ACCQPrep HP150

Operation Guide

TELEDYNE ISCO
Everywhereyoulook™

69-5233-899

Rev C, January 2025

Safety Overview

Before installing, operating, or maintaining this equipment, it is imperative that all hazards and preventive measures are fully understood. While specific hazards may vary according to location and application, heed the following general warnings:

⚠ WARNING

Liquids associated with this instrument may be classified as carcinogenic, biohazard, flammable, or radioactive. Should these liquids be used, it is highly recommended that this application be accomplished in an isolated environment designed for these types of materials in accordance with federal, state, and local regulatory laws, and in compliance with your company's chemical/hygiene plan in the event of a spill.

⚠ WARNING

Avoid hazardous practices! If you use this instrument in any way not specified in this manual, the protection provided by the instrument may be impaired.

⚠ WARNING

If you are using flammable solvents or chemicals with this system, vapor concentration levels may exceed the maximum exposure levels as recommended by OSHA Guide 1910.1000. To reduce those levels to a safe exposure, Teledyne ISCO recommends that you place the system in a laboratory hood designed for the purpose of ventilation. This hood should be constructed and operated in accordance with federal state and local regulations. In the event of a solvent or chemical spill, your organization should have a plan to deal with these mishaps. In all cases, use good laboratory practices and standard safety procedures.

Hazard Severity Levels

This manual applies *Hazard Severity Levels* to the safety alerts. These three levels are described in the sample alerts below.

⚠ CAUTION

Cautions identify a potential hazard, which if not avoided, may result in minor or moderate injury. This category can also warn you of unsafe practices, or conditions that may cause property damage.

⚠ WARNING

Warnings identify a potentially hazardous condition, which if not avoided, could result in death or serious injury.

⚠ DANGER

DANGER – limited to the most extreme situations to identify an imminent hazard, which if not avoided, will result in death or serious injury.

Hazard Symbols

The equipment and this manual use symbols used to warn of hazards. The symbols are explained in the table below.

Hazard Symbols	
Warnings and Cautions	
	The exclamation point within the triangle is a warning sign alerting you of important instructions in the instrument's technical reference manual.
	The lightning flash and arrowhead within the triangle is a warning sign alerting you of "dangerous voltage" inside the product.
Symboles de sécurité	
	Ce symbole signale l'existence d'instructions importantes relatives au produit dans ce manuel.
	Ce symbole signale la présence d'un danger d'électocution.
Warnungen und Vorsichtshinweise	
	Das Ausrufezeichen in Dreieck ist ein Warnzeichen, das Sie darauf aufmerksam macht, daß wichtige Anleitungen zu diesem Handbuch gehören.
	Der gepfeilte Blitz im Dreieck ist ein Warnzeichen, das Sei vor "gefährlichen Spannungen" im Inneren des Produkts warnt.
Advertencias y Precauciones	
	Esta señal le advierte sobre la importancia de las instrucciones del manual que acompañan a este producto.
	Esta señal alerta sobre la presencia de alto voltaje en el interior del producto.

For Additional Information

Technical assistance for the Teledyne ISCO ACCQ*Prep* HP150 can be obtained from:

Teledyne ISCO
4700 Superior St.
Lincoln NE 68504

Phone: (800) 775-2965 or (402) 464-0231
Fax: (402) 465-3001
E-mail: IscoService@teledyne.com

ACCQ*Prep* HP150

Table of Contents

Section 1 Introduction

Section 2 Configuration

Section 3 Operation

Section 4 PeakTrak

Section 5 Maintenance

Appendix A

ACCQ*Prep* HP150

Section 1 Introduction

1.1 Overview

This Operation Guide provides the following:

- Safety information
- Instrument configuration options
- A basic system operation guide
- Networking instructions to connect the ACCQ*Prep* HP150 with Windows and iOS operating systems
- Certification and warranty information

1.2 Product Overview

The Teledyne ISCO ACCQ*Prep* HP150 chromatography system combines high resolution and productivity in a small footprint. It features easy to use software, programmable gradients, UV detection and peak collection, and automatic detection of collection tube racks. Its small size makes it a great personal system and well-suited for operation within chemical hoods and other limited indoor spaces. The extended pressure capability supports operation of columns at high flow rates for maximum throughput or added tolerance for high back pressures as columns age.

⚠ WARNING

Avoid hazardous practices! If you use this instrument in any way not specified in this manual, the protection provided by the instrument may be impaired; this may increase your risk of injury.

The ACCQ*Prep* HP150 is available in different configurations to meet your needs. An optional Solvent Selector Valve Module supports up to three choices for solvent A and three different choices for solvent B. Detection options include UV, UV/Vis, ELSD, and a mass spectrometer capable of detecting your compounds with up to four individual mass signals, including a range of masses. Automation options include the AutoInjector, the AutoSampler, and the Column Selector modules.

AutoInjector Module

The AutoInjector is an optional module for the ACCQ*Prep* system. The AutoInjector module is necessary for stacked injections. When installed, it performs automated, repetitive compound injections completely unattended. This allows you to automatically purify larger amounts of compound than can be purified with a single separation run, increasing productivity. And, when the optional column switcher and solvent switching valves are installed, you can test different chromatographic conditions for a single sample. Following PeakTrak's on-screen instructions for the AutoInjector eliminates carry-over between samples.

AutoSampler Module

The AutoSampler is an optional module for the ACCQ*Prep* system. When installed, it automates purification when you have several different samples that require multiple injections and different chromatographic conditions. It can be used unattended: it moves its probe robotically and automatically washes it between samples to eliminate carry-over. The AutoSampler uses RFID rack swapping technology to allow you to replace completed racks.

Column Selector Module

The Column Selector module supports up to four Prep HPLC columns ranging from 4.6 mm up to 50 mm inner diameter (Larger columns may be used at less than optimum flow rates.) PeakTrak software operates the module's column selector valve.

Figure 1-1 ACCQPrep HP150

ACCQ*Prep* HP150 – This Prep HPLC system has flow rates from 1–150 mL/min, with up to 6000 psi capability. A two component gradient can be formed from up to six solvents. An additional third solvent modifier pump is available to pump a fixed percentage of modifier. The system includes active solvent level sensing and detection of a full waste container. The base system includes two fraction collection racks. Two different AutoSampler options extend the number of collection racks on the system to four or six respectively. The RFID tagged racks can be replaced while in operation with new racks for practically limitless fraction collection.

ACCQ*Prep* HP150 with optional ELSD – This system has the same high performance features as the ACCQ*Prep* HP150 system but also includes an internal evaporative light scattering detector (ELSD). During operation, this detector can be combined with the UV (200–400 nm) or UV-vis (200–800 nm) detection to isolate visible and UV absorbing compounds as well as compounds with little or no chromophores.

ACCQ*Prep* HP150 with optional PurIon Mass Spectrometer – This system has the same features as the ACCQ*Prep* HP150 but also includes a mass spectrometer with a detection range of 10 to 1200 Daltons (Da) (PurIon S) or 10–2000 Da (PurIon L systems). During purification, this system can be combined with the UV (200–400 nm) or UV-Vis (200–800 nm) detector to isolate visible and UV absorbing compounds as well as compounds with specific molecular weights or mass ranges.

1.3 Operating Overview

The ACCQ*Prep* HP150 system is equipped with a capacitive touchscreen display for local control.

The system also supports TCP/IP communication. This allows direct control of the system by an external computer between Ethernet ports of the ACCQ*Prep* HP150 system and the computer.

TCP/IP communication also allows remote control of the system via an established network. Remote controlling devices on the network can be Windows or Apple desktop and laptop computers or Apple iOS mobile devices (iPod Touch, iPhone, and iPad).

> ☑ ***Note***
>
> Teledyne recommends that you obtain assistance from your Information Technology department before attempting direct or network connections. See *Technical Note 28 Networking Guidelines* on the Teledyne LABS website for more information.

1.3.1 Multiple Control Possibilities

The system can be accessed from the built-in touch panel and by up to ten network devices. The touch panel shares control with all connected devices. The system performs the most recent command from any control input.

1.3.2 File Storage

To support operation from a variety of direct and network connections, the software and all files are stored in the ACCQ*Prep* HP150. This ensures that your compound purification methods and run history files can be viewed from any connection. Optionally, run files may be saved to a USB flash drive, a networked controlling computer, or a network drive.

1.4 Safety Components

Before installing, operating, or maintaining this equipment, it is imperative that all hazards and preventive measures are fully understood. Refer to the Safety Overview section at the front of this operation guide for general safety information.

1.4.1 Power disconnects

The power cord is a safety disconnect for the ACCQ*Prep* (Figure 1-2).

