used to separate the sample.

1.5 WHERE TO GO FOR FURTHER INFORMATION

Additional information about Flash Chromatography and optimizing the use of your Reveleris™ system can be obtained from your local Grace Davison Discovery Sciences representative or at www.discoverysciences.com.

2. INSTALLATION

2.1 WHAT YOU WILL NEED

2.1.1 FACILITY REQUIREMENTS

The facility should meet the following environmental conditions:

- *The temperature range of the laboratory should be between 18°C and 30°C (64°F - 86°F). The system should be installed in an area in which the temperature range is fairly constant (the suggested temperature variation is +/- 2°C/hr (+/- 5°F/hr)). The relative humidity of the area should meet the ASHRE standard for human comfort.*
- *The system should not be placed near a window, air conditioning duct, etc. or any device in which significant temperature changes may occur (e.g. an oven).*
- *The system should not be positioned so that it is difficult to operate the disconnect switch or access the power plug.*

⚠WARNING It is strongly recommended that the system be operated in a fume hood with a minimum face velocity of 100 fpm. If the instrument is not operated in a fume hood, the user must ensure that a suitable ventilation system is employed.

- *Open flames must be prohibited in the laboratory*
- *Corrosive vapors, solvents and dust should not be allowed in the laboratory as these can affect system performance.*

2.1.2 SPACE REQUIREMENTS

The dimensions of the Reveleris™ are 21.50" (54.61 cm) W 16.75" (42.54 cm) H x 19.75" (50.16 cm) D.

A minimum of 6"(15 cm) should be provided at the rear of the unit to allow for proper ventilation if the system is not in a hood.

If flammable solvents will be employed, ensure that all sources of heat are kept at least 6' (2 m) from the system.

2.1.3 ELECTRICAL REQUIREMENTS

The Reveleris™ instrument includes an automatic voltage selector (85-265 V, 50/60 Hz) and has a power consumption of 1200 W. The system should be connected to the main power line and should be connected to a safety earth. The system should not be connected to a power line that is susceptible to significant changes in power requirements such as a power line that is connected to a compressor, oven or other source of heat generation. If significant changes in the power may occur, a constant voltage transformer may be required.

The system should not be installed near equipment that generates strong electromagnetic fields. If the power line is noisy, it may be necessary to install a noise filter.

2.1.4 CARTRIDGE AND MOBILE PHASE

A cartridge and HPLC-grade mobile phase solvents that are capable of separating the compounds of interest are required and isopropanol is required for the operation of the ELSD detector. If you are uncertain about the cartridge and/or mobile phase to use, please contact your Grace Davison Discovery Sciences representative or the Grace Davison Discovery Sciences Technical Support Group for assistance. A listing of the available cartridges is provided in Section 7.5 and at www.discoverysciences.com

2.2 UNPACKING

The Reveleris™ Flash Chromatography system and its accessories are shipped in the same container. Unpack components carefully, making sure all items in the list below have been included and are in good condition. Save the container and packing material for future use.

The Reveleris™ Flash Chromatograph system shipping container contains

- The Reveleris™ Flash Chromatography system
- A Fraction Collection Tray
- An accessory kit,which includes the following items:

Flash Solvent Tubing Assemblies	1
Flash Waste Tubing Assemblies	1
Flash Carrier Pump Tubing Assembly	1
Flash Instrument Tool Kit	1
Flash Solvent GL-45 Bottle Caps (5pk)	1
Flash Solvent 38-430 Bottle Caps (5pk)	1
Flash Touch-Screen Stylus	1
Sample and Cartridge Bypass Columns (2pk)	1
Corrugated Tubing Assembly	20
Fittings	1
Power Cord	1
Adjustable Solid Loader Plunger 5 Gram	1
Fraction Collector Tray Assembly 18x150mm (2pk)	1
Flash Instrument User Manual	1
Plastic Syringe 30mL Luer Tip (5pk)	1
GraceReveleris™ 12g Silica Cartridge	1
GraceReveleris™ 40g Silica Cartridge	1
GraceReveleris™ 120g Silica Cartridge	1
5 Amp Fuse	2
10 Amp Fuse	2
Filled Solid Loader 25G	1

TABLE 2-1: SHIPPING CONTAINER CONTENTS	
Qty	**Product Description**
1	Grace Reveleris Flash Liquid Chromatography System
1	Flash Instrument Accessory Kit
4	Solvent Tubing Assemblies: 4 sets of paired 3/16" black antistatic tubing wrapped together with 1/8" Teflon tubing (includes nuts, ferrules, labels and Stainless Steel filter).
2	Waste Tubing Assemblies: Two 6ft sections of 3/16" black anti-static tubing and one 6ft length of 1/8" Teflon tubing wrapped together (includes nuts, ferrules, labels and Stainless Steel filter).
1	Carrier Pump Tubing: One 6ft length of 1/8" black anti-static tubing and one 6ft length of 1/8" Teflon tubing (includes nuts, ferrules, labels and Stainless Steel filter).
10	Solvent Bottle Caps: Five solvent bottle caps with drilled holes to allow solvent lines to pass thru (euro and US standard size caps)
1	Solid Sample Loader: Solid sample loader with two high pressure sleeves and Luer tip attachment (25g Adjustable Solid Loader)
2	By Pass Columns; Sample and Cartridge Bypass columns
2	Fraction Collector Trays; Two fraction collector trays 18 x 150mm
1	Power Cord: 110V and 220V add at country destination, use cord door on side of box
3	Syringe: Plastic syringe for priming
2	Manual: Instrument Manual and documentation in plastic bag with CD or Grace flash drive
1	Stylus
1	ELSD Hose
1	ELSD Exhaust Fitting
	Extra Ferrules and Nuts
	Extra Tubing
1	Ethernet cable to allow instrument to connect to the network or a PC
1	Sample kit of solid loaders and cartridges
1	Tool kit
1	Solid Loader Plungers: 25g (75 mL tubes)
1	GraceReveleris 12g Silica Cartridge (20pk)
1	GraceReveleris 40g Silica Cartridge (15pk)
1	GraceReveleris 120g Silica Cartridge (10pk)

The Reveleris™ Flash Chromatography system has been carefully packed and shipped to ensure that it is received in proper condition. Any damage to the container or its contents should be reported immediately to your local distributor or to Grace Davison Discovery Sciences. Please refer to Section 7.3-, Warranty, Returns, and Repairs, for more information.

If replacement parts are needed, please refer to Section 7.2, Replacement Parts for part numbers.

2.3 CONTROLS AND FEATURES

2.3.1 FRONT PANEL AND CONTROLS

Figure 2-1: Front Panel

2.3.2 REAR PANEL

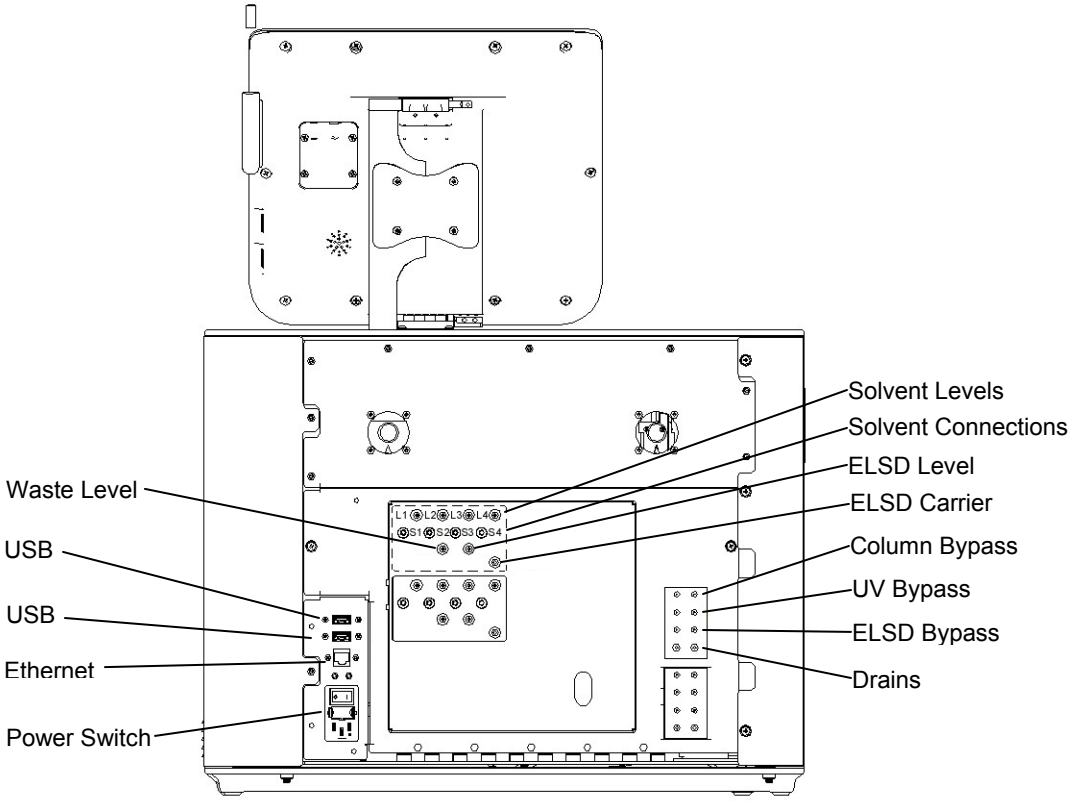


Figure 2-2: Rear Panel

2.4 INSTALLING THE INSTRUMENT

2.4.1 UNPACK THE INSTRUMENT

Remove the Reveleris™ instrument from its shipping container and position it on a level surface near or in a fume hood. Make sure there is free flow of air to the bottom of the unit and to the cooling fan at the back panel. Allow the instrument to warm to ambient temperature if necessary.

NOTICE The instrument is typically installed by a Grace Davison Discovery Sciences factory trained person.

NOTICE Save the shipping container for future use.

2.4.2 LOCATING THE INSTRUMENT IN THE LABORATORY

⚠WARNING It is strongly recommended that the system be operated in a fume hood with a minimum face velocity of 100 fpm. If the instrument is not operated in a fume hood, the user must ensure that a suitable ventilation system is employed.

⚠WARNING When flammable solvents or chemicals are used with the Reveleris™ in strument, it is possible that the concentration of the vapor may exceed the maximum exposure level recommended by OSHA Guide 1910.0000 (or other regulatory organizations). If the solvent vapor level may exceed this level, the system must be placed in an appropriate fume hood.

2.4.3 ELECTRICAL CONNECTIONS

1. Plug the power cord provided with the unit into the power module on the back panel of the Reveleris™ instrument (Figure 2-2) and plug the power cord into the mains. The system automatically detects the input voltage (85-265V).

⚠CAUTION Only use the power supply cord recommended by the manufacturer.

2. Plug in any auxiliary devices such as a printer or a personal computer. Any device that is connected to the system must meet CE (or equivalent) requirements.

2.5 FLUID CONNECTIONS

2.5.1 SOLVENT LINES

Connect all solvent lines and the solvent level lines to the manifold on the rear panel (Figure 2-2).

⚠WARNING Many solvents and samples that are used by the system may present a potential hazard to the user. Protection appropriate to the hazard must be provided for users and others working in the area.This protection should be in accordance with all applicable federal, state and local laws and regulations and in compliance with your organization's chemical safety program.

Fill the solvent reservoirs with the appropriate solvent and place them on top of the instrument. Insert a delivery line with a filter into each bottle and firmly attach the bottle cap to each bottle.

2.5.2 DRAIN SETUP

Attach the drain tubing and clamp included with the system to the DRAIN OUTLET on the rear of the instrument. Extend the tubing to a drain waste container (not included) either at bench or at floor level and make certain that the container is sealed to prevent solvent fumes from escaping. The waste container will contain solvents from your mobile phase and should be disposed of properly.

2.6 EXHAUST CONNECTIONS

If the instrument is **not** installed in a hood:

- *Connect the exhaust on the rear panel (Figure 2-2) to a fume hood. Install a condensate trap in the exhaust line to prevent solvent condensate from being pulled into the ventilation system.*

- *An exhaust hose should be placed over the solvent bottles to remove vapors.*

2.7 MOVING THE INSTRUMENT

If the instrument is to be moved, remove all liquids and the solvent bottles from the chromatograph. Ensure that the waste line is empty and secure it. Unplug the system from the mains and disconnect all auxiliary devices (e.g. printer, mouse and keyboard).

⚠CAUTION **The system weighs 34 kg (75 lb). At least two people should be used to move the instrument.**

3. THE USER INTERACTION PROGRAM

3.1 OVERVIEW

When the unit is powered up, a number of self-test procedures are performed and the system is initialized. A welcome screen is presented during the start-up period and the *Setup* window (Figure 3-1) is presented at the conclusion of the initialization procedure (Section 3.2).

The *Setup* window is used to enter operating parameters and initiate a separation. When a run is initiated, the *Run in Progress* window (Section 3.3) is displayed. If desired, the operator can view previous runs via the *Past Run* window (Section 3.4). The active window can be selected via the *View* menu.