To remove power from the ACCQ*Prep,* remove the power cord by pulling it straight out from the power inlet connector. The circuit breaker is located adjacent to the power inlet connector. If an internal circuit fault occurs, this breaker will trip. It can be reset by pressing the end of the rocker labeled "|". In addition, switching the breaker manually to the "O" position removes power from the internal operating components.

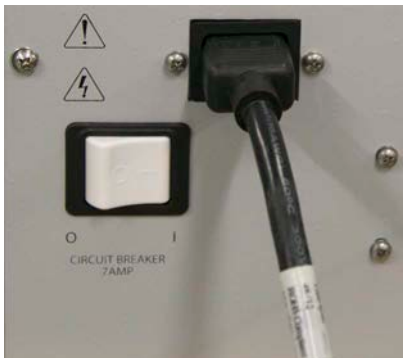


*Figure 1-2 Location of power cord for the ACCQ*Prep

1.5 Specification

Table 1-1 ACCQ*Prep* HP150	
Dimensions of ACCQ*Prep* (Footprint without column installed)	27.5 x 14.0 x 20.0 in (69.9 x 35.6 x 50.8 cm)
Dimensions of ACCQ*Prep* AS 2x1	18.9 x 14.0 x 19.3 in (48.0 x 35.6 x 49.0 cm)
Weight (Including AutoInjector, Column Selector Valve Module, Solvent Selector Valve Module, and column cover)	93.2 lbs (42.3 kg) 106 lbs (48 kg) with optional Evaporative Light Scattering Detector (ELSD)
Weight of ACCQ*Prep* AS 2x1	27.3 lbs (12.4 kg)
User Interface	15" touchscreen
Power	Input voltage range from 100 to 240 VAC, 300 VA maximum. Line cord is the disconnect device. Power connection is via IEC 60320 C14 power inlet.
Line Frequency	50/60 Hz
Ambient Temperature	20 to 40 °C (maximum temperature must be at least 15 °C below the boiling point of the solvent)
Humidity (when connected to power)	90% relative humidity maximum at 20 to 40 °C (non-condensing)
Flow Rate Range	1-150 mL/min, ELSD fraction collection limited to 4-150 mL/min
Flow Rate Accuracy (tested with water at 6.9 bar or 100 psi)	± 2%
Pressure Limit	414 bar (6000 psi) derated linearly from 6000 psi at 75 mL/min to 3000 psi at 125 mL/min; and to 2000 psi at 150 mL/min.
Pressure Accuracy	5% of full scale
Gradient Formation	Binary gradient 2 solvent inlets. Optional Solvent Selector Valve Module expands to 3 solvents each for A & B. Optional Solvent Modifier Module allows addition of third solvent for ternary gradient formation.
Gradient Accuracy:	±1% of full scale (typical)
Peak Detection Modes	Slope or threshold
Flow Cell Pathlength	0.3 mm, ±25% (Standard, other pathlengths available)

Table 1-1 ACCQ*Prep* HP150	
UV Detection Wavelength	200 to 400 nm, optional 200 to 800 nm UV-Vis
UV Lamp Source	Deuterium
Wavelength Accuracy	±5 nm
Fraction Accuracy	±[2mL + (flow rate ÷ 60)]
Optional ELSD	
Gas Inlet Pressure	60 to 70 psig
Gas Consumption	<2.5 SLPM
Spray Chamber Temperature	Setting range: 10 to 60 °C limited to minimum of 5 °C below ambient
Drift Tube Temperature	Setting range: 30 to 90 °C Must be 5 °C above spray chamber temperature Minimum temperature is 5 °C above ambient Maximum temperature is 60 °C above ambient
Electrical Safety per EN 61010-1	
Pollution Degree	2
Installation Category	II
Maximum Altitude	2000 meters
Note 1. All specifications are subject to change.	

Table 1-2 ACCQ*Prep* HP150 with PurIon System	
Dimensions (H x W x D)	ACCQ*Prep*: 27.5 x 14.0 x 20.0 in (69.9 x 35.6 x 50.8 cm) Mass Spectrometer 26 x 11 x 22 in (66 x 28 x 56 cm) Roughing Pump 10 x 9 x 18 in (26 x 23 x 46 cm)
ELSD Detection	Option that can be combined with either UV or UV-Vis
Mass Spectrometry Detection	10 – 1200 Dalton for S model, 10-2000 for L model, 1 Dalton Resolution
	Electrospray Ionization (ESI) or optional Atmospheric Pressure Chemical Ionization (APCI)
	Simultaneous positive and negative ionization for both S and L models.
Note 1. All specifications are subject to change.	

Table 1-3 Component Materials List	
Chromatographic Tubing	316 stainless steel tubing, PEEK, and Fluoropolymer
Drain Tubing	Vinyl with FEP liner
Chromatographic Valves	PEEK, PPS, perfluorelastomer (FFKM)
Flowcell	303 SST, Type ES Quartz, SIMRIZ® SZ485®
Chromatography Pump	316 Stainless Steel, UHMWPE, ETFE, ruby, sapphire, and zirconia

Table 1-3 Component Materials List	
Pressure Transducer	316 and 17-4 PH stainless steels and perfluorelastomer (FFKM)
AutoSampler Wash	Pharm-A-Line™, polypropylene

ACCQ*Prep* HP150

Section 2 Configuration

2.1 Configuration of the ACCQ*Prep*

The CONFIGURATION window (TOOLS > CONFIGURATION) groups its settings on tabs placed along its top.

Solvent settings, the default tube volume, the system name, and the time zone setting (Figure 2-1) can be found under TOOLS > CONFIGURATION > INSTRUMENT CONFIGURATION.

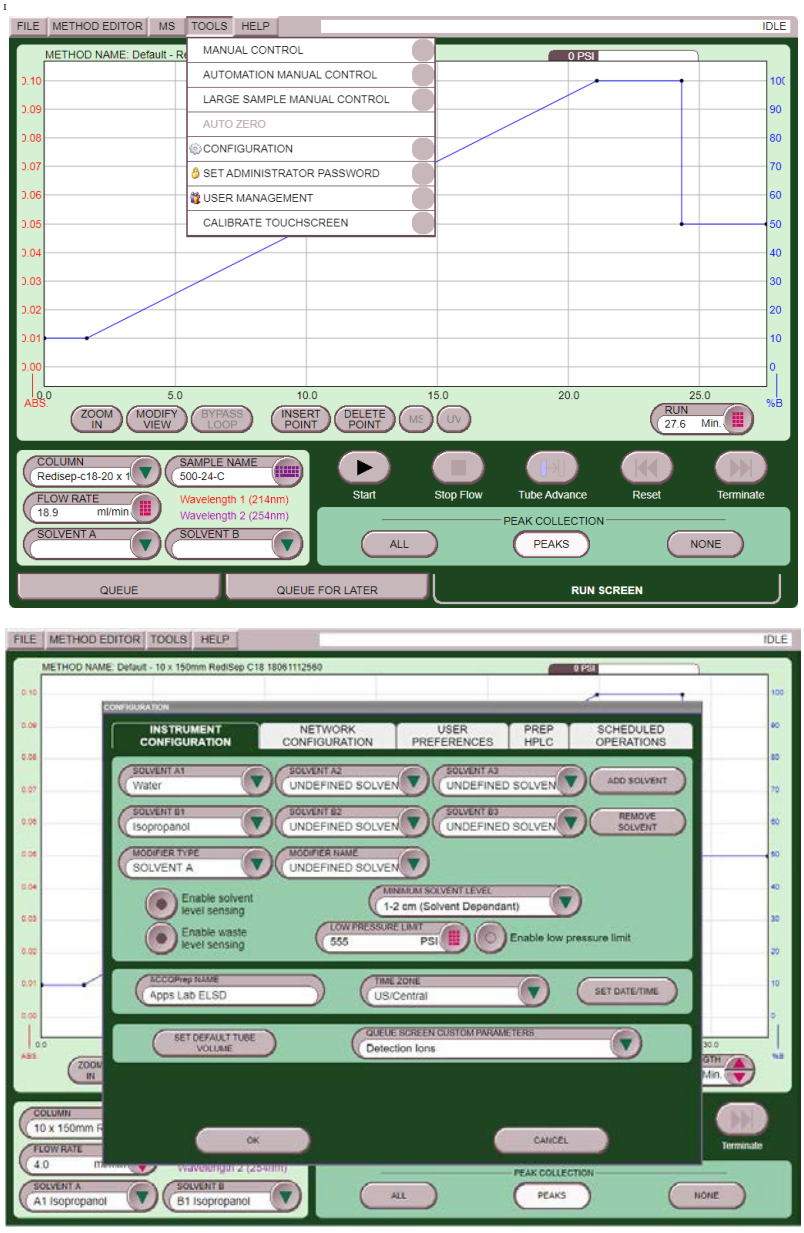


Figure 2-1 Instrument Configuration

> **☑ Note**
>
> You can back up and restore instrument settings and data using Help > Backup / Restore (Section 4.2.6).

When the Solvent Selector Valve Module is present, you can select the solvents for both SOLVENT A and B from the INSTRUMENT CONFIGURATION tab (Figure 2-1). If the valve is absent, the solvents are selected on the MAIN RUN screen, and these options are not shown in the CONFIGURATION window. INSTRUMENT CONFIGURATION will still show the ADD SOLVENT and REMOVE SOLVENT buttons to edit the list.

If you do not see your solvent name on the list, select ADD SOLVENT, then type in the solvent name. You can also REMOVE SOLVENT names that you no longer use.

Modifier Type (ACCQPrep HP150 with 3rd Solvent Modifier Module) – This allows you to choose which solvent your modifier is dissolved in.

Solvent Level Sensing –

- ENABLE SOLVENT LEVEL SENSING monitors the solvent level in each of the supply containers.
- ENABLE WASTE LEVEL SENSING stops the system when the waste level reaches approximately one inch above the sensing line.

Low Pressure Limit – A pressure (in psi). In a high pressure system, if the pressure is below this level for a significant time, it is likely that there is a leak somewhere. When ENABLE LOW PRESSURE LIMIT is selected, the system will shut down when the low pressure limit is not reached.

ACCQPrep Name – A label for the system. Reports printed by the system have this label, so a unique system name identifies the source of the report. Useful when more than one system is in use.

Set Date/Time – The date and time, including a time zone. All separations not named previously are tagged with the date and time of operation. Usually the local date and the current time are entered. If the selected time zone observes daylight savings time, the system automatically changes the time as required. SET DATE/TIME requires the administrator password.

Set Default Tube Volume – Determines how much is collected in each test tube. A suggested amount is preset, but if you prefer another level of fluid in each test tube, you can enter the tube volume you would like to use. This amount becomes the default used for new methods, but it can be changed within the METHOD EDITOR.

Queue Screen Custom Parameters – The parameter chosen here displays a column of editable parameters on the QUEUE tab of the MAIN screen.

- UV/UV-VIS THRESHOLD sets the threshold for detection.
- DETECTION IONS selects the ions for the PurIon mass spectrometer.

If data isn't entered, the default threshold is used, or the mass spectrometer will not collect data unless a method that has these parameters entered is used.

2.2 Network Configuration

TOOLS > CONFIGURATION > NETWORK CONFIGURATION is used to connect the system to a network. While not required, doing so allows you to remotely view the user interface for monitoring or controlling the system, print to a network printer, or automatically save data files directly to the network.

To connect to a network, you need access to IP addresses and network information from your IT department. For more information about networking, see *TN28 Networking Guidelines for CombiFlash Products* available at www.teledynelabs.com.

See 4.3.12 *Network Configuration Options* for more information about using PeakTrak to network the ACCQ*Prep* HP150.

2.3 User Preferences

TOOLS > CONFIGURATION > USER PREFERENCES sets display units and the language for the logged-in user.

> **Note**
>
> You can back up and restore instrument settings and data using Help > Backup / Restore (Section 4.2.6).

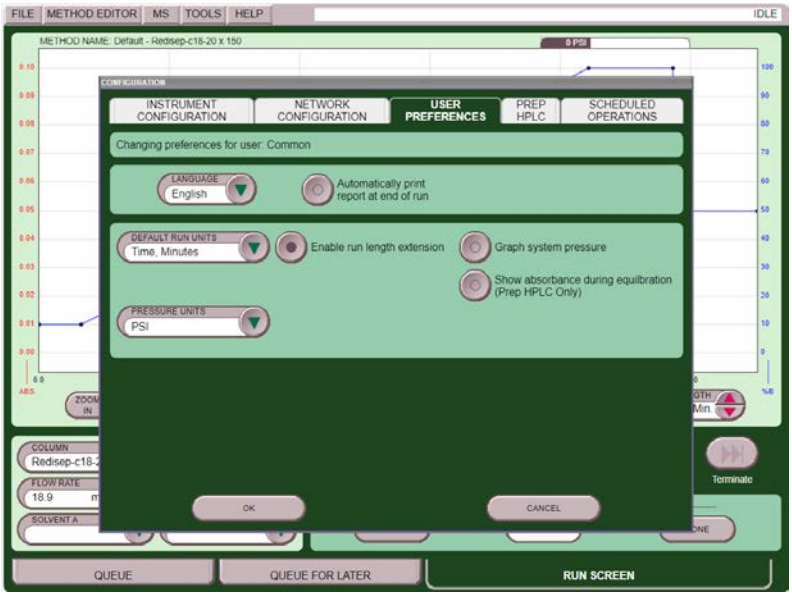


Figure 2-2 User Preferences configuration

Language – Sets the language for the user interface; several languages are available. If you have set up the system for multiple users, each user can select a preferred language.

Automatically Print Report – Prints a report at the end of a run if you are connected to a network.

Graph System Pressure – Shows the system pressure during a separation. This information is useful for troubleshooting a system that doesn't appear to operate as expected.

For normal operation, selecting GRAPH is not recommended. This information is always stored with the run data for later review, so if a problem is suspected, this parameter can be selected and the suspect separation opened to review the pressure profile during the separation.

The pressure trace doesn't have a vertical scale with pressure. To view the actual pressure at a point in time, refer to the status bar in the upper right corner of the screen during a separation. If you are reviewing a previous separation, this information isn't available on the screen, but the profile of the trace is often adequate for trouble-shooting.

Show Absorbance During Equilibration – Shows absorbance of the system during equilibration to verify baseline stability.

Pressure Units – Instructs the system to display pressure as PSI or Bar.

2.4 Prep HPLC

Columns and methods can be defined and deleted using TOOLS > CONFIGURATION > PREP HPLC (Figure 2-3).

> **Note**
>
> You can back up and restore instrument settings and data using Help > Backup / Restore (Section 4.2.6).

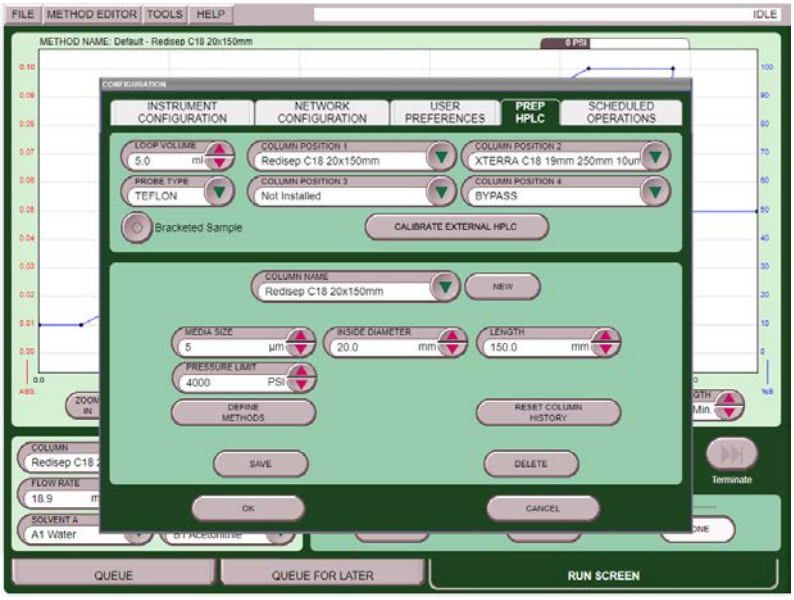


Figure 2-3 Prep HPLC configuration

Loop Volume – The ACCQ*Prep* uses the installed loop volume for several calculations within the software, including ELSD peak alignment and focused gradient optimization. It is important to update this value if loops are changed on the

system. It ensures that the samples entered do not exceed the loop volume amount per injection. For manual injections, a warning displays if half of the loop volume is exceeded, but it does not limit the injection amount that can be programmed. When using an AutoInjector or the AutoSampler module, the injection volume error will only indicate when sample may be lost to the injector. This warning can be ignored.

Column Position – This allows you to define a column(s) used on the instrument. If you do not have the Column Selector Valve Module (CSV-4), you can define multiple columns and select which column is currently connected. If you have the CSV-4 installed, you can configure the columns that are installed. The name of the column used for the separation is recorded on the report. This name can be descriptive for the column, or identify it exactly using the column serial number.

Probe Type – The probe type, described by material. The standard PTFE probe supplied with the instrument is the default. You can also select the optional "Steel" needle available for piercing vials with an aluminum foil cover to prevent evaporation. This optional probe is NOT able to pierce silicone septa. The steel probe has a slightly different internal volume that requires a different compensation volume.

Bracketed Sample – ""Brackets" the sample injection with air and a user supplied solvent to address potential solubility issues when injected. When selected, each separation defaults to using a bracketed sample injection protocol that can still be overridden for individual samples. Further details can be found in Section 3.3.8.