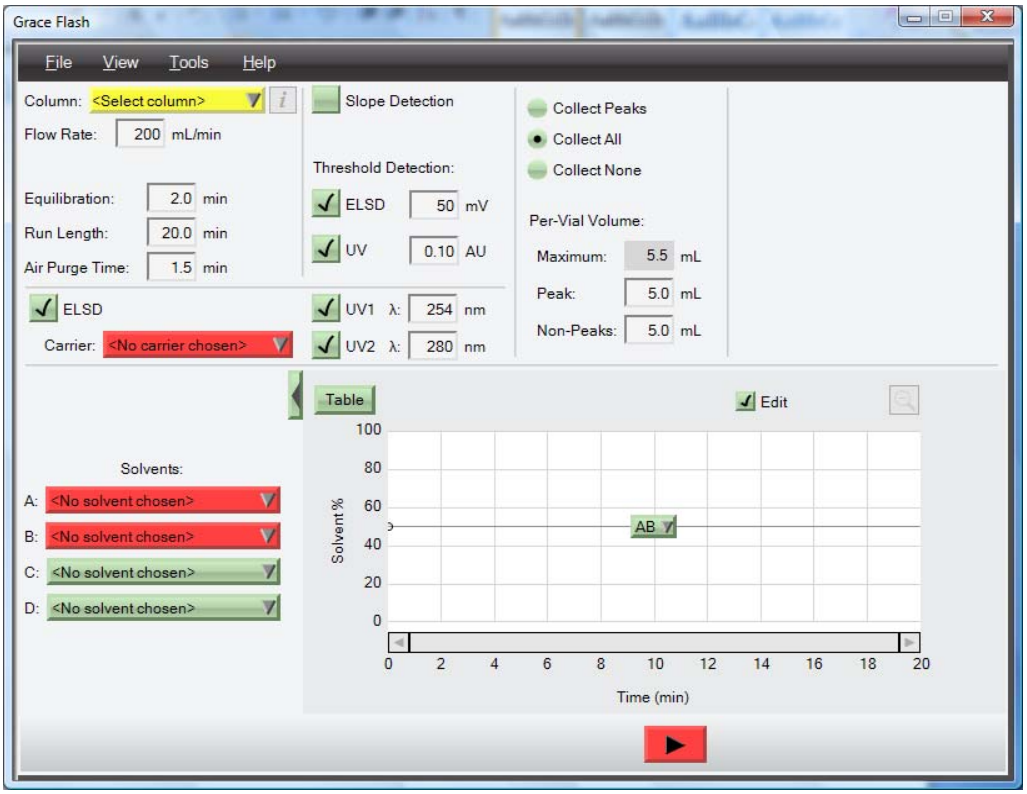


Figure 3-1: The Setup Window

This chapter describes the various features of the user interaction scheme. It should be used in conjunction with Chapter 4, which describes how the operator separates a sample.

3.2 THE SETUP WINDOW

The *Setup* window (Figure 3-1) is used to enter the conditions for the separation of a sample and consists of the following regions:

- *Chromatographic Conditions (Section 3.2.1)*
- *Detector Selection (Section 3.2.2)*
- *Collection Criteria (Section 3.2.3)*
- *Fraction Collection Information (Section 3.2.4)*
- *Solvent Selection (Section 3.2.5)*
- *Mobile Phase Composition (Section 3.2.6)*

3.2.1 CHROMATOGRAPHIC CONDITIONS

Column: The *Column* field indicates the column that is installed in the chromatograph. The column type is determined by the column RFID sensor. If a column that does not have a RFID sensor, the system detects the column by its height.

Flow Rate: The suggested flow rate for the selected column is indicated in the *Flow Rate* field and can be edited by the user (range is 1-200 mL/min). To edit the flow rate, click in the field to present a numeric keypad (Figure 3-2), enter the desired value and press *OK*.

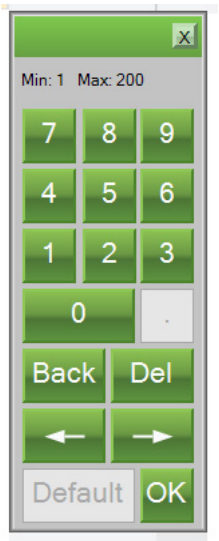


Figure 3-2: Numeric Keypad

NOTICE If desired, the operator can enter information via a standard Windows keyboard connected to the system via a USB port. If this option is employed, the desired value can be selected by moving the cursor to the appropriate field and typing the value in via the keyboard.

Equilibration: The *Equilibration* field is used to indicate the period of time that the mobile phase should be permitted to flow through the column before the sample is injected (range 0.0-20.0 min) and is edited in the same way as the *Flow Rate*.

NOTICE The minimum recommended equilibration time is dependent on the flow rate and column size to fully equilibrate the UV and flush the fraction collector arm.

Run Length: The *Run Length* field is used to indicate the time for the separation of the sample (range 0.0 to 99.9 min) and is edited in the same way as the *Flow Rate*.

Air Purge: The *Air Purge* field is used to indicate the period of time that air should be passed through the column after the separation to remove mobile phase (range 0.0-20.0 min) and is edited in the same way as the *Flow Rate*.

3.2.2 DETECTOR SELECTION

The Reveleris™ system includes a two-channel UV detector and an evaporative light scattering detector (ELSD) to monitor the eluted sample and determine if/when a fraction should be collected.

To employ one or more detectors, place a check mark adjacent to the desired detector(s).

If a UV channel is selected, enter the desired wavelength in the same way as the *Flow Rate*.

If the ELSD is selected, click on the *Carrier* field and select *Isopropanol*.

NOTICE A red field indicates an invalid entry that must be edited before a separation can be initiated.

3.2.3 COLLECTION CRITERIA

The collection of a sample can be started or terminated by either slope detection or the intensity of the signal.

- *To use Slope Detection to trigger the onset or termination of sample collection, place a check mark in the **Slope Detection** check box and select the detector that should be used (and the wavelength if a UV detector is selected).*
- *To use Threshold Detection to trigger the onset or termination of sample collection place a check mark in the appropriate check box and indicate the desired threshold.*

If either *Slope Detection* or *Threshold Detection* is selected, ensure that the *Collect Peaks* option is employed as the fraction collection option (Section 3.3.3)

NOTICE If two or three criteria are selected for peak collection, it is possible that the different criteria will be met at slightly different times in the separation. As an example, assume that the absorbance threshold is set at 0.1 AU and the wavelengths are set at 254 nm and 280 nm. If the absorbance at a given time at 254 nm is 0.11 AU and the absorbance at 280 nm is 0.08 AU, so peak collection will begin if the *Collect Peaks* option is employed as the fraction collection option (Section 3.3.3). A few seconds later, the absorbance at 254 nm is 0.19 AU and the absorbance at 280 nm is 0.11 AU, so another peak collection event will occur, moving the fraction collector to a new tube.

3.2.4 FRACTION COLLECTION OPTIONS

Select the desired option for fraction collection via the radio buttons. The *Maximum* field in the *Per-Vial* area is dependent on the tray(s) in the system as determined by the RFID sensor and is not editable by the user.

The *Peak* and *Non-Peaks* fields in the *Per-Vial* area are editable by the user in the same manner as the *Flow Rate.* The range is zero to the maximum value for the tubes in the tray.

3.2.5 SOLVENT SELECTION

The Reveleris™ system can deliver isocratic and binary gradients that are generated from four solvent bottles. When the system is initially installed, the solvent fields (lower left corner) will indicate *<No solvent chosen>* and it is necessary to select the solvents to be used in the separation.

To select a solvent for Solvent A:

1. Press *<No solvent chosen>* to present the message and drop down menu shown in Figure 3-3.

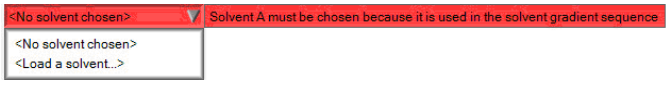


Figure 3-3: Solvent Selection Menu

2. Select *<Load a Solvent…>*to present the *Solvent Loading* dialog box (Figure 3-4).

Figure 3-4: Solvent Loading Dialog Box

The *Level* field indicates the height of solvent in the bottles connected to the various lines.

3. Press *Manage* ▶> to present a menu in which the only active entry is *Load*.

4. Press *Load* to present the *Load Solvent Line* dialog box (Figure 3-5).

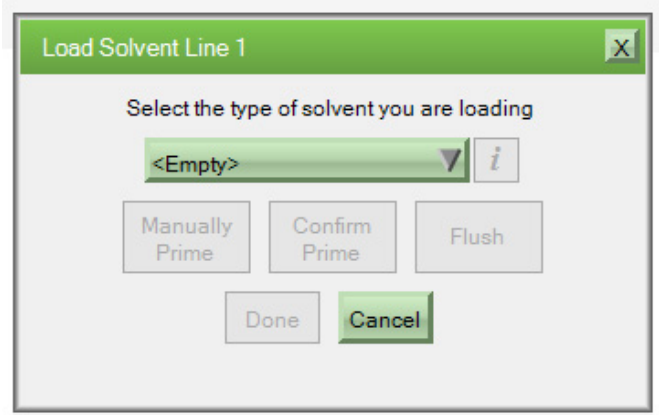


Figure 3-5: Load Solvent Line Dialog Box

5. Press the *Empty* field to access a list of solvents that are commonly used for flash chromatography and select the solvent that is the bottle associated with line 1 (e.g. Ethyl Acetate). The *Manual Prime* and *Flush* buttons will be activated, allowing the user to perform those activities. After you have primed/flushed the line, the *Done* button will be activated to allow for return to the *Setup* window, which will now indicate the name of the solvent and solvent line (e.g. Ethyl Acetate (SL1).

NOTICE Line 1-4 refers to the solvent that is withdrawn by line 1-4 of the system, while A-D refers to its definition with respect to the gradient that is formed (Section 3.2.5)

6. Repeat steps 1-5 for each solvent. A typical solvent region with four solvents selected is presented in Figure 3-6.

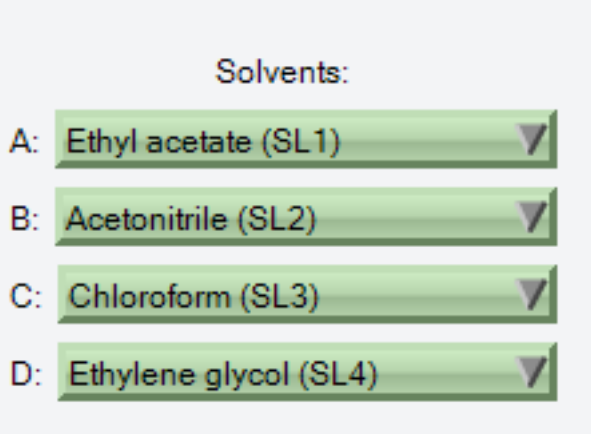


Figure 3-6: Complete Solvent Field

NOTICE Two solvents must be defined for operation of the system (this does not require that two different solvents are required, you can set one solvent to 100% and the other to 0% for an isocratic separation). If desired, you can use two or more solvent lines to deliver the same solvent, thereby reducing the frequency of refilling bottles.

3.2.6 MOBILE PHASE DESIGN

The composition of the mobile phase as a function of time can be defined by entering a table or graphically using the plot in the lower right corner of the *Setup* window.

3.2.6.1 TABULAR METHOD

To enter a gradient table:

1. Press the *Table* button to present the *Gradient Table* (Figure 3-7).

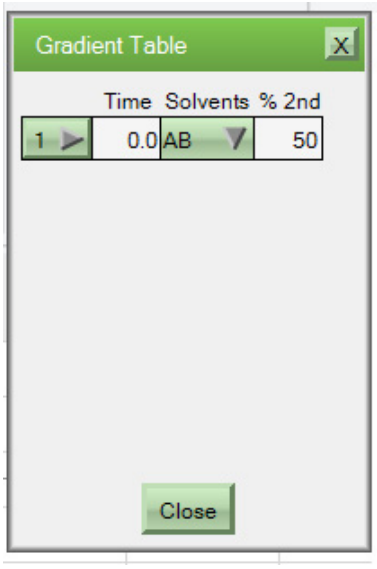


Figure 3-7: Gradient Table

2. Click on the AB▼ field to access a list of the various solvent line combinations (AB, AC, AD, etc.) and select the two solvents that you want to use at time = 0.0. The letters A, B, C, D refer to the solvents indicated to the left in the *Solvent* field. .

3. Click on the *% 2nd* field and enter the fraction of the second solvent that you want to use. As an example, if you want the mobile phase at time 0 to be 20 % Ethyl Acetate/80 % Acetonitrile, you would enter 80 in this field.

4. To add additional lines to the *Gradient Table*, press the number field (1►) to present a menu to add the next line above or below the selected line. The inserted line will be editable in the same manner as the line for time = 0. As you generate a gradient table, make certain that:

   – there is a line for t = 0,
   – no time is duplicated
   – no time is greater than the run length time

5. When you have completed the gradient table, press *Close*. The gradient will be presented in the plot (e.g. Figure 3-8).

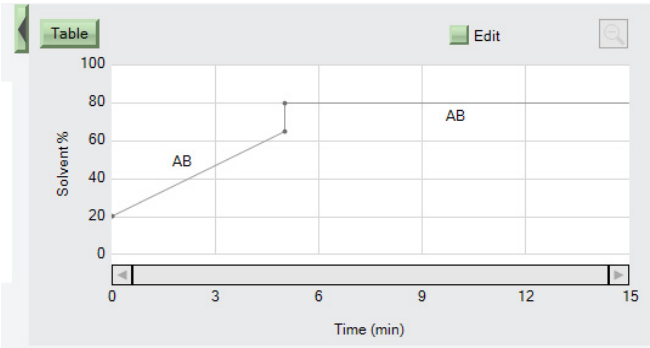


Figure 3-8: Typical Gradient

3.2.6.2 GRAPHICAL METHOD

As an alternative, the gradient can be generated or edited directly via the plot. To employ this mode:

1. Place a checkmark in the *Edit* box.

2. Click on the line at the time for which you want to edit the gradient and drag it to the desired %B, then remove the stylus (as an alternative, you can use a mouse). As an example, the %B was increased to 100% by pointing the mouse at the 15-minute mark and dragging the line to 100 %B (Figure 3-9).

3. To remove a step, drag it the point to the baseline.

Figure 3-9: Edited Gradient

NOTICE The boxes that indicate AB in Figure 3-9 are used to select the solvents to be used in the gradient in the same manner as the *Gradient* table (Figure 3-7).

3.2.7 SAVING AND RETRIEVING METHODS

Once you have generated a method, it can be stored and retrieved.

To save a method, press *Save a method as*: on the File menu to present the *Save Method as* dialog box (Figure 3-10), click in the *Enter Method Name* field to present a QWERTY keyboard, enter the desired name and press *OK*.

NOTICE The *Save Method* entry on the *File* menu will save the method with the same mane.

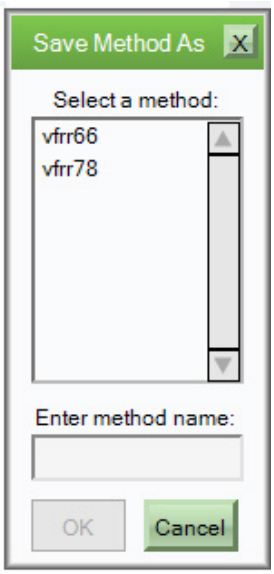


Figure 3-10: Save Method As Dialog Box

To open a stored method, press *Open Method* on the *File* menu, which presents a list of existing methods. Click on the desired method and press *OK*.