2.4.1 Define a Column

Column size and type parameters are important to update for the system to work optimally. The calculated column volume from these parameters are used in several key features of the system including the focused gradient calculator and ELSD peak alignment.

To define a column at CONFIGURATION > PREP HPLC:

1. Select NEW. If you are accessing CONFIGURATION from the touchscreen, an on-screen keyboard opens.
2. Enter the name of the column. Select ENTER (touchscreen) or OK (remote computer).
3. To complete the column definition, enter the MEDIA SIZE and COLUMN DIMENSIONS.
4. Select the column's INSIDE DIAMETER. A flow rate for a default method is selected based on scaling up the linear velocity of a 4.6 mL analytical column running at 1 mL/min.
5. Select the LENGTH of the column. This provides the suggested default method with about 10 column volumes for the gradient portion of the separation, along with an initial isocratic portion, a strong solvent portion to wash the column, and a portion that returns the column to a suggested storage fluid concentration.

6. The PRESSURE LIMIT is set by default to 3000 psi (207 bar), which is a typical limit for preparative HPLC columns, but this can be changed for each column to a suitable pressure not to exceed the system pressure limit of 6000 psi.
7. SAVE the default method associated with the column.

To customize the new method while at CONFIGURATION > PREP HPLC, select DEFINE METHODS, then EDIT to view and modify all the method parameters.

2.4.2 Delete a Column

To delete a column at CONFIGURATION > PREP HPLC:

1. Be sure that the column to be deleted is not selected in a COLUMN POSITION field.
2. Select the column you would like to delete from the COLUMN NAME list.
3. Select DELETE.
4. If you get an error message stating that the method is currently in use, close the CONFIGURATION window. Go the to RUN screen and select a different COLUMN.

2.4.3 Creating Other Methods

Methods created at CONFIGURATION > PREP HPLC are associated with a particular column when created. If multiple methods are associated with a single column, these methods can be easily accessed whenever a particular column is selected. These methods are be shown in the column selection lists in the order the methods were created.

To create the new method:

1. Select TOOLS > CONFIGURATION. The CONFIGURATION window opens.
2. On the PREP SFC tab, select the Column Name that you want to associate with the new method.
3. Select DEFINE METHODS (Figure 2-4). The PREP HPLC tab hides column options and shows method options.

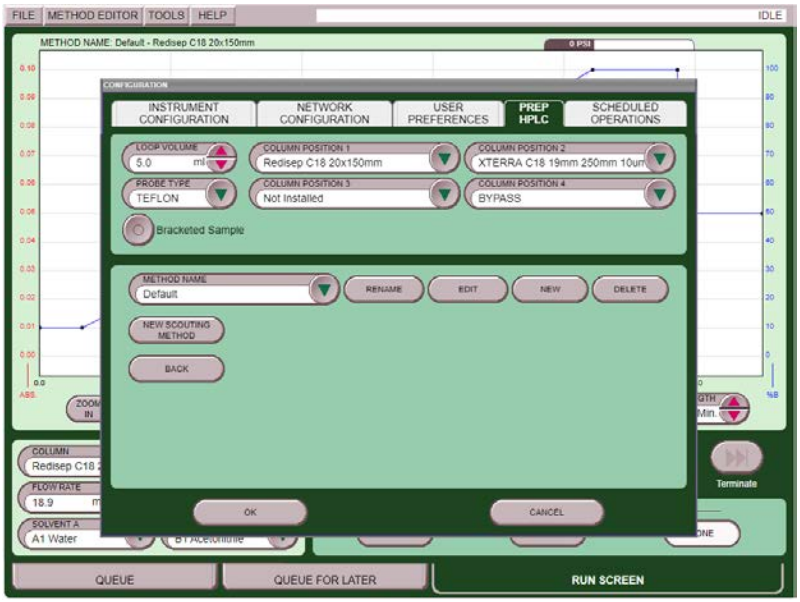


Figure 2-4 Define Methods

4. Select NEW. If you are accessing CONFIGURATION from the touchscreen, an on-screen keyboard opens.
5. Enter in the name you have chosen. Select ENTER (touchscreen) or OK (remote computer).
6. The newly created method uses the default method parameters. To modify the new method:
   a. Select EDIT. The METHOD EDITOR opens.
   b. Make changes as needed in the METHOD EDITOR.
   c. EXIT when completed.

2.4.4 Creating Scouting and Focused Gradients

PeakTrak offers an integrated tool for method optimization called the Focus Gradient Generator. This feature runs a scouting gradient on your sample to find the ideal focused gradient conditions for your separation. Focused gradients offer a quick way to greatly improve resolution around the peaks of interest.

To use the Focus Gradient Generator feature, you must first configure your column to use a Scouting Gradient:

1. Select TOOLS > CONFIGURATION. The Configuration window opens.
2. On the PREP HPLC tab, select the COLUMN NAME that you want to associate with a scouting gradient.

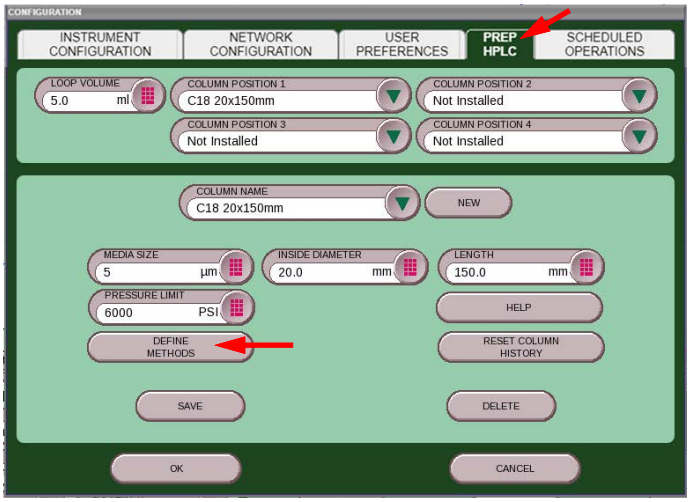


Figure 2-5 Column Configuration

3. Select DEFINE METHODS. Options for defining methods are then made available on the PREP HPLC tab.
4. Select NEW SCOUTING METHOD. This opens the SCOUTING METHOD FOR FOCUSED GRADIENT window (Figure 2-4).
5. Define the FLOW RATE, the INITIAL %B, and the FOCUS RANGE, and assign a METHOD NAME to the scouting method. This name is then listed among the methods that can be selected to run on the column that you selected in Step 1.

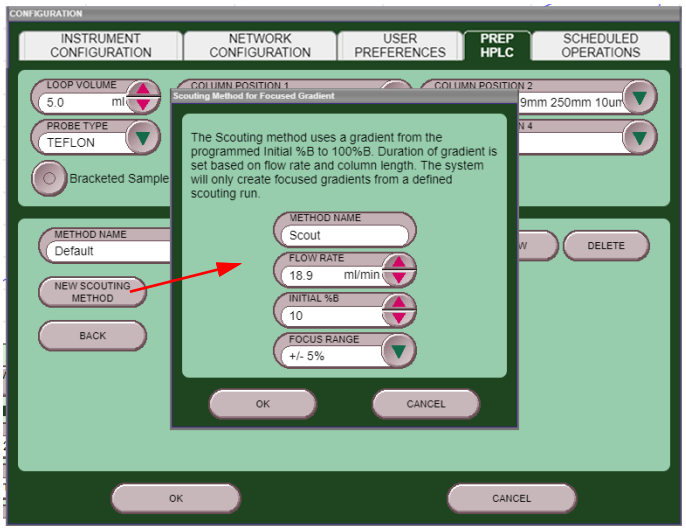


Figure 2-6 Scouting Method definition window

After a scouting gradient has been defined, load the column and the scouting method:

1. In the Main screen, select the column that you want to use for the run.

2. Select the scouting method you previously defined for that column (Figure 2-7).

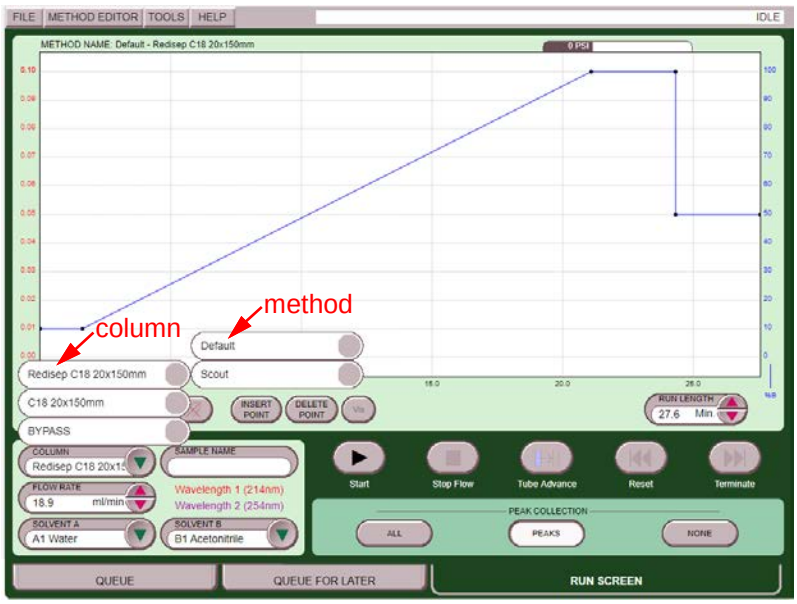


Figure 2-7 Loading the Scouting method

3. After the column and method are selected, select START.
4. Input your injection parameters as usual, and let the run proceed (Figure 2-8).

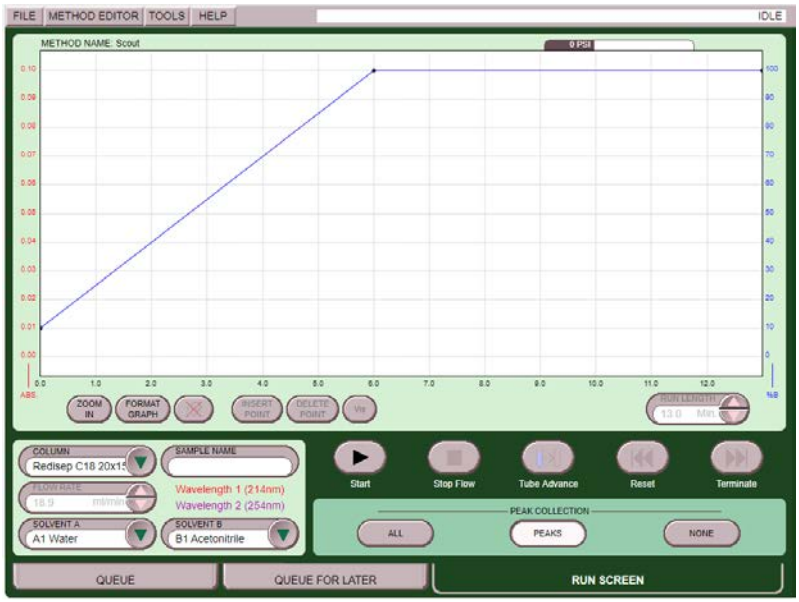


Figure 2-8 Scouting Run screen

A RUN screen appears upon completion of the run:

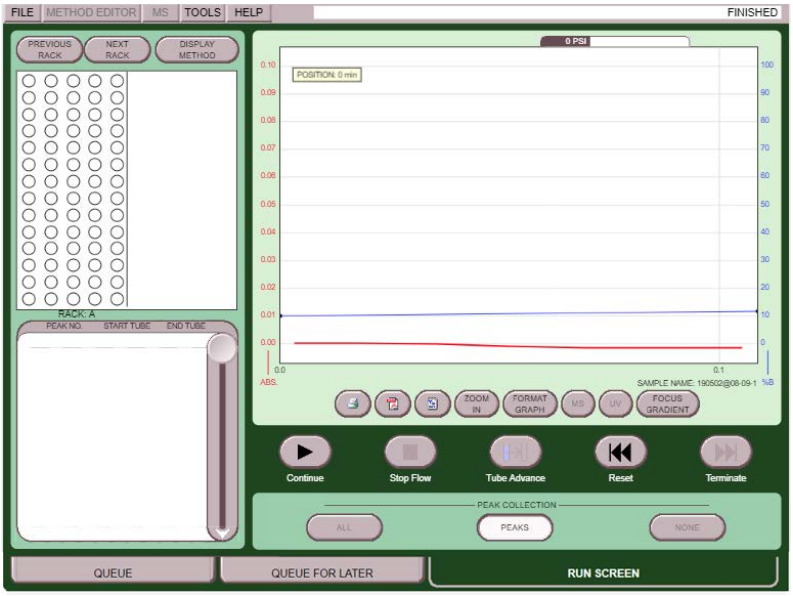


Figure 2-9 Run screen at end of run

Next, define the focused gradient:

1. Select FOCUS GRADIENT from the bottom right of the Run screen (Figure 2-9). The FOCUSED GRADIENT DEFINITION window appears (Figure 2-10).

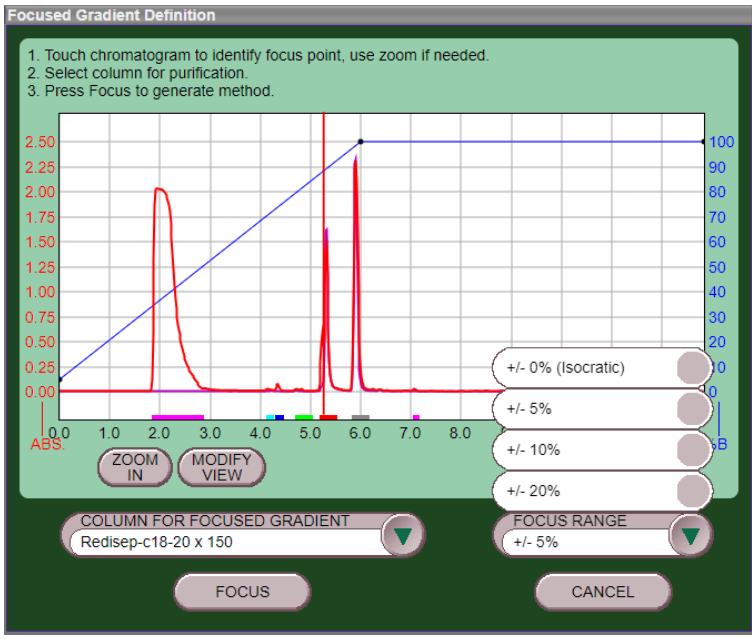


Figure 2-10 Focused Gradient Definition window

2. Select the peak at which you would like your purification to be optimized. Using a finger on the touchscreen, you may move the vertical red line designating this peak as needed.

3. Select the column for which to generate the optimized focused gradient from the list at the bottom of the window.
4. Select a FOCUS RANGE (±0% to ±20%).
5. Select FOCUS. An optimized method centered on the selected peak is generated.

A possibility: a dialog warns you that the target compound eluted too early or too late, so the generated method may not be truly optimal. Possibly, early elution could be remedied by using another solvent system or column. See Section 3.4 *Operation Troubleshooting* (*Peaks elute too early* and *Peaks elute too late*) and the Teledyne's Technical Note *Focused Gradients—What Do You Mean the Compound Eluted Too Early or Too Late?!* (TN61).

When the run is finished, a RUN VIEWER window opens to show the focused gradient.

A RUN VIEWER window displaying a previous scouting run (Section 4.4.7) may also make the FOCUS GRADIENT button available.

2.4.5 Calibrating HPLC

Calibrating an analytical HPLC system to the ACCQ*Prep* HP150 can increase method development efficiency.

To calibrate the external HPLC, run universal test mix on both the external HPLC and the ACCQ*Prep* with matching chemistry columns. Refer to Teledyne LABS *Chromatography Technical Note 52* for that procedure. After the parameters are determined, enter them into the ACCQ*Prep* HP150:

1. CONFIGURATION > PREP HPLC tab > CALIBRATE EXTERNAL HPLC. The CALIBRATE EXTERNAL HPLC window opens (Figure 2-11).
2. Select ENABLED.
3. Enter settings to calibrate the analytical HPLC system to the preparative HPLC system, then select OK to dismiss the CALIBRATE EXTERNAL HPLC window.
4. Select OK to dismiss CONFIGURATION and to reveal the MAIN screen.

To use the custom method, you must select it when you select a column for a separation:

1. On MAIN, select the RUN SCREEN tab.
2. Select a COLUMN. When a column is selected, a method list appears.
3. Select HPLC FOCUS from the list. The HPLC FOCUS window opens (Figure 2-12).
4. Enter the RETENTION TIME from your analytical system.
5. Enter a FOCUS RANGE (+/- 0, 5, 10, or 20%), then select OK.

The Calibrate External HPLC window

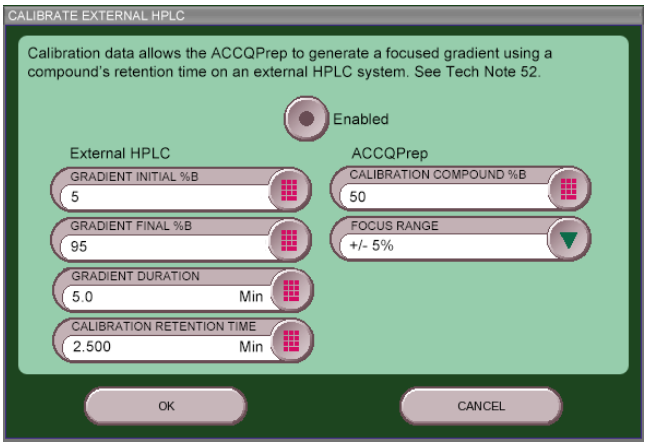


Figure 2-11 The Calibrate External HPLC window

Enabled - – Provides columns with an HPLC Focus method using the gradient and calibration options selected on the Calibrate window.