If desired, you can annotate a method by pressing *Add Method Notes* on the *File* menu or pressing the  button adjacent to the method name on top of the window to present a dialog box where you can add any relevant information.

3.3 THE RUN IN PROGRESS WINDOW

3.3.1 ACCESSING THE RUN IN PROGRESS WINDOW

When the ► button on the *Setup* window is pressed, a dialog box is presented to allow the user to assign a run name. The default name is yyyy-mo-da_hh-mm-ss (e.g. 2008-12-12_08-23-45) and the user can enter any desired run name.

When a file name has been entered, the user will be prompted to lower the sample arm (Figure 3-11). Press the appropriate button so that the arm just touches the top of the sampling device and press *OK* to initiate the run (Figure 3-12).

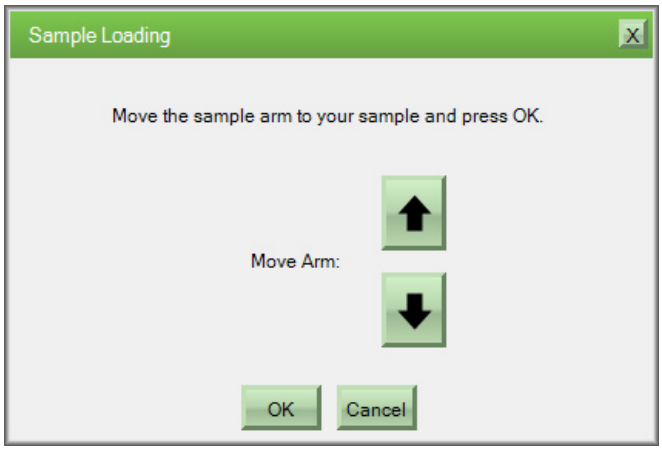


Figure 3-11: Sample Loading Dialog Box

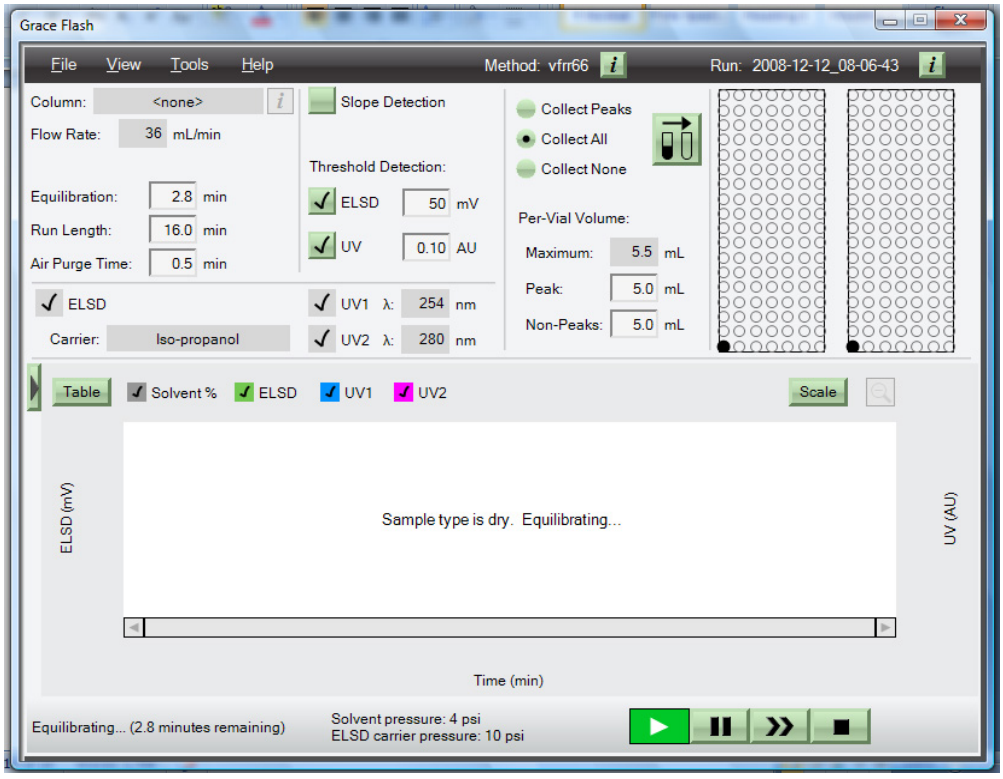


Figure 3-12: The Run Window

3.3.2 COMPONENTS OF THE RUN WINDOW

The *Run* window presents the pattern for the fraction collector trays detected by the RFID sensors in the upper right corner and the status of the system in the lower half. During equilibration, a series of messages describing the operation is presented (e.g. Figure 3-12). When the sample is being separated, the chromatogram is presented (Figure 3-13) and the fraction collection depiction indicates that tubes are being filled in.

Figure 3-13: The Run Window During a Run

The check boxes immediately above the chromatogram are used to display/remove the indicated traces from the chromatogram and the *Table* button presents the gradient table). The gradient cannot be edited during a run.

The  button to the left of the chromatogram can be pressed to present the list of solvents (Figure 3-6) and a smaller chromatogram. When the solvent list is present, a larger arrow will appear which can be used to return to the full-scale chromatogram.

If desired, you can change the ordinate scale of the detector display via the *Scale* button, which presents a dialog box (Figure 3-14).

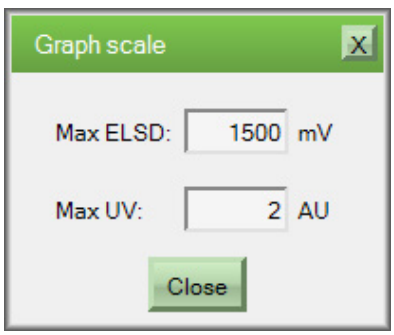


Figure 3-14: The Graph Scale Dialog Box

A region of the chromatogram can be expanded by placing the cursor at one of the corners of the desired region and dragging the mouse to the far corner of the desired region. To return to the full plot, press the  button.

When a fraction is being collected, the stripe immediately below the X-axis of the chromatogram corresponding to the collection period will present a color, and the position in the tray will correspond to the indicated color. If the fraction is collected in two or more tubes, the color will remain the same, if a different fraction is collected, the color will change (white indicates that no fraction is being collected).

The  button, which is adjacent to the method name and run name access the information windows where you can annotate the method or run as desired.

The *Run* panel at the lower right corner of the screen is used to control the operation of the system.

 The PLAY button is used to re-start the present operation if the system has been paused.

 The PAUSE button is used to stop the present operation. If the system is paused due to an error, this button will change to yellow.

 The ADVANCE button is used to advance to the next operation

 The STOP button is used to terminate the operation of the system.

3.4 THE PAST RUN SCREEN

After a run is complete, the system presents a dialog box that instructs the user to remove the sample and column equipment from the column and press Close. The *Past Run* screen (Figure 3-15) is then presented.

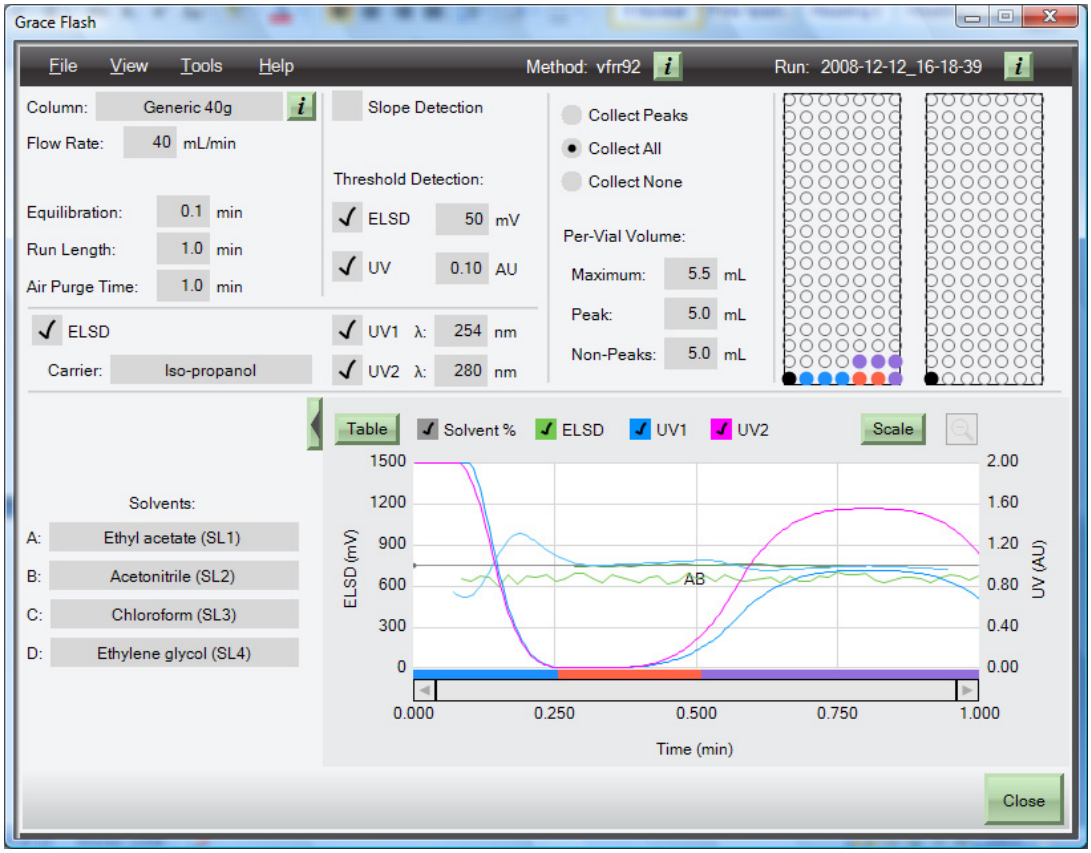


Figure 3-15: The Past Run Screen

On the *Past Run* screen, you can access a dialog box to annotate information about the method or the run by pressing the  button or selecting *Edit a Run* or Edit a Method on the File menu.

The *Past Run* screen is presented if you open a previously collected run from the *Open a Run* command on the File menu.

3.5 THE SOLVENT LOADING DIALOG BOX

The *Solvent Loading* dialog box (Figure 3-16), which is accessed by selecting *Solvent Loading* on the *Tools* menu or via the *Solvent Selection* area of the main window is used to introduce solvents into the system.

Figure 3-16: The Solvent Loading Dialog Box

The *Manage* button leads to a drop down menu with four options:

- *Load – active if there is no assigned solvent*
- *Refill – used when you want to refill a bottle*
- *Change – used when you want to change solvent for a line*
- *Flush/Prime – used when you want to flush or prime a bottle*

This dialog box is discussed in detail in Section 4.3.1.1.

3.6 THE SOLVENT DEFINITION DIALOG BOX

The *Solvent Definition* dialog box (Figure 3-17) is add or delete solvents to the list of solvents that can be used with the system.

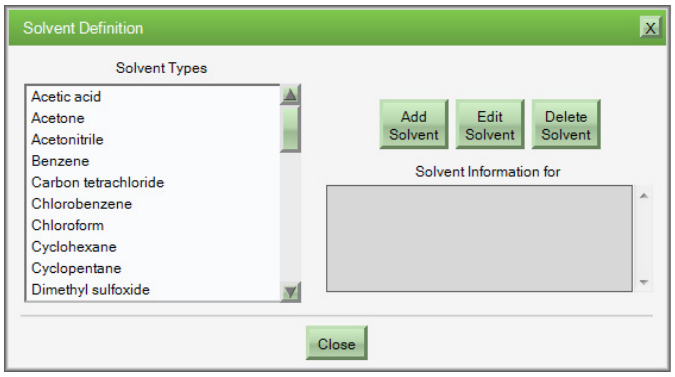


Figure 3-17: The Solvent Definition Dialog Box

The *Add Solvent* button present a dialog box (Figure 3-18) in which you can enter the name of the solvent and any descriptive information. If you click in either field, a QWERTY keyboard will be presented.

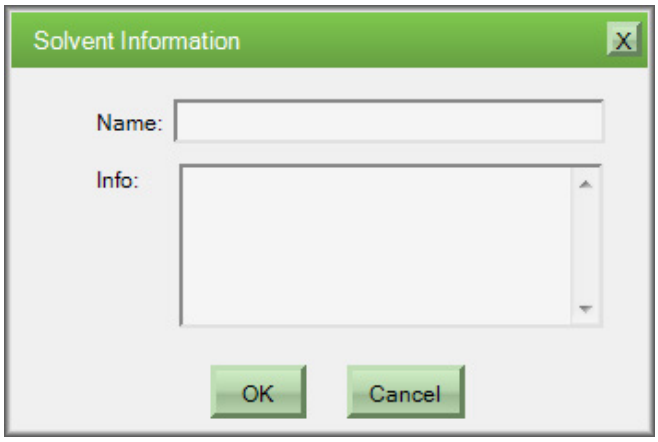


Figure 3-18: Solvent Information Dialog Box

To exit an existing solvent, move the highlight to it and press *Edit Solvent* to present a similar dialog box, with name of the solvent and any descriptive information that has been entered.

To delete a solvent, move the highlight to it and press *Delete Solvent.* A message will be presented to determine if you are certain that you want to delete the solvent.

If desired, you can export a solvent list via the *Solvent Definition /Export* dialog box (Figure 3-19) which is accessed via the *Solvent Definition Import/Export* command on the *Tools* menu. The data is exported as an .xml file which can be viewed in Microsoft Excel.

Figure 3-19: Solvent List Export Dialog Box

To import a solvent list, press the *Import Solvents* radio button to present the Solvent List Import dialog box (Figure 3-20).

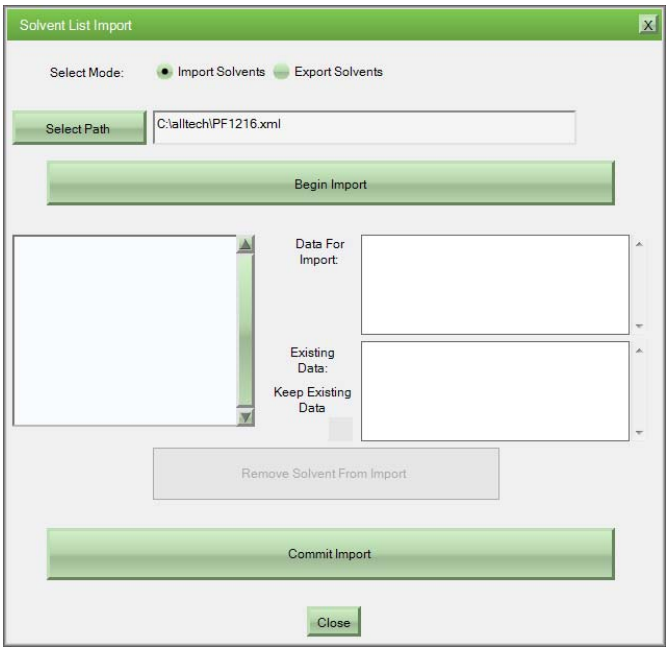


Figure 3-20: Import Solvents Dialog Box

4. SEPARATING A SAMPLE

4.1 OVERVIEW

This chapter is designed to lead the operator through the separation of a sample via the Reveleris™ Flash Chromatography system and includes information about sample handling, preparation of the system for operation and related topics. A detailed discussion of the operating system is presented in Chapter 3.

4.2 POWERING UP THE SYSTEM

The system includes two power switches, one on the lower right corner of the rear panel and a push button on the display. It is recommended that the switch on the rear panel be left on at all times and the push button on the display should be used to power up the system.

When the unit is powered up, a welcome screen on the display is presented while a number of self-test and initialization processes occur. When these processes are complete, the *Setup* window (Figure 4-1) will be presented.

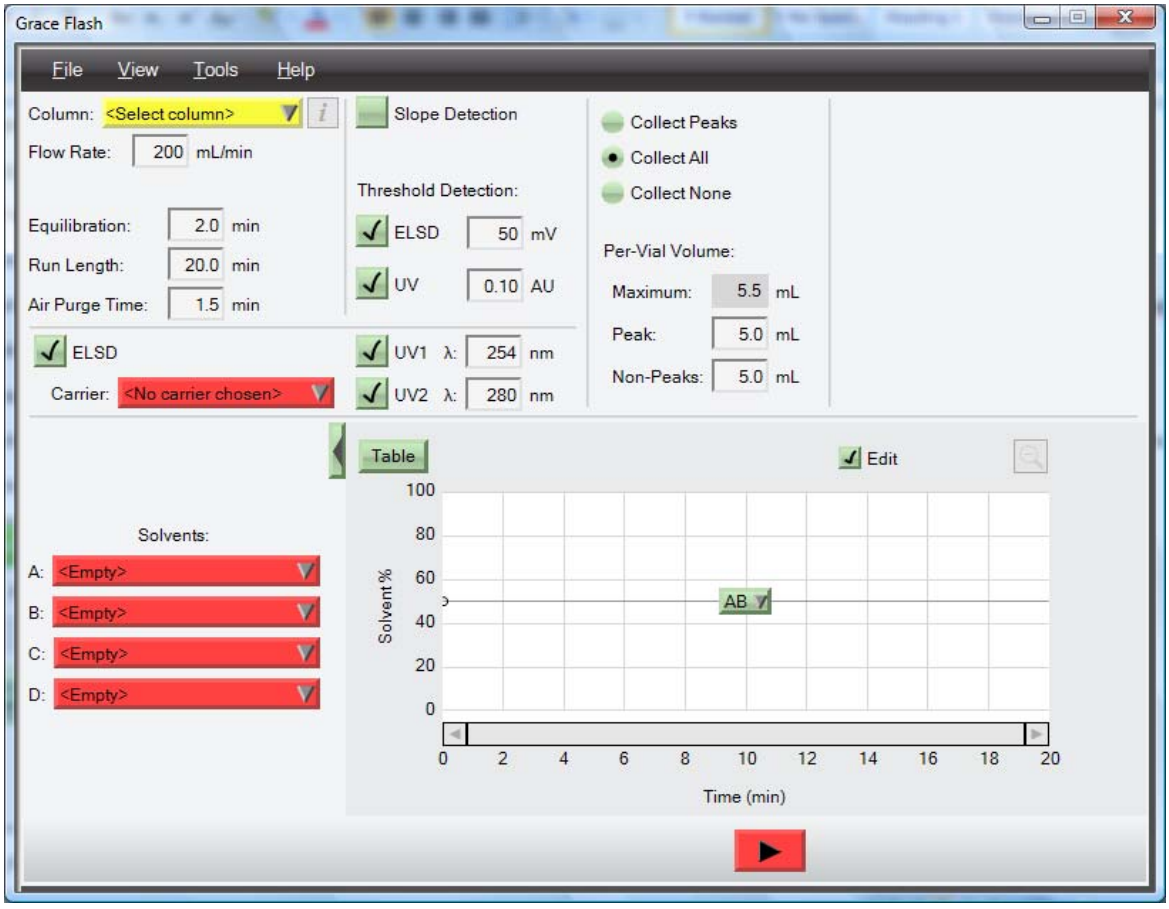


Figure 4-1: The Setup Window

4.3 PREPARING THE SYSTEM FOR OPERATION

NOTICE The discussion in this section is based on the development of a method of a separation using a system that has been powered up (i.e. the display appears as shown in Figure 4-1). If the system is powered up and solvents have been selected, it will not be necessary to perform all of the steps described below.

4.3.1 SELECTING SOLVENTS AND PRIMING/FLUSHING THE SYSTEM

The Reveleris™ system includes four solvent lines that can be used to generate a binary gradient. The solvents used to define the gradient can be altered during the separation (e.g., you could use a gradient consisting of A and B for the first 10 minutes of a separation and a gradient consisting of A and C for the last 5 minutes). Alternatively, you could place the same solvent in two or more solvent bottles to reduce the frequency of refilling of solvent lines.

⚠WARNING The temperature of the laboratory should be maintained at least 25°C below the fire point of the solvents that are used for the separation. The quantity of flammable solvent that is kept in the immediate vicinity of the system should be minimized to prevent the spread of fire. All local and institutional regulations regarding the care and handling of flammable solvents as well as Good Laboratory Practies should be strictly obeyed.

4.3.1.1 ASSIGNING SOLVENTS TO SOLVENT LINES

The *Solvent Loading* dialog box (Figure 4-2), which is accessed via the *Tools* menu, is used to indicate the solvent delivered by each solvent line and to prime the fluidics. The *Level* column indicates the present solvent level for each line.

Figure 4-2: Solvent Loading Dialog Box

To load the solvent in *Line 1* (the process is the same for all solvent lines):

1. Press the *Manage* ► button for the line to access a drop down menu in which *Load* is the only active entry.

2. Press *Load* to present the *Load Solvent Line 1* dialog box (Figure 4-3).

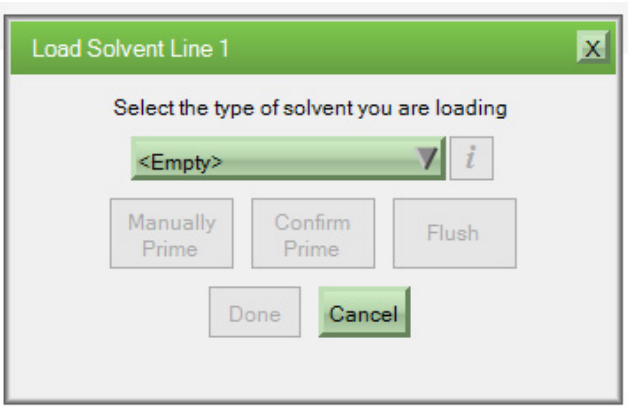


Figure 4-3: Load Solvent Line 1 Dialog Box

3. Press *<Empty>* to access a list of solvents that are commonly used in flash chromatography.

4. Select the desired solvent. The solvent name will be indicated in the green box and the *Manually Prime* and *Flush* buttons will be activated (Figure 4-4).

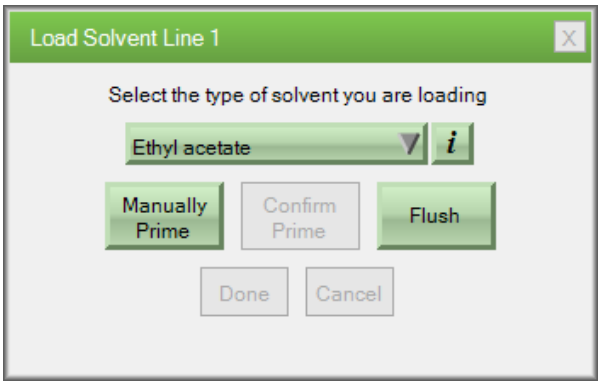


Figure 4-4: Load Solvent Line 1 Dialog Box after a Solvent has been Selected

5. Press the *Manually Prime* button. The display will present the dialog box shown in Figure 4-5.

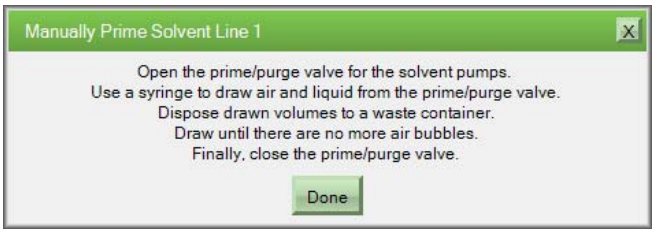


Figure 4-5: Manually Prime Solvent Line 1 Dialog Box

6. Attach a syringe to the prime port (the bottom port) on the front panel of the detector behind the door (Figure 4-6).

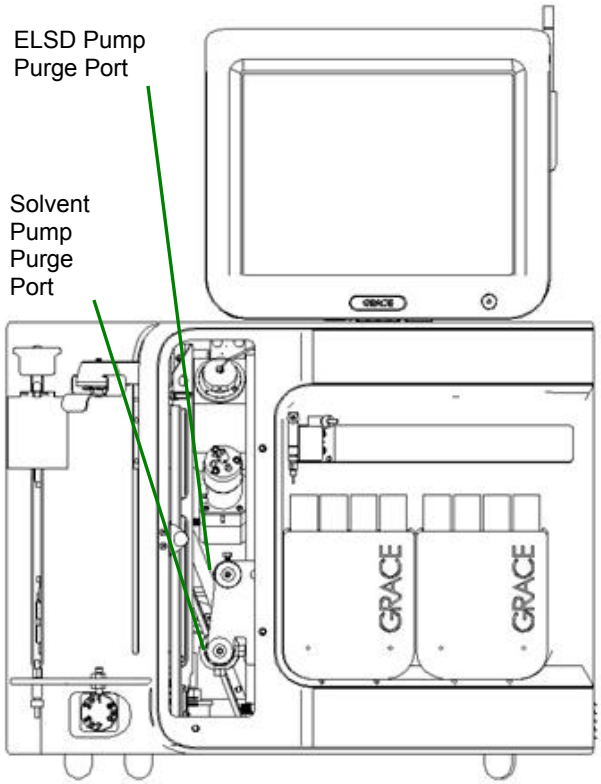


Figure 4-6: Front View of Reveleris™ Flash Chromatography System

7. Withdraw solvent from the bottle using the syringe until no air bubbles are seen coming from the system. This will typically require the withdrawal of approximately 60 mL, but will depend on the nature of the solvent. After you have primed the system, press *Done* (Figure 4-5). The system will present Figure 4-4 again with the *Done* and *Cancel* buttons activated.

8. Press *Done.*

9. Repeat steps 1-8 for each solvent that you will use for the separation.

10. If you intend to use the ELSD, click on <*No carrier chosen* ▼> adjacent to Carrier below ELSD to present a drop down menu and select <*Load a carrier*> to present Figure 4-2).

11. Select isopropanol in the same manner as the solvent line entries and prime the system. The prime port for the ELSD detector is the top port on the front panel.

12. Close door to re-engage interlock.

4.4 SELECTING OR DEVELOPING A METHOD

4.4.1 SELECTING AN EXISTING METHOD

If a method exists that contains the conditions that you expect to use, select *Open Method* on the *File* menu to present a list of the methods (Figure 4-7). Click on the desired method and press *OK* to load the method.

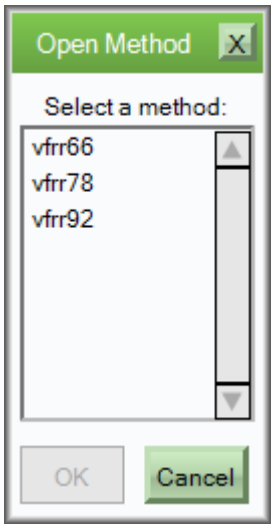


Figure 4-7: Open Method Dialog Box

All parameters of the method will be loaded except for the *Column* selection that is automatically entered when the column is placed in position. The Per-Vial volume information in the method will be entered but it may be may changed when the fraction collection tray(s) is installed.

The method can be edited; if the method is edited, it is suggested that the new method be saved via the *Save Method As* command on the *File* menu using a different file name.

If the method is not saved, an "*" will be displayed in front of the method name. The "*" will be displayed until the method changes are saved.

A typical setup screen from a retrieved method is shown in Figure 4-8.