External HPLC options

- GRADIENT INITIAL %B – The starting percentage of B solvent.
- GRADIENT FINAL %B – The ending percentage of B solvent.
- GRADIENT DURATION – The length of the gradient. It is *not* the total run time.
- CALIBRATION RETENTION TIME – Specifically, the retention time of the calibration compound.

ACCQPrep options

- CALIBRATION COMPOUND %B – The %B that causes the calibration compound to elute at the correct retention time.
- FOCUS RANGE – +/- 0, 5, 10, or 20%.

The HPLC Focus window

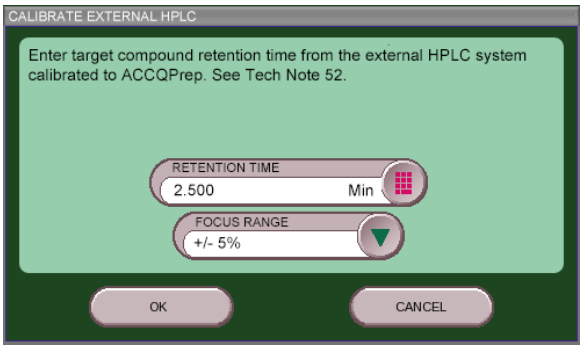


Figure 2-12 The HPLC Focus window

2.5 Scheduled Operations

Scheduled operations run sample queues at predetermined days and times. They are run after any samples in an existing queue are completed.

Scheduled Operations are useful to

- wash all installed columns at the end of the day to remove buffers and modifiers, potentially improving column life,
- wash strongly retained samples from columns, and
- periodically run reference standards to test retention times and peak shapes.

Scheduled Operations require sample queues saved to the system (Section 3.3.5). Each queue file must contain at least on operation. Entries from the file are added to the active queue and are run after queued samples.

Scheduled Operations can be found under TOOLS > CONFIGURATION > SCHEDULED OPERATIONS tab (Figure 2-3). Here, up to three routine operations can be scheduled to be run as needed.

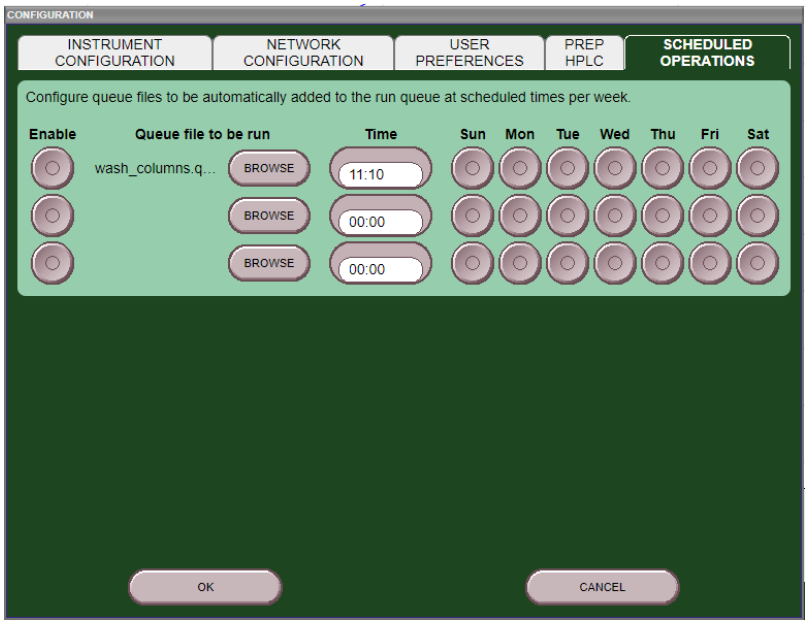


Figure 2-13 Scheduled Operations

To schedule an operation,

1. BROWSE to select a queue file to run.
2. Select ENABLE. Toggling ENABLE off disables the operation but retains its settings for later use.
3. Enter a TIME in a 24:00 format. The TIME ZONE set in CONFIGURATION (Section 2.1) is used by the scheduler.
4. Select at least one day. For example, column cleaning can be done every day, while column testing may be done once a week.

Before a scheduled operation is run, make sure that the required solvents and samples are in place. Samples must be placed in the sample rack at the location listed in the saved sample queue. Prime pumps with the appropriate solvents when an operation such as column testing is scheduled.

ACCQ*Prep* HP150

Section 3 Operation

3.1 Introduction

The ACCQ*Prep* HP150 is unique in the Preparative HPLC market. It provides several methods of operation that you can select to fit your needs.

Methods contain all of the information used during a separation of a single sample, such as gradient profile, detection and peak cutting parameters, and fraction size. Repetitive sample injection is programmed as needed and doesn't require creation of a separate file.

One of the unique features of the ACCQ*Prep* is that a defined method file is not required for operation. A default or existing method can be loaded and modified for the current separation without the need to save the newly modified method.

In addition, any of the method parameters can be modified during the separation. None of these changes will affect the original method unless you choose to save the changes to the original method. All method and injection parameters used for starting the separation or modified during the separation are stored with the chromatographic data, so no information is lost.

In addition, the method and injection parameters used during a separation can be extracted from the data file and saved as a named method for future reuse.

3.2 Method Selection

There is more than one way to select a method:

- Use a pre-programmed column that has methods associated with it. After the column is selected, select one of the associated methods. This is especially convenient if you typically only use a few predefined methods.
- Use a method file that has been saved before. Go to FILE > OPEN. A FILES selection window opens. Sort for the method file (*.pmtd) that fits your needs and select it.
- Extract a method from a previous run. Go to FILE > OPEN. A FILES selection window opens. OPEN the run file (*.run) from a previous separation, then select EXTRACT on the lower portion of the Run file window.

If the method selected is adequate, select START to continue. If the method is not exactly as needed, the method can be edited. Edit the common parameters of any method either by adjusting the points on the graph, changing major parameters on the main screen, or by selecting the METHOD EDITOR. After it is edited, the method can be used directly for the immediate separation(s) or saved with a name for future reuse.

3.3 Starting a Separation

To separate a single sample, select START to start a separation. The MINIMUM RUN REQUIREMENTS window opens. Select an injection technique and select where the fractions will be collected (the starting rack and tube position). The system projects the amount of solvent required and the separation duration. To proceed, select START EQUILIBRATION.

For an ELSD

If an ELSD is installed on the system, MINIMUM RUN REQUIREMENTS provides the option to use the ELSD or disable it. Typically, ELSD method parameters are determined by the solvents used and are therefore somewhat universal when doing preparative separations of nonvolatile samples. Semi-volatiles may require optimization of the ELSD parameters. Peak delay compensation is based on using water and methanol or water and acetonitrile and is selected on the basis of the names assigned to the solvents.

> ☑ ***Note***
>
> Use the METHOD EDITOR to change the ELSD parameters.

For a PurIon

If a PurIon mass spectrometer is connected, the MINUMUM RUN REQUIREMENTS and DETECTION OPTIONS windows allows some customization of the PurIon operating conditions. This allows the use of default or commonly used methods while allowing customization of the separation for both masses used for fractionation or mass loading of sample sent to the PurIon for detection. (Low mass load is the default and sends the minimum amount of sample to the PurIon for detection. Because of the high concentrations of sample in a preparative separation, this is adequate for most samples with good ionization.)

PurIon detection options can also be accessed from the METHOD EDITOR. Further information about these options be found in Section 4.3.4 *Method Editor* (under *Peak Detection*).

If No AutoInjector or AutoSampler are Connected

If you do not have an AutoInjector or AutoSampler, the ACCQ*Prep* gives you the option of injecting the sample immediately or after column equilibration.

- LOAD NOW — If your sample is ready, you can inject it into the syringe port on the side. Select START EQUILIBRATION. No further intervention will be required on your part.
- LOAD AFTER EQUILIBRATION — This gives your column time to equilibrate while you finish preparing your sample. When the equilibration is complete, the system will pause and prompt you to inject your sample.

The next section covers starting a separation when the additional modules are set up.