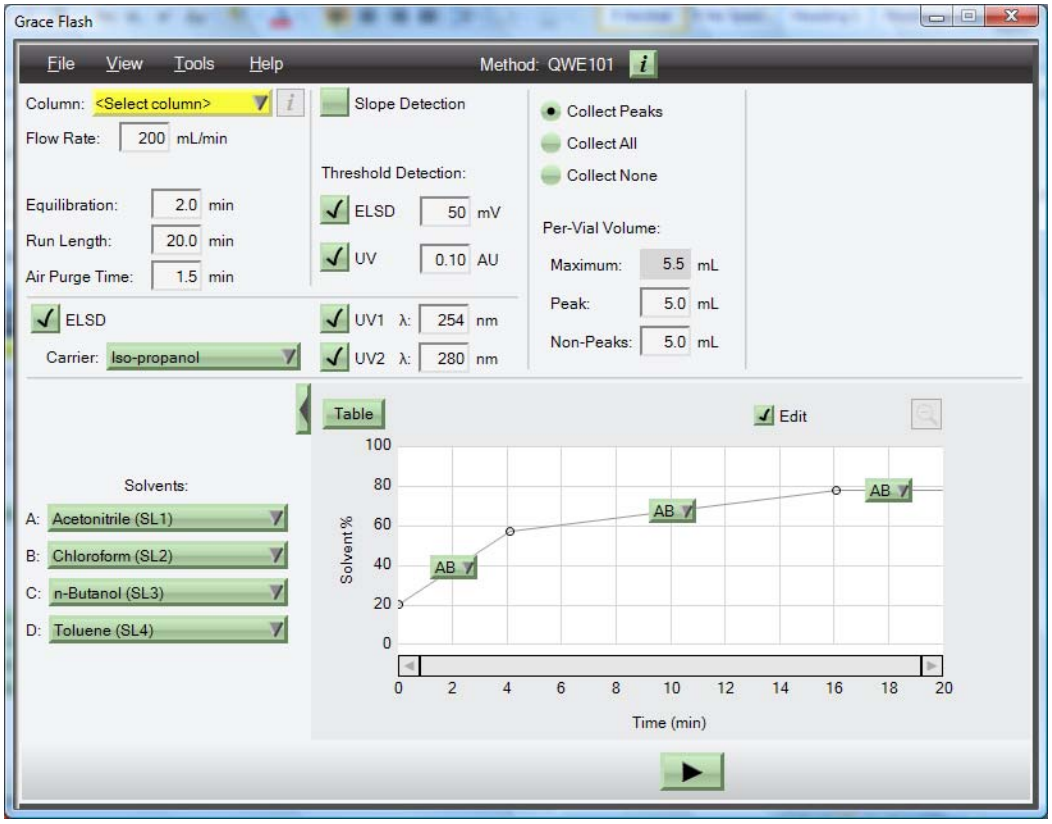


Figure 4-8: A Typical Setup Window

4.4.2 GENERATING A NEW METHOD

NOTICE The discussion that follows presents the steps that must be performed if a new method is to be designed (i.e. the instrument has just been powered up and Figure 4-1 is presented on the display).

To generate a new method:

1. Select the desired flow rate and time for each step (Section 3.2.1)

2. Select the detector(s) to be employed (Section 3.2.2). If the ELSD is chosen, press the <*No carrier field*> adjacent to *Carrier* and select *Isopropanol*)

3. Select the detection option(s) to trigger fraction collection (Section 3.2.3).

4. Select the fraction collection protocol (Section 3.2.4). This is dependent on the tray that is employed.

5. Select the solvent for each of the solvent lines by pressing on the line adjacent to the letter that you will use to define the solvent concentration in the gradient (Section 3.2.5).

6. Generate the desired gradient (Section 3.2.6).

4.5 PLACING A COLUMN IN THE CHROMATOGRAPH

The flash chromatography column to be used is placed in the column area (Figure 4-6). As you place the column into the instrument, ensure that it is positioned so that the RFID sensor detects the column size and the size is correctly indicated in the *Column* field of the *Setup* window. The arm on the top of the column is fitted by gravity and should be fit snugly on the column.

Grace Davison provides a broad range of flash chromatography columns that are listed in Section 7.5. As new columns are developed, they will be listed in the Grace Davison Discovery Sciences website (www.Discoverysciences.com). Table 4-1 contains a listing of the recommended maximum sample size for each column.

TABLE 4-1: MAXIMUM SAMPLE SIZE FOR FLASH CHROMATOGRAPHY COLUMNS

Column Size	Sample
4g	0.8 g
12g	2.4 g
40 g	8.0 g
80 g	16 g
120 g	24 g
330 g	65 g

The arm on the top of the column is gravity driven and should be snug on the column.

4.6 PLACING COLLECTION TUBES IN THE FRACTION COLLECTION TRAYS

NOTICE Do not move the collection arm when installing the trays. If the arm is moved manually, it is possible that the tracking of the arm will be disturbed and fractions will not be collected in the appropraite tubes.

Place sufficient collection tubes in the collection trays and place the trays in the collection area. The RFID sensor should detect the tube size and the maximum per vial volume field should indicate the tube volume.

4.7 PLACING A SAMPLE IN THE CHROMATOGRAPH

4.7.1 LIQUID SAMPLES

Liquid samples are introduced into the system via a syringe that is placed in the syringe arm (Figure 4-6). The arm will be moved to the top of the syringe when you start the separation (see Section 4.8). The arm contains a switch that detects if a solid sample or a liquid sample is to be separated.

4.7.2 SOLID SAMPLES

Solid samples are introduced into the system via the solid sample assembly shown in Figure 4-9. To access the sample loader, turn the outer cylinder so that the flange on the cylinder corresponds to the gaps in the cap. Repeat this action with the sample loader. After you have filled the chamber with the sample, reattach the sample chamber and the outer cylinder.

Figure 4-9: Solid Sample Assembly

Place the solid sample accessory in the sample area (Figure 4-6). The arm will be moved to the top of the solid sample assembly when you start the separation (see Section 4.8). The arm contains a switch that detects if a solid sample or a liquid sample is to be separated.

4.8 STARTING THE SEPARATION

Press the  button to start the separation. The display will prompt for a sample name (Figure 4-10).

Figure 4-10: Set Run Name Dialog Box

The default run name is *yyyy-mo-da_hh-mm-ss* (e.g. 2008-12-21_15-07-25) and it will appear in the upper right hand corner of the window. Any desired run name can be entered (if you click on the *Enter run name* field, a QWERTY will be displayed).

When the OK button is pressed, the display will instruct the user to move the sample arm to the sample and press *OK* (Figure 4-11).

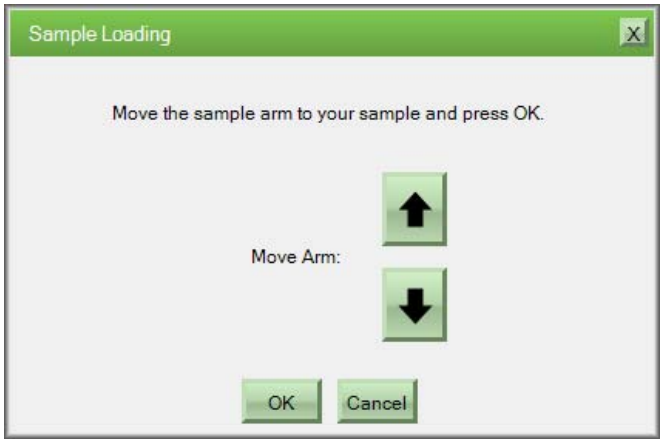


Figure 4-11: Sample Loading Dialog Box

Use the up and down arrows to move the sample arm to the top of the sample syringe or assembly. The bottom of the arm should be in contact with the top of the sample syringe or assembly.

NOTICE A switch in the arm is provided to determine if a solid sample holder or a syringe is employed .

When the arm is in position, press *OK* to initiate the method. The display will appear as shown in Figure 4-12. The message in the chromatogram window and the information at the bottom of the window will update as relevant.

-

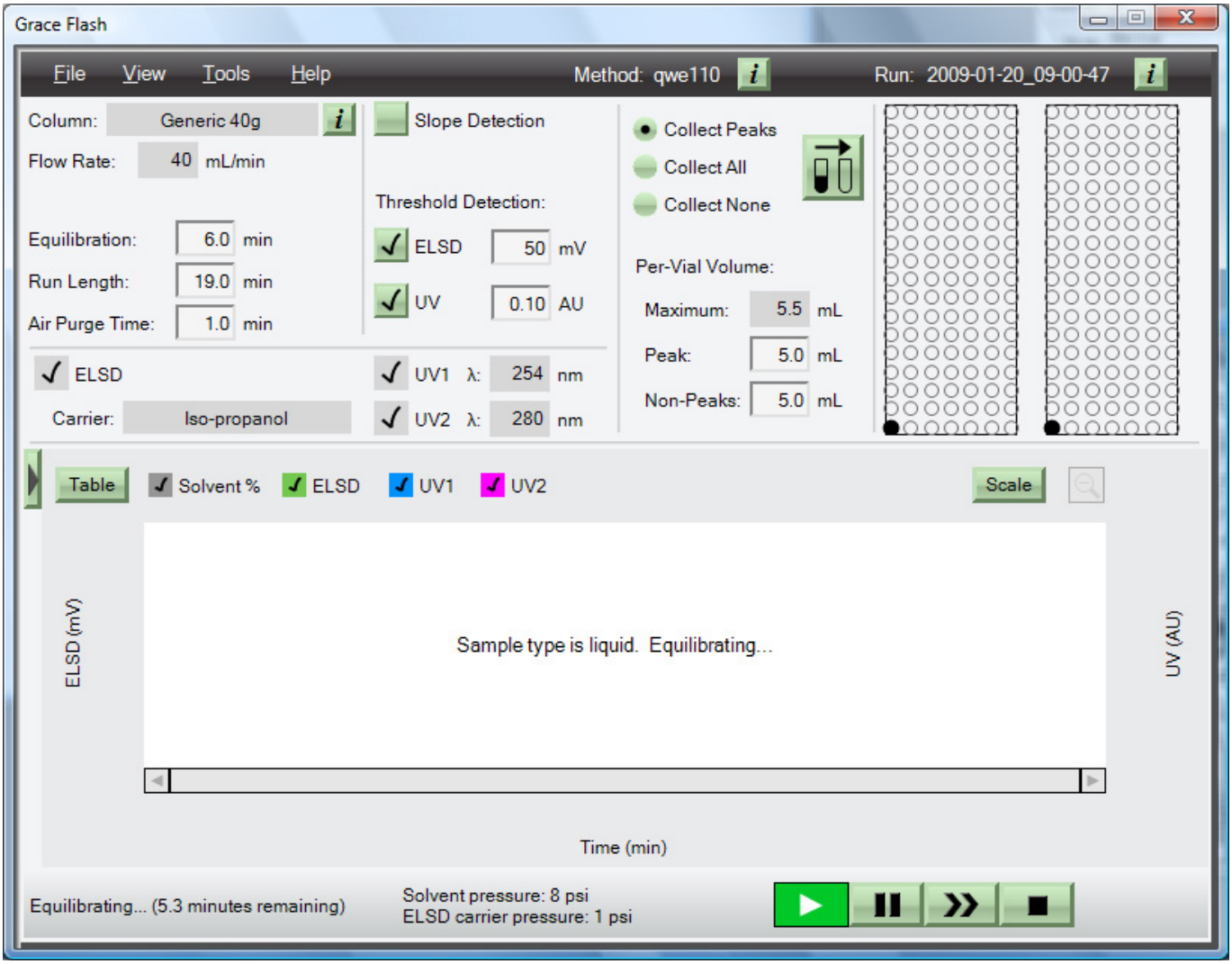


Figure 4-12: The Run Window

At the end of the equilibration, the separation will start and the chromatogram will be displayed (Figure 4-13). A detailed discussion of the *Run* window is presented in Section 3.3.2.

The maximum pressure for the system is 200 psi with an RFID column and 45 psi for a column without an RFID tag. The system will try to reduce the flow rate when the system pressure is reaching the pressure limit. After reducing the flow rate, the run length will be extended automatically.

Figure 4-13: The Run Window During a Run

The *Run* panel at the lower right corner of the screen is used to control the operation of the system.

 The PLAY button is used to re-start the present operation if the system has been paused.

 The PAUSE button is used to stop the present operation.

 The ADVANCE button is used to advance to the next operation.

 The STOP button is used to terminate the operation of the system.

After the separation and air purge is complete, a prompt to remove the sample and column equipment will be presented (Figure 4-14). During a run, any parameter indicated in green (e.g the detection threshold(s)) can be edited except for gradient steps that have already occurred and you can turn on/off any detection trace or the gradient line from the plot.

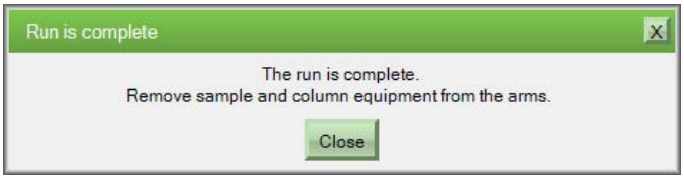


Figure 4-14: The Run is Complete Prompt

When the *Close* button is pressed, the *Post Run* window is presented (Figure 4-15).

NOTICE The data is saved only after the *Close* button is pressed.

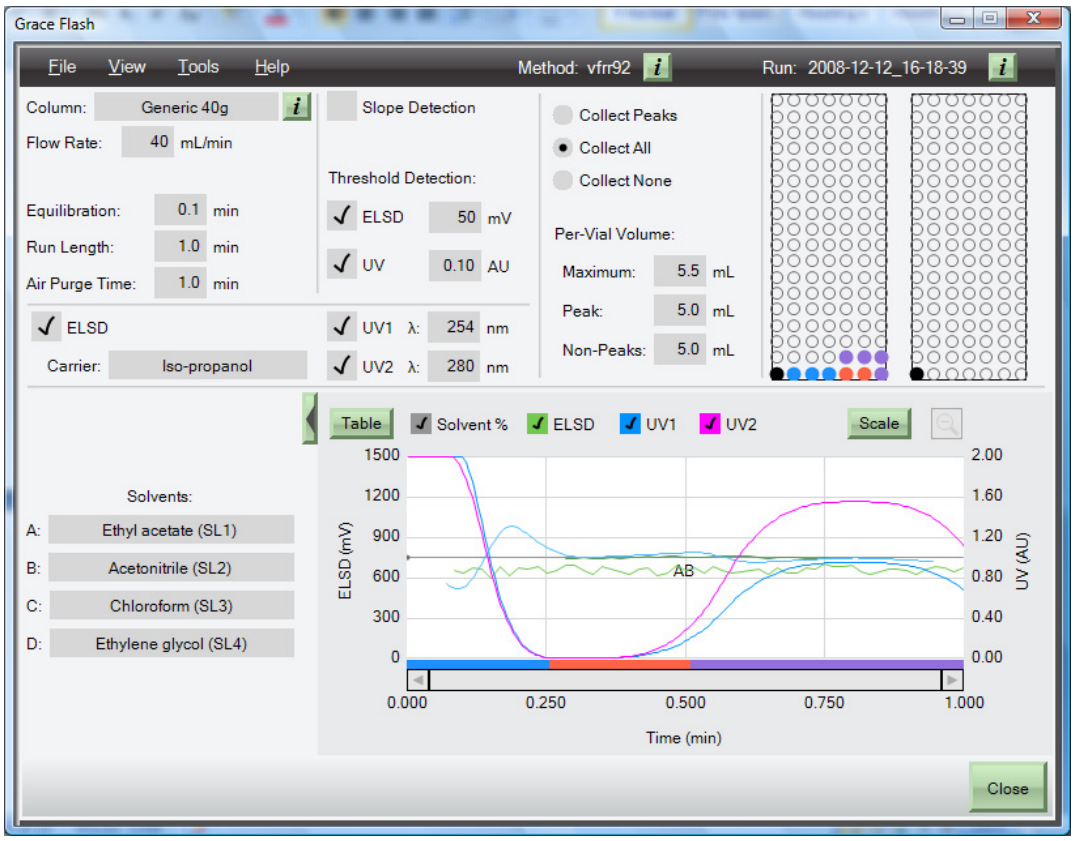


Figure 4-15: The Post Run Screen

To annotate the run or the method, press the  button by the run name or method name.