3.3.1 Separation with an AutoInjector

To use the AutoInjector module:

1. Place the sample probe into your sample container.
2. Select START to start a separation as you would for an individual separation. The MINIMUM RUN REQUIREMENTS window opens; in front of that, the SAMPLE OPTIONS window opens.
3. Select the SAMPLE TYPE.
   a. If you want to automatically perform multiple injections (or to perform a single injection using the AutoInjector Module), select AUTOINJECTOR as the SAMPLE TYPE (Figure 3-1). This is the default selection if an AutoInjector Module is present.
   b. If an AutoSampler module is present, select AUTOSAMPLER.

   If an AutoSampler or AutoInjector is not installed, they will not be listed as a SAMPLE TYPE.

☑ Note

If you want to manually inject your sample without using the AutoInjector Module, replace the sample probe fitting with a Luer injection port (supplied) or 22 gauge needle port (optional).

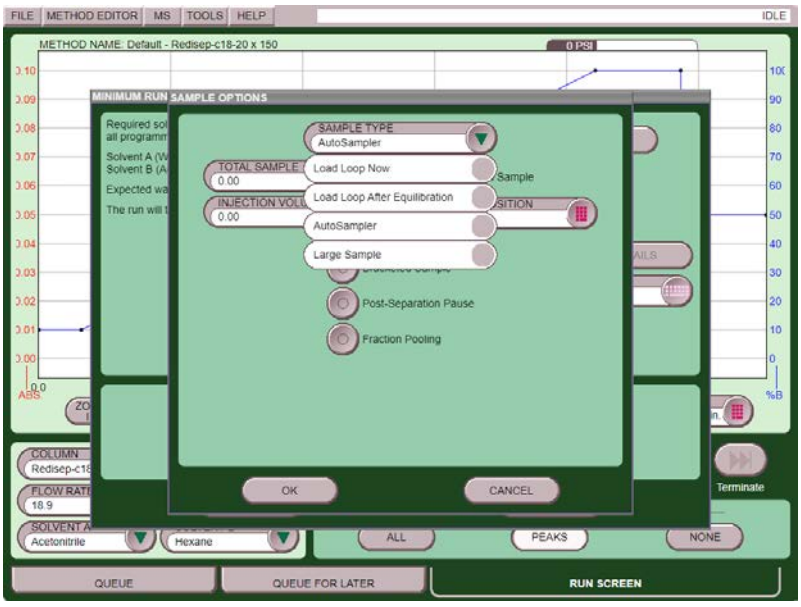


Figure 3-1 Choosing an Injection Method

4. In the dialogue box, enter the TOTAL SAMPLE volume and the INJECTION VOLUME (Figure 3-2). PeakTrak divides the total volume by the injection volume to determine the number of injections for each separation. The number of

injections that these two volumes will yield is listed on the window.

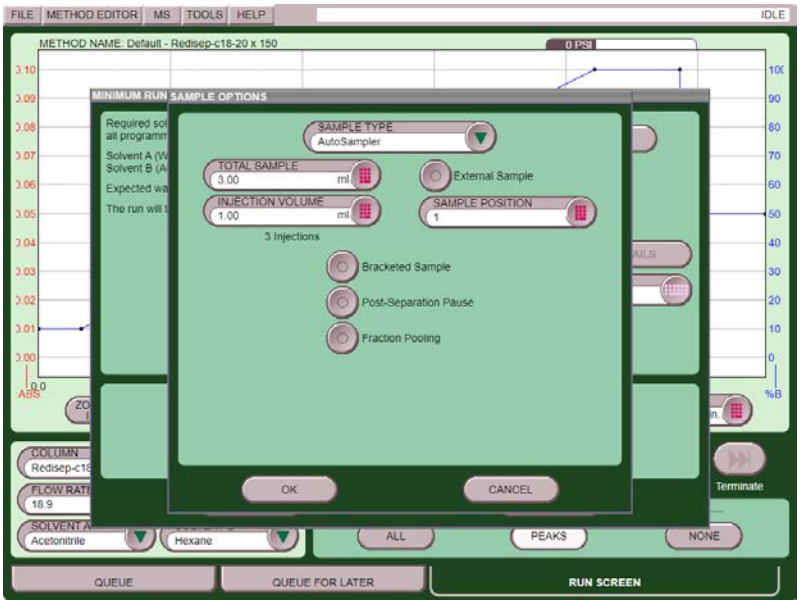


Figure 3-2 Selecting the sample volume and number of injections

If the INJECTION VOLUME exceeds 50% of the loop volume, a warning message recommends a change in the parameters. This message can be ignored at your discretion. PeakTrak performs exactly as programmed. It also compensates for the volume of tubing used for the supplied sample probe. If the sample probe is ever lost or damaged, replace it with the AutoInjector Needle Assembly (PN 60-5234-657).

During the programmed separation sequence, any changes made to the method are included on any of the remaining injections. This includes automatic changes such as using the peak hold feature or automatic run length extension. For the most reliable operation, it may be best to disable these automatic features. Mid-separation flow rate changes will occur at the beginning of the next separation. Note that changes of flow rate do not automatically affect the overall run length.

5. After the last injection is performed, a sample probe wash screen is shown, prompting you to wash the probe with a strong solvent followed by a weak solvent. Perform these duties as requested.

☑ Note

A weak solvent is used following the usage of the strong solvent to inhibit poor separation due to excessive strong solvent in the probe tubing. It is important to perform Step 5 to ensure there is no carryover into the next separation.

6. The final separation results are displayed at the completion of the sequence. To view previous separations, select RESET to return to the MAIN screen.
7. Select FILE > OPEN to view previous separations.

If a sample had multiple injections, the run sequence is displayed under a single name preceded by a "+" symbol. This symbol indicates there are multiple injections with the same base file name. Names are appended with "-I(#)" to signify the numerical order of the separation. For example:

- If you have a single injection run, it can be named A01.
- For multiple injections, the first injection can be named A03-I1, the second can be A03-I2, and the third can be named A03-I3.

8. Select the file you want to display.
9. The remaining injections can be immediately viewed by pressing the left or right arrows at the lower left and right of the file viewer screen. This function can also be used to immediately view files before or after the currently viewed file even if they are not part of the same injection sequence.

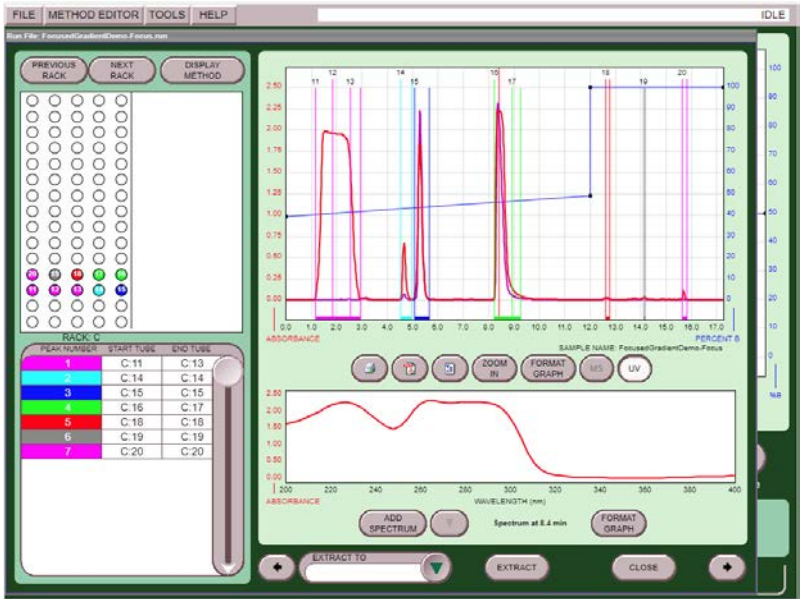


Figure 3-3 Viewing injections

3.3.2 AutoInjection Operating Steps

This section outlines the steps performed by the AutoInjector during sample injection.

1. You are prompted to place the sample probe into the sample container before the separation begins.
2. During column equilibrium, the sample loop is first placed into the run position. This position passes the column equilibration fluid through the loop to wash out any left-over fluid from a previous separation and fills the loop with

the initial gradient conditions from the current separation. This prevents any strong solvent remaining in the loop from affecting the current separation.

3. Near the end of the equilibration, the inject valve moves to the sample load position.
4. The AutoInjector Module aspirates enough sample to fill the loop with the programmed amount and to compensate for the volume of the sample probe.
5. After the equilibration has completed, the injection valve moves to the separation position, and the separation process continues.
6. After the first separation has completed, the next separation begins with the equilibration as described in steps 2–5. During this process, the AutoInjector Module continues to aspirate the programmed injection volume.

   When the sample is aspirated for the final injection of the sequence, the total volume of all the sample injections will match the programmed amount.

   - If the sample container held less sample than programmed, the final injection will aspirate a small amount of air. Small amounts of air will not damage the HPLC column.
   - If the sample container held more sample than programmed, there may be some sample remaining in the probe after the final injection. For this reason, we recommend programming the injection sequence with about 0.5 mL more sample volume than the actual sample amount provided in the sample. This will ensure all of your sample is processed.
   - If some sample remains in the sample probe (probe volume is approximately 0.43 mL), you can recover it by loosening the sample probe fitting at the injection valve to allow the sample to drain back into the sample container.