4.9 PERFORMING ADDITIONAL SEPARATIONS

After a separation has been completed, additional separations can be performed by pressing the *Close* button on the Post Run window, which will present the *Setup* window again. To perform another separation, simply press the  button. The system will use the existing method to separate the sample. Alternatviely, another method can be generated or recalled as described above.

The *Solvent Loading* dialog box (Section 4.3.1.1) assists the user in maintaining the system. If a solvent has been defined, the drop down menu obtained by pressing the solvent name allows for refilling the solvent bottle, changing the solvent or flushing/priming the system.

Refill is used if you want to use a new solvent bottle of the same sample type. This command serves to reinitialize the solvent level information and presents the dialog box shown in Figure 4-16. Follow the directions indicated on the dialog box.

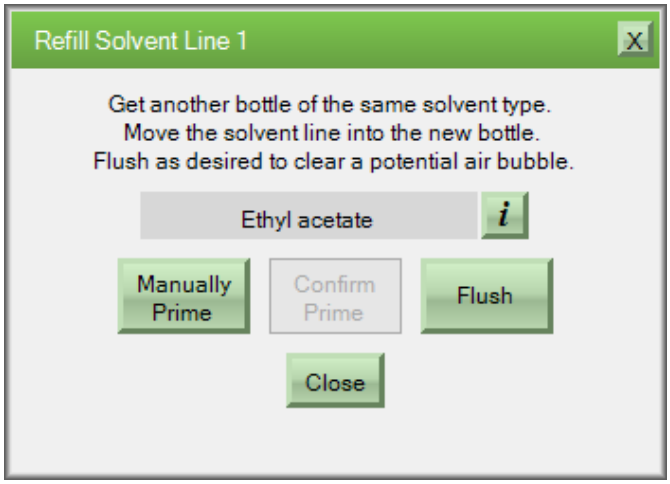


Figure 4-16: Refill Solvent Line Dialog Box

Change presents a dialog box similar to the *Load Solvent Line 1* dialog box (Figure 4-3). Select the solvent in the same manner as for an empty solvent entry and prime the system in the same manner.

NOTICE Ensure that the new solvent is miscible with the old solvent. If the two solvents are not miscible, use an intermediate solvent such as isopropanol to remove the original solvent.

Flush/Prime presents a dialog box to the *Load Solvent Line 1* dialog box (Figure 4-3) with the difference that you cannot select a solvent.

5. TROUBLESHOOTING

5.1 INTRODUCTION

Troubleshooting refers to the steps to determine the cause of a problem. The Reveleris™ includes number of systems (e.g. solvent delivery system, sample delivery system UV detector, ELSD, fraction collector) and an important aspect of troubleshooting is to determine which system is responsible for the problem. As an example of this point, if the peaks from a sample are smaller than what is expected, the following problems might be the cause:

- *The UV detector cell might be dirty.*
- *The syringe did not introduce the desired amount of sample into the cartridge.*
- *The injector valve is not delivering the entire sample.*

The most efficient method of determining the cause of the problem is to perform a systematic investigation into each potential cause.

While some problems are instrumental in nature, others are sample related. As part of a troubleshooting protocol, it is useful to develop a standard sample and separate it on a periodic basis. The results should be compared to previously obtained results; if small differences are obtained; it may be possible to highlight a problem before it becomes serious. As an example of this point, a small decrease in the signal from what is expected might be due to the contamination of the cell in the UV detector. An additional benefit of the use of a standard sample is that it can help distinguish between instrumental problems and sample/mobile phase issues.

If any operating parameter appears to differ significantly from that which was obtained in a previous run, the cause for the difference should be looked into before additional runs are performed.

5.2 SOFTWARE AND FIRMWARE ERRORS

In the event of a suspected firmware or software error:

- *Log any error messages*
- *Reboot the instrument and then attempt to operate the system*
- *Contact Grace Davison Discovery Sciences if rebooting the system does not correct the problem.*

When you contact Grace Davison Discovery Sciences, please provide as much information as possible relating to the problem, including what the system was doing before the problem was observed, any error messages, any abnormal events that were noted, anything which you feel may be of assistance in troubleshooting, the system serial number and software revision. If the error message or problem can be reproduced, please indicate all actions that were done to reach the message or problem.

5.3 TROUBLESHOOTING STRATEGIES

5.3.1 TROUBLESHOOTING CHECKLISTS

The following series of troubleshooting checklists is provided to assist the operator in determining the cause of a problem and suggests possible remedies. The problems described below are selected on the basis that they are likely occurrences in normal operation that can be readily resolved by the user. It is not an all-inclusive list, if you encounter problems that cannot be resolved using this troubleshooting section, please contact your local Grace Davison Discovery Sciences technical support representative.

NOTICE The operating program of the Reveleris™ includes a large number of internal procedures that automatically alert the user to an unacceptable situation and will not allow the system to start. As an example of this point, if the user tries to start the system without having a column installed, the green *Start* arrow on the bottom of the display will not be active.

5.3.2 INSTRUMENTAL ISSUES

5.3.2.1 THE SYSTEM DOES NOT POWER UP OR SHUTS DOWN AUTOMATICALLY

Problem	Remedy
Power is not being supplied to the system	Verify that the line cord is plugged into the line
	Check the circuit breaker that provides power to the line is turned on.
	Line power voltage, amperage or frequency is outside of system specifications
System shuts down automatically	Connect system to line via a UPS (Major fluctuations in the line power are present)

5.3.2.2 DISPLAY PROBLEMS

Problem	Remedy
The display does not power up	Make sure that the display cable is securely attached to the real panel of the unit
Poor resolution, brightness	Make sure that the display cable is securely attached to the real panel of the unit

5.3.2.3 CANNOT INTERFACE WITH EXTERNAL COMPUTER

Problem	Remedy
Ethernet cable is faulty	Obtain a new cable
Incorrect IP Address used	Verify the IP address

5.3.3 SOLVENT/PUMP PRESSURE PROBLEMS

5.3.3.1 THE PUMP IS NOT DELIVERING THE DESIRED MOBILE PHASE

Problem	Remedy
A solvent bottle is empty	Fill bottle
A solvent filter is clogged	Replace solvent filter
A solvent tube or cap has become dislodged	Check the cap on the solvent bottle and the connection to the system
Incorrect solvent or gradient information was entered	Re-enter solvent or gradient
Poorly mixed mobile phase	Check solvent preparation method
Column not recognized by RFID sensor	Remove column and replace
Mobile phases are immiscible	Select different solvents or gradient

5.3.3.2 AIR BUBBLES IN SOLVENT

Problem	Remedy
Pump was not primed	Prime pump
Level sensor malfunction	Replace level sensor
Solvent filter malfunction	Replace solvent filter
Incorrect mixing of solvents	Remix solvents
Aqueous solvent not degassed	Degas aqueous solvent
Mobile phases are immiscible	Select different solvents or gradient

5.3.3.3 MOBILE PHASE LEAKS

Problem	Remedy
System Clogged	Flush with solvent to remove blockage
Defective tubing/cartridge	Replace tubing
A solvent tube or cap has become dislodged	Check the cap on the solvent bottle and the connection to the system

5.3.3.4 THE PRESSURE IS HIGHER THAN EXPECTED

Problem	Remedy
Some component of the system is blocked due to impure solvent	Remove blockage, filter all solvents (especially buffers)
Some component of the system is blocked due to precipitation of sample	Replace column or purge with a strong solvent. Review sample purification steps and mobile phase to ensure solubility.
Some component of the system is blocked due to microbial growth (in buffer)	Clean column and use freshly prepared solvent. If separation permits, add additional organic solvent and/or growth inhibitor.
Some component of the system is blocked due to foreign matter (precipitation, dust, etc.)	Remove blockage
Tubing is deformed or bent	Change tubing
Algae formation in line (if aqueous solvent is used)	Flush lines

5.3.3.5 THE PRESSURE IS LOWER THAN EXPECTED

Problem	Remedy
There is a leak in the system	Check and tighten all fittings.
Incorrect solvent selected	Replace solvent

5.3.3.6 PARTICULATES OBSERVED IN FLUIDIC LINES

Problem	Remedy
Defective solvent filter	Clean or replace solvent filter heck and tighten all fittings.
Sample or solvent component precipitates due to insolubility as gradient changes	Change solvent
Some component of the system is blocked due to microbial growth (in buffer)	Clean column and use freshly prepared solvent. If separation permits, add additional organic solvent and/or growth inhibitor.

5.3.4 CHROMATOGRAPHIC ISSUES

5.3.4.1 THE PEAK HEIGHT IS SMALLER THAN EXPECTED/NO PEAK DETECTED

Problem	Remedy
Flow cell is dirty (UV detector)	Clean cell (flush with appropriate solvent)
Lamp is aged	Replace lamp
Sample is dilute	Increase sample concentration
Sample retained by column	Change column and/or mobile phase
Injector did not inject the appropriate quantity	Check injector (The syringe is defective)
Incorrect wavelength selected (UV detector)	Change wavelength
Not all of the sample dissolved (solid sample loader)	Change mobile phase
Nebulizer, drift tube and/or optics are dirty	Clean the optical components
Compound is too volatile for ELSD detector	
Poor evaporation of solvent (ELSD detector)	Change mobile phase

5.3.4.2 DRIFT IN THE DETECTOR SIGNAL

Problem	Remedy
Detector has not thermally stabilized	Allow detector to stabilize. Maintain system is an area where the temperature is fairly constant
If a gradient is used, one of the components of the gradient absorbs light (UV Detector)	Select a wavelength where the mobile phase does not absorb light
Tightly retained compounds from previous run eluting from column	Use a strong solvent to clean column. It is suggested that a new column be employed for each run
Contamination in UV cell	Flush system with appropriate solvent to remove contamination

5.3.4.3 SPIKES IN CHROMATOGRAM

Problem	Remedy
Air in pump or lines	Purge system

5.3.4.4 NOISY UV CHROMATOGRAM

Problem	Remedy
Aged Lamp	Replace Lamp
Tightly retained compounds from previous run eluting from column	Use a strong solvent to clean column. It is suggested that a new column be employed for each run
Low concentration of compound	
Incorrect wavelength	Select appropriate wavelength

5.3.4.5 NOISY ELSD CHROMATOGRAM

Problem	Remedy
Drift tube/optics dirty	Clean nebulizer
Tightly retained compounds from previous run eluting from column	Use a strong solvent to clean column. It is suggested that a new column be employed for each run
Low concentration of compound	

5.3.4.6 RETENTION TIMES DIFFER FROM EXPECTATION

Problem	Remedy
Pump is delivering solvent at a different flow rate	Check pump flow rate
Incorrect cartridge or defective cartridge	Check cartridge
Incorrect solvent	Choose correct solvent
Cartridge overloaded	Select larger column or smaller sample
Change in column temperature	Ensure that temperature of environment does not change

5.3.4.7 POOR RESOLUTION

Problem	Remedy
Pump is delivering solvent at a different flow rate	Check pump
Incorrect cartridge or defective cartridge	Check cartridge
Wrong mobile phase	Select more appropriate mobile phase
Column overloaded	Select larger column or smaller sample

5.3.4.8 THE SAMPLE IS NOT SEPARATED BY THE COLUMN (POOR RECOVERY)

Problem	Remedy
An incorrect mobile phase is used	Use a different mobile phase (depends on the characteristics of the compounds to be separated)
The mobile phase is incorrectly defined or generated	Verify that the proper mobile phase is employed. If the mobile phase is not properly generated, contact service.
Sample Overloading	Use smaller sample or larger column
The stationary phase is not suitable for the separation	Use a different stationary phase (see Section 7.4).

5.3.4.9 BROAD PEAKS

Problem	Remedy
Sample Size too large (column overloaded)	Reduce sample size
Poor chromatographic resolution	See Section 5.3.4.6
The stationary phase/mobile phase is not suitable for the separation	Use a different stationary phase/mobile phase

5.3.4.10 GHOST PEAKS

Problem	Remedy
Tightly retained compound from previous run is eluted)	Use a new column or clean column and employ a strong solvent between runs
	Select a new mobile phase and/or stationary phase

5.3.5 FRACTION COLLECTION ISSUES

5.3.5.1 DESIRED FRACTIONS ARE NOT BEING COLLECTED/LOW YIELD OF FRACTIONS

Problem	Remedy
Detector parameters not correctly set	Change detector parameters (e.g. wavelength for trigger)
Sample not separated by column	Use appropriate column and solvent
Injector did not inject appropriate quantity	Check injector, clean if necessary
Sample not totally dissolved in solvent (solid sample)	Change solvent
Nebulizer/drift tube optics dirty (ELSD)	Clean detector
Sample is too dilute	Use more concentrated sample
Compound is too volatile (ELSD)	
Distribution arm does not move	Power system down to reinitialize
Poor solvent choice for ELSD (high boiling point)	Change solvent

5.3.5.2 TEST TUBES OVERFLOW

Problem	Remedy
Per vial volume is incorrect	Adjust parameter
Incorrect Vials are used	Use appropriate vials
Distribution arm does not move	Power down instrument and power up again

5.3.5.3 FRACTION COLLECTION ARM DOES NOT MOVE

Problem	Remedy
Distribution arm is jammed due to obstruction	Power down instrument and power up again
Manual repositioning of arm	Power down instrument and power up again

5.3.5.4 SYSTEM STOPS AFTER ONE TRAY IS FILLED

Problem	Remedy
RFID tag on tray is not read properly	Remove tray and replace
Only one tray in system	Add second tray

5.3.6 COLUMN EVACUATION PROBLEMS

Problem	Remedy
Column is plugged	Change solvent to a solvent that can dissolve material
Injector plugged	Change solvent to a solvent that can dissolve material

6. MAINTENANCE

6.1 OVERVIEW

This chapter describes a series of activities that should be performed on a routine basis to optimize the performance and ensure long term, safe operation of the Reveleris™ system. The suggested frequency for each activity indicated below is based on the "typical operation" of the system and is dependent on a variety of factors such as the nature and purity of the mobile phase and the nature of the sample. Over time, it is likely that the user will determine an appropriate schedule that meets the specific application of the flash chromatography system.

6.2 CLEANING THE SYSTEM

The exterior surfaces of the system should be cleaned with a cloth using water with a mild detergent on a periodic basis. If mobile phase or sample is accidentally spilled on the system, it should be removed immediately. If a mild detergent is not able to remove the foreign material, an organic solvent such as methanol or isopropanol can be used.

The collection racks and trays are manufactured from a conductive plastic and the presence of foreign material such as dirt, film, and foreign coatings may prevent their ability to dissipate static electricity. Clean the racks and trays on a monthly basis with water containing a mild detergent.

6.2.1 ROUTINE INSPECTION

On a daily basis, the following activities should be performed:

- *Check for leaks. If a leak is observed, resolve the issue before continuing.*
- *Inspect fittings; if solid material is deposited on a fitting, tighten (replace) the fitting before continuing.*
- *Inspect all tubing. If defective tubing is found (e.g. kinks, bends, discoloration or deterioration), replace the tubing.*
- *Empty the drain bottle (if the drain bottle contains more material than you expect, determine the source of the liquid).*
- *Check all drains to ensure that liquid can flow thru them to the drain bottle (i.e. there is no particulate matter blocking the drain).*

On a weekly basis, the following activities should be performed:

- *Check the filters in the solvent bottles and clean if necessary. They can be cleaned by placing them in an ultrasonic bath with a suitable solvent for a few minutes.*

6.3 ACCESSING SYSTEM COMPONENTS

⚠WARNING Make certain that the Reveleris™ system is powered down the power cord is unplugged from the unit before the side or front panel is removed. Failure to unplug the system will expose the user to dangerous electrical current.

The left side panel can be removed to access the pump, UV Detector, and the pump for the ELSD (Figure 6-1).

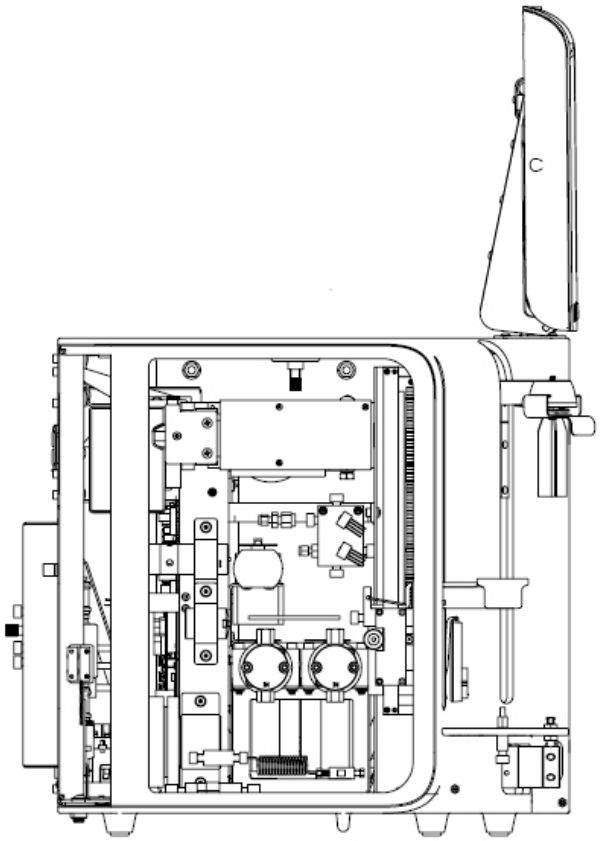


Figure 6-1: The Left Panel of the Reveleris™, Cover Removed

The front panel can be removed to access the ELSD (Figure 6-2).

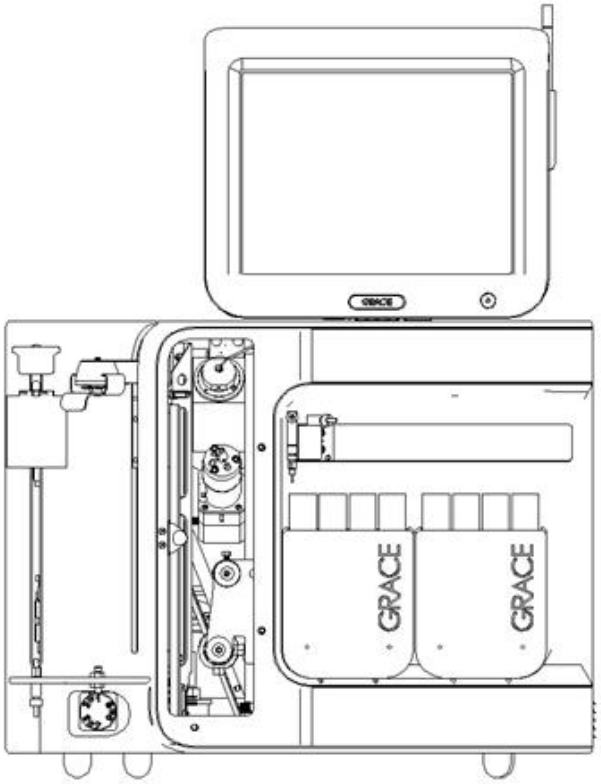


Figure 6-2: The Front Panel of the Reveleris™, Cover Removed

6.4 PUMP MAINTENANCE

NOTICE If lower than normal pressures, pressure variations, or leaks in the pumping system are observed, it is likely that that the piston seal, piston, or check valves need to be cleaned or replaced. As a first approximation, the piston seals may need to be replaced after 1000 hours of running time.'

⚠ DANGER The pumps are accessed via the left door of the instrument. Power down the system and unplug it before you remove the left panel.

6.4.1 REPLACING PISTON SEALS (SOLVENT PUMP)

Materials Needed:

- *Open-end wrench, 1/4" x 5/16"*
- *Seal Insertion/Removal Tool (supplied with Seal Kit)*
- *Ultrasonic Bath*

To replace the piston seals:

1. Remove the nut that secures the tubing on the inlet check valve that draws solvent from the mobile phase reservoir manifold (Figure 6-3) from the inlet check valve that (from the mobile phase reservoir manifold). This nut can normally be loosened by hand.

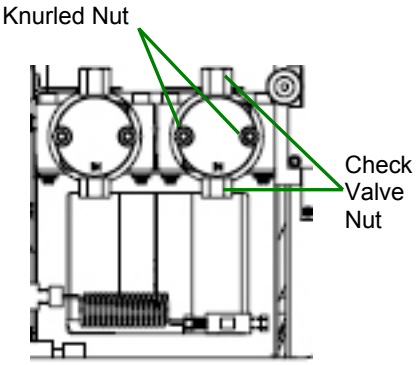


Figure 6-3 Solvent Pumps

2. Remove the nut that secures the tubing from the outlet check valve (to the mixer). This nut will normally require a wrench.

3. Carefully remove the two knurled nuts at the front of the pump head.

4. Carefully separate the pump head from the pump. Move the pump head straight out from the pump and remove it from the piston. Remove the seal from the piston if it did not stay in the pump head.

NOTICE Be careful not to break the piston when removing the pump head. Twisting the pump head can cause the piston to break

NOTICE If you remove the piston seal, install a new seal after cleaning the head. It is not recommended that you reinstall the used piston seal since it is likely to be scratched and damaged during removal and will not provide a reliable seal if reused.

5. Remove the seal using the flanged end of the plastic seal removal tool supplied with the seal replacement kit as shown in Figure 6-4. Avoid scratching the sealing surface in the pump head.

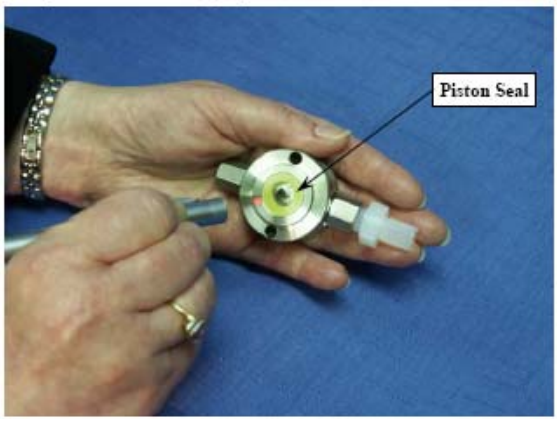


Figure 6-4: Removing the Piston Seal

6. Inspect the piston seal cavity in the stainless steel pump head. Remove any foreign material using a cotton swab or equivalent. Avoid scratching the sealing surfaces during the cleaning process and take care to ensure that no fibers from the cleaning swab remain in the components.

7. The pump head may be cleaned using a laboratory grade detergent solution in an ultrasonic bath for at least 30 minutes, followed by rinsing for at least 10 minutes in distilled water. Be sure that all particles loosened by the above procedures have been removed from the components before reassembly.

6.4.2 CLEANING THE PISTON

Materials Needed:

- *Scouring pad (supplied with Seal Removal Kit)*
- *Lint free cloth saturated with Methanol*

Use the scouring pad included in the seal replacement kit to clean the piston. Gently squeeze the piston within a folded section of the pad and rub the pad along the length of the piston (it is not necessary to remove the piston from the housing to clean the piston). Rotate the pad frequently to assure the entire surface is scrubbed.

NOTICE Do not exert pressure perpendicular to the length of the piston, as this may cause the piston to break.

After scouring, use a lint-free cloth, dampened with alcohol, to wipe the piston clean.

6.4.3 REPLACING THE SEAL

Materials Needed:

- *Seal Insertion/Removal Tool (supplied with Seal Kit)*
- *Isopropanol/Water (50/50) or Methanol/Water (50/50)*

To replace the seal:

1. Place a high-pressure replacement seal on the rod-shaped end of the seal insertion/removal tool so that the spring is visible when the seal is fully seated on the tool.

2. Insert the tool into the pump head so that the open side of the seal enters first, facing the high-pressure cavity of the pump head. Be careful to line up the seal with the cavity while inserting.

3. Withdraw the tool, leaving the seal in the pump head. When you look into the pump head cavity, only the polymer portion of the seal should be visible.

4. Attach the pump head in the reverse order of removal.

5. Condition the seal by run the pump with a 50:50 solution of isopropanol (or methanol) and water for 30 minutes at the back pressure of about 10 psi or higher and a flow rate of about 50 mL/min using the column bypass feature.

NOTICE Use water and organic solvents to break-in new seals. Buffer solutions and salt solutions should never be used to break-in new seals. If buffers or salt solutions are employed, the seals will not set properly and have s short useful life.

6.4.4 CHECK VALVE CLEANING AND REPLACEMENT

Materials Needed:

- *Liquid Laboratory Detergent*
- *Seal Insertion/Removal Tool (supplied with Seal Kit)*
- *Isopropanol/Water (50/50) or Methanol/Water (50/50)*
- *Open-end wrench, 1/4" x 5/16"*
- *Torque wrench*

Check valve problems are frequently the result of small particles that are present in the mobile phase (e.g. dust and non-dissolved buffers and salts). Most problems can be solved by pumping a strong solution of liquid laboratory grade detergent through the check valves at a rate of 20 mL/min for one hour. If the pump is used for delivering solvents that are not miscible with the laboratory detergent, pump an intermediate solvent such as isopropanol for a few minutes before introducing the detergent solution. After washing with detergent, pump distilled water through the pump for 15 min. The output should be direct to a waste beaker during this operation. If cleaning the check valve does not solve the problem, it should be replaced.

To replace the check valve:

1. Remove the check valve holder from the pump head. The check valve capsule will then be accessible as shown in Figure 6-5.

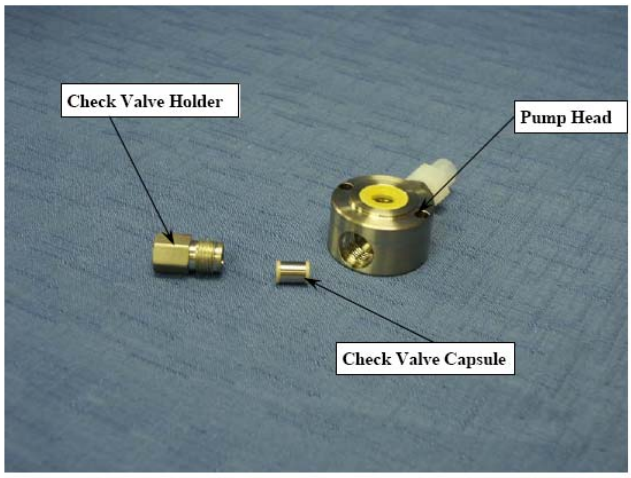


Figure 6-5: Check Valve and Check Valve Holder

2. The replacement check valve kit should be used to replace the defective valve. Ensure that the replacement check valve capsule is installed in the same orientation as the old check valve.

   Replace the check valve capsule in the prime purge valve in the same manner as those in the head.

3. To complete the installation, torque the check valve holder to 75 inch-pounds.

6.5 UV FLOW DETECTOR

6.5.1 CLEANING THE FLOW CELL

A noisy UV signal may be due to the accumulation of extraneous materials in the flow cell. Cleaning the flow cell can be done by bypassing the column and pumping a strong solvent such as methanol at a flow rate of 50 mL/min for a few minutes.

6.5.2 REPLACING THE LAMP

⚠ DANGER The lamp is accessed via the left door of the instrument. Power down the system and unplug it before you remove the left panel.

To replace the Lamp for the UV detector:

1. Remove the three screws on the shield covering the lamp (Figure 6-6).

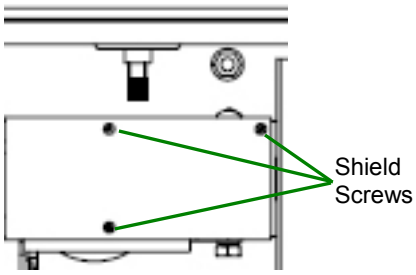


Figure 6-6: Lamp Shield Mounting Screws

2. Remove the Shield.
3. Unplug the lamp from the UV board.
4. Remove setscrew from lamp mount.
5. Pull old lamp from mount.
6. Install new lamp.
7. Tighten setscrew on lamp mount.
8. Plug in the new lamp to the UV board.
9. Attach shield and install the three screws that affix it to the system

NOTICE When a new lamp is installed, allow approximately 24 hours of use to stabilize the output. The lamp output will be noisy for the first 24 hours of use.

6.6 ELSD DETECTOR

6.6.1 NEBULIZER CLEANING PROCEDURE

The nebulizer can become blocked over time due to deposition of sample and particulate matter from the mobile phase. A dirty or blocked nebulizer can cause increased baseline noise and decreased sensitivity.

To clean the nebulizer:

1. Power off the unit and disconnect the power cord.
2. Remove the front door by gently pulling it towards you from the handle and set aside
3. Disconnect the stainless steel liquid inlet line from the nebulizer using a 1/4” wrench (Figure 6-7).

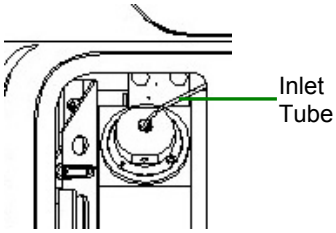


Figure 6-7: ELSD Detector

4. Remove the nebulizer from the drift tube by turning it firmly counterclockwise.
5. Remove the orange wear band from the nebulizer and set aside.
6. Disconnect the gas inlet (with gas tubing attached) from the nebulizer using the 5/16" wrench and set aside.
7. Place the nebulizer in a beaker filled with a methanol: water (50:50) solution.
8. Sonic ate the nebulizer in an ultrasonic bath for 10 minutes.

NOTICE Do NOT sonicate the wear band or nebulizer gas inlet.

9. If the nebulizer is still completely blocked, connect a high-pressure airline to the nebulizer inlet to help remove the blockage.
10. If the nebulizer is permanently blocked or cannot be cleaned, it should be replaced.
11. Replace the nebulizer wear band and nebulizer gas inlet.
12. Replace the nebulizer back into the unit by aligning the grooves and turning clockwise until the nebulizer locks firmly into place.
13. Replace the liquid and gas inlet lines to the nebulizer.
14. Replace the front door.

6.6.2 DRIFT TUBE CLEANING PROCEDURE

The drift tube can become dirty over time from sample and particulate matter from the mobile phase. A dirty drift tube can cause an increase in baseline noise and a decrease in sensitivity.

Materials Needed:

- *Open-end wrench, 1/4" x 5/16"*
- *Hex ball driver, 3/32" (long)*

The following procedure can be used to clean the drift tube:

⚠WARNING Make certain that the Reveleris™ system is powered down and unplug the power cord from the unit. Failure to unplug the system will expose the user to dangerous electrical current.

1. Allow the detector to cool for at least 30 minutes.
2. Remove the nebulizer if it has not already been removed. Refer to Section 6.6.1, Steps 2 - 5 for instructions.
3. Use the hex ball driver to remove the two screws on the removable cartridge on the front panel of the unit. Remove the cartridge and set aside.
4. Once the drift tube has been cleaned, reinsert the impactor cartridge and tighten the screws. Make sure the drain hole is located on the bottom of the tube as it is inserted.

NOTICE The drain hole located on the bottom of the impactor cartridge must be aligned with the drain hole inside the unit when the cartridge is reinserted. Otherwise, flooding could occur inside the unit.

7. APPENDICES

7.1 SPECIFICATIONS

REVELERIS SPECIFICATIONS	
Mode of Operation	Gradient Flash Chromatographic System
Fluidics	
Solvent Delivery System	4 Independent channels (4 L)
Gradient Formation	Binary Gradient with High Pressure Mixing
Flow Rate	4-200 mL/min
Flow Rate Accuracy	+/- 3% (10-125 mL/min)
Maximum Pressure	200 psi
Gradient accuracy (at 40 mL/min)	+/- 1.5 %
Gradient precision (at 40 mL/min)	< 1%
Sampling	
Sample Modes	Liquid or Solid Sample Modes, detected via micro switch
Sample Loader/Syringe Interface	Sample loaders between 5 g to 70 g, syringes from 10 mL to 140 mL can be employed
Sample Loader Plunger	Four sizes (15 mL, 25 mL, 75 mL, and 150 mL). Includes sleeve with locking mechanism to ensure safe use at pressures up to 200 psi.
Absorbance Detector	
Wavelength Range	200-360 nm
Number of Wavelengths monitored	2 wavelengths can be monitored and used to trigger fraction collection
ELSD Detector	
Light Source	Laser 1 mW
Detector Element	Silicon Photodiode
Mobile Phase Flow rate	50 µL- 3 .0 mL
Product Class	Class 1 laser product
Fraction Collection	
Triggers	UV signal, ELSD signal or Time Based
Collection Racks	Multi position racks for 12, 13, 16, 18, 25 m tubes, 480 mL bottles
Rack Size	5” x 12”, 2 racks for tubes, 1 rack for bottles)

Communications:	
Operating Parameter Selection & Display:	Windows-Based Graphical LCD with full QWERTY keyboard
Power Requirements:	120/240V, 50/60Hz, 10 A
Dimensions:	16.75" H x 21.50" W x 19.75" D (42.54cm H x 54.61 cm W x 50.16 cm D)
Weight:	75 lbs (34.1 kg)
Environmental Operating Temperature:	15 to 40^{0}C
Relative Humidity:	10 – 90%, Non-Condensing
Maximum Altitude:	2000m

7.2 REPLACEMENT PARTS

REPLACEMENT PARTS	
Part No.	**Description**
Chromatography Sorbents	
5146130	GraceReveleris™ 4g Silica Cartridge (20/pack)
5146131	GraceReveleris™ 12g Silica Cartridge (20/pack)
5146132	GraceReveleris™ 40g Silica Cartridge (15/pack)
5416133	GraceReveleris™ 80g Silica Cartridge (12/pack)
5416134	GraceReveleris™ 120g Silica Cartridge (10/pack)
5416135	GraceReveleris™ 330g Silica Cartridge (4/pk)
8621250	GraceReveleris™ Silica TLC Plates 20x20cm (25/pack)
Fraction Collector Trays	
8623401	Fraction Collector Trays, 12mm OD x 75mm H (126 vials per tray)
8623402	Fraction Collector Trays, 13mm OD x 100mm H (112 vials per tray)
8623403	Fraction Collector trays, 16 x 125mm tube racks (84 vials per tray)
8623404	Fraction Collector trays, 16 x 150mm tube racks (84 vials per tray)
8623405	Fraction Collector trays, 18 x 150mm tube racks (60 vials per tray)
8623406	Fraction Collector trays, 25 x 150mm tube racks (36 vials per tray)
8623400	Fraction Collector trays for French Square Bottles (9 vials per tray)
Fraction Tubes	
8623413	12x75mm (1000, 4 pkg. of 250)
8623414	13x100mm (1000, 4 pkg. of 250)
8623415	16x125mm (1000, 4 pkg. of 250)
8623416	16x150mm (1000, 4 pkg. of 250)
8623410	18x150mm (1000, 4 pkg. of 250)
8623411	25x150mm (1000, 4 pkg. of 250)
8623412	480 mL French Square Bottles (24)

Part No.	**Description**
Sample Delivery Tools	
5148510	Adjustable Solid Loader Plungers, 5 g (15 and 25 mL tubes)
5148511	Adjustable Solid Loader Plungers, 25g (75 mL tubes)
5148512	Adjustable Solid Loader Plungers, 60g (150 mL tube)
5142050	Empty Solid Loaders, 15mL (100/pack)
5142051	Empty Solid Loaders, 75mL (100/pack)
5147808	Empty Solid Loaders, 150mL (1000/pack)
5142035	Filled Solid Loaders, 5g (15mL) (20/pack)
5142034	Filled Solid Loaders, 25g (75mL) (16/pack)
5147772	Filled Solid Loaders, 60g (150mL) (12/pack)
5148507	Plastic Luer Lock Syringe, 5mL (rubber free plunger) (5/pack)
5148500	Plastic Luer Lock Syringe, 10mL (rubber free plunger) (5/pack)
5148504	Plastic Luer Lock Syringe, 30mL (rubber free plunger) (5/pack)
5148505	Plastic Luer Lock Syringe, 50mL (rubber free plunger) (5/pack)P

7.3 CONTACT INFORMATION

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7.4 WARRANTY, RETURNS, AND REPAIRS

WARRANTY

Grace Davison Discovery Sciences (Grace) warrants its products against defects in workmanship or material under normal use or service for one year from delivery. All obligations or liabilities under this warranty are limited to repair or replacement, at Grace's option, F.O.B. Deerfield, IL, of parts that are returned, freight prepaid, and which are accepted as being defective upon inspection by Grace. The remedies herein provided are the sole and exclusive remedies for product defects.

Components and/or parts that are subject to normal wear and/or are scheduled for routine replacement within the warranty period and which are subjected to effects of corrosion or deterioration by chemical or other action are excluded from the above warranty if Grace determines that the corrosion or deterioration by chemical or other action is caused by inadequate facilities, operating conditions, or utilities.

Equipment and components may only be returned with Grace's prior approval and must bear a Grace Return Authorization Number to be returned. Call the Grace Customer Service Department to obtain a Return Authorization Number.

The warranties for products, equipment, and accessories manufactured by others and distributed by Grace shall be limited to the manufacturer's warranties, which will be passed along whenever possible to the buyer directly or through Grace.

Any modification made to equipment covered by this warranty that is made without written permission from Grace voids the warranty. Grace reserves the right not to honor this warranty if the products are obviously mishandled by the user. Grace disclaims all other warranties, express and implied, not included herein, INCLUDING IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. Grace assumes no responsibility for consequential, economic, or incidental damages of any nature. Grace assumes no responsibility for on-site reinstallation costs arising out of any future alleged failure of any of its products or their accessories. The buyer assumes all risk and liability for results obtained by the use of Grace products.

This warranty supersedes any and all previous warranties unless otherwise agreed in writing by Grace at the time of sale.

DAMAGED SHIPMENTS

The Interstate Commerce Commission has held that carriers are responsible for both concealed and visible damage occurring during transit. Unpack the shipment upon receipt and check for concealed damage even if no visible damage is apparent. If concealed damage is discovered, stop unpacking the unit, request an immediate inspection by the local carrier agent, and obtain a written report of the findings to support a claim. This request must be made within 15 days of receipt; otherwise, the claim will not be honored by the carrier. Do not return damaged goods to Grace without first obtaining an inspection report and calling Grace for a Return Authorization Number.

FILING OF CLAIMS

After a damage inspection report has been obtained, Grace will cooperate in replacing damaged goods and in handling of claims, which have been initiated by either party.

RETURNS

If it is necessary to return any material to Grace, please call Grace's Customer Service Department for a Return Authorization Number and forwarding instructions. No returns may be made without a Return Authorization Number.

REPAIRS

Grace is the only organization that is authorized to service or repair the Reveleris™ instrument. Any repairs performed without notifying Grace will void the warranty. To obtain repair service, call Grace's Customer Service Department for instructions.

7.5 MOBILE PHASES AND STATIONARY PHASES FOR FLASH CHROMATOGRAPHY

⚠CAUTION Users should be aware of the hazards associated with their mobile phase. Always use appropriate personal protective equipment such as safety goggles or glasses, Lab coat and gloves when the instrument is in operation or when handling mobile phase. Refer to the manufacturer's Material Safety Data Sheet (MSDS) for detailed hazard information."

A typical flash chromatographic separation is performed using a cartridge containing a stationary phase such as 40-63 µm silica and a mobile phase such as hexane: ethyl acetate (70:30) at a flow rate of 40 mL/min at a moderate pressure (typically less than 100 psi). Flash Chromatography provides the ability to separate gram size samples (e.g. that might be obtained from an organic synthesis) into its various components in a short period of time. A typical flash chromatographic separation is shown in Figure 1-1, where 9.2 g of a mixture of dimethyl phthalate and toluene was separated with baseline resolution in approximately four minutes.

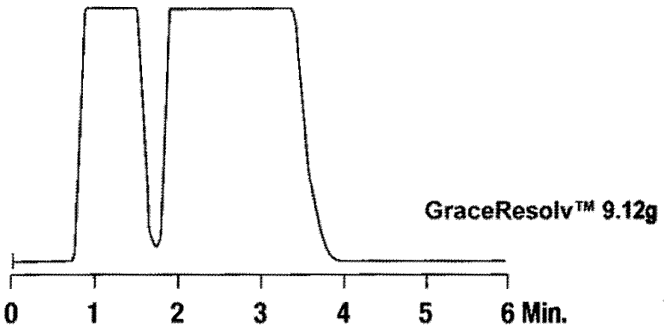

Figure 7-1: Typical Flash Chromatography Separation (Sample = 9.12 g of a mixture of dimethyl phthalate and toluene, Mobile Phase = Hexane: Ethyl Acetate (70:30), Flow rate = 40 mL/min.

In flash chromatography, as in analytical or preparative HPLC, the separation is a function of the relative interaction of the various compounds in the sample with the stationary phase and the mobile phase. In the example shown above, the stationary phase is more polar than the mobile phase and the polar compound (dimethyl phthalate), which has a stronger interaction with the stationary phase than with the mobile phase will be more strongly retained by the column and will have a longer retention time than the non-polar compound (toluene) . This mode of chromatography is commonly termed "normal phase chromatography".

A broad range of solvents are commonly used in flash chromatography (see Table 7-1). In many cases, preliminary separations are developed using small samples and thin layer chromatography plates to determine to optimum solvent for a sample.

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