After completion of the final injection, the system washes the sample probe to prevent sample carryover during the next separation. To perform the wash, the system will prompt you to follow the proper wash steps.

The wash sequence proceeds as follows:

1. The system prompts you to place the probe in a strong wash solvent. This solvent should be capable of completely washing the sample from the probe.
2. 10 mL of strong solvent is aspirated through the probe to wash away any remaining sample from the probe.
3. The system prompts you to place the probe in a weak wash solvent.
4. 10 mL of weak solvent is aspirated through the probe to wash away any remaining strong solvent from the probe so that it cannot interfere with future separations.

3.3.3 Separation using an AutoSampler Module

Verify that the wash station contains fluid. If it doesn't, go to TOOLS > AUTOMATION MANUAL CONTROL and select START WASH. After the wash station is primed, select STOP WASH. Verify that the wash fluid supply container has sufficient clean wash fluid for the planned separations.

In addition, if the separations will use a solvent bracketed injection, ensure there is sufficient bracket solvent for the planned separations. This solvent is placed into an 18 mm test tube located to the left of the wash station. The system uses about 100 µL per injection. The probe can only aspirate fluid down to the level of the top of the tube holder, so the tube must be filled to a level above that point.

To begin a separation sequence with the AutoSampler Module, you can create a sample queue entry or, to start with a single sample, select START. Accessing the RUN tab results in a screen and operation much like the standard ACCQ*Prep* without an installed AutoSampler Module. Selecting START opens the MINIMUM RUN REQUIREMENTS window. There, you can perform a manual injection by removing the Luer fitting from the inject loop, or you can perform multiple injections on a single sample from the AutoSampler module. A queue is created automatically. Selecting the QUEUE tab allows you to set up a separation sequence for multiple samples.

> ☑ ***Note***
>
> If you are performing a manual injection, the Luer fitting or a needle port must be reinstalled on the injection valve.

3.3.4 Using the Dilute Sample Load Pump

Large samples, sometimes greater than a column volume, can be loaded onto columns using the ACCQ*Prep*. Installation of the dilute sample loading pump is described on the instruction sheet accompanying the module. After it's installed, follow the directions below to use the pump.

To prime the dilute sample load pump:

1. Make sure that your ACCQ*Prep* HP150 is primed.
2. Place the dilute sample load pump's inlet line into a weak solvent.
3. Verify that the sample load pump's valve is set to RUN.
4. Place an empty syringe on the prime port, then open the prime port by turning it counterclockwise.
5. Press the P button on the pump while drawing air out of the system (Figure 3-4). Repeat until no more air is drawn via the syringe
6. Turn the prime valve clockwise until closed, and press P to stop priming.

Figure 3-4 Loading Pump P button

The dilute sample load pump can be ran manually through the pump face interface or set up to run automatically (with user prompts) with a programmed volume and flow rate. With the manual valve, you do not need to be present for user-prompted action to turn the valve as needed.

To avoid issues of clogging the lines of the system and pump longevity, properly filtering the sample before loading it is strongly recommended. This will also help extend the life of your column.

When you are ready to inject:

1. Ensure that the dilute sample load pump is primed as described above.
2. Place your sample in the cartridge or the inlet line (or syringe with sample) into a vessel with your sample.
3. Select the START button on the MAIN screen. The SAMPLE OPTIONS window (Figure 3-5) opens above the MINIMUM RUN REQUIREMENTS window.
4. Choose "Large Sample." Select other parameters, then select OK to dismiss the SAMPLE OPTIONS window.
5. Choose your parameters on MINIMUM RUN REQUIREMENTS window, then select START EQUILIBRATION.
6. When the equilibration is started, the LARGE SAMPLE INJECTION window (Figure 3-6) appears to allow observation of the baseline, adjust flow rates, and to provide a means to stop loading after the sample saturates the column.

Figure 3-5 Dilute Sample Loading Pump Automatic Loading window

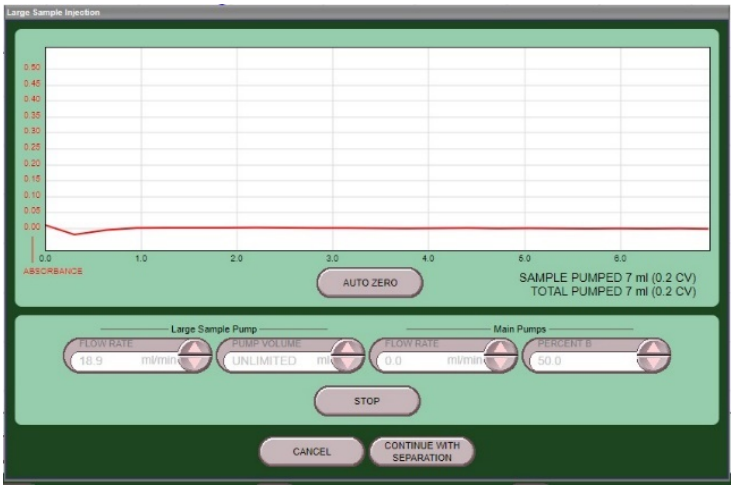


Figure 3-6 The Large Sample Injection window

By default, the flow rate is the same as that for the column. However, the HPLC pumps can also be run at a different flow rate to provide a desired solvent composition to prevent phase collapse on the column or to prevent the sample from precipitating.

- AUTO ZERO – Zeros the detector before starting the separation.

Pump control options:

- FLOW RATE – Type or select the pump's flow rate in mL/min.

- PUMP VOLUME – Type or select the pump's volume in mL, or choose UNLIMITED.
  - UNLIMITED – The pump runs until you stop it. The pumps can be restarted to allow for flask washings to be loaded.

  The main pumps can also be run from this window. Running 5% B solvent can be useful for preventing phase collapse when working with aqueuous compounds.
- CONTINUE WITH SEPARATION – Notifies you that the gradient and peak detection parameters can be changed before starting the run. Afterward, you may CONTINUE.
- CONTINUE – Starts the purification.
- CANCEL – Stops the purification.

3.3.5 Using the Queue Tab

Sample queues are lists of samples, purification methods, detection parameters, and injection instructions to run in sequence. The queues can be appended with samples and saved for running later. Saved queues are also used for scheduled operations (Section 2.5).

Creating a sample queue

To create a sample queue, an AutoSampler or AutoInjector must be installed.

1. Install a sample vial rack before opening the QUEUE tab. This allows the software to limit sample programming to the positions available for sample vials.
2. Select the QUEUE tab at the bottom of the MAIN screen (Figure 3-7).
3. Do any of the following:
   - Select an empty row to create a sample entry. A new row is populated with the COLUMN and its default METHOD displayed on the MAIN screen.
   - Select a sample to edit its column entries. (See Section 3.3.6 for a detailed discussion of the QUEUE tab's column options.)
   - Populate the tab with samples that you have earlier saved to a file using the IMPORT SAMPLE LIST INTO QUEUE button (described below).
4. (Optional) The samples list can then be exported to a plain text file (.txt) for future use by selecting EXPORT COLUMN/METHOD LIST. Later, you can IMPORT SAMPLE LIST INTO THE QUEUE to populate the QUEUE tab.

Running a sample queue

Because sample queues allow the system to run unattended for extended time periods, you should verify that the system is ready to run the samples that will be in the queue. Although the ACCQ*Prep* system will stop the pumps for situations such as empty solvent bottles, a filled waste container, or when all fraction containers used, no samples will be run until the condition stopping the run is resolved. Make sure that the sample is in the correct position in the rack and that the vial cap is removed

To run a queue, do one of the following:

- Select the RUN tab, then select START.
- Select the "≡" menu on the first unfinished run, then select START RUN.

In either case, MINIMUM RUN REQUIREMENTS opens. Accept or modify the starting rack or tube for fraction collection.

While a separation is in process, the QUEUE tab can be accessed to add samples to the queue. Samples that have been completed cannot be edited. However, they can be viewed by touching the sample name corresponding to that run.

To perform continuous separations, during the separation remove completed fraction racks when full and replace with empty racks.

If a single rack containing fractions is removed and then replaced into the rack position, PeakTrak displays a prompt to determine if the rack contains empty tubes.

a. If you respond that the tubes are empty, PeakTrak considers this rack available for future separations.

b. Otherwise, PeakTrak continues to mark this rack as full of samples and not available for fraction use.

This feature allows you to remove a rack to obtain a sample for verification while leaving the rack in the instrument for convenience and later removal.

After the entire queue has completed, a RESULT window is displayed showing the last separation.

To view completed separations, do one of the following:

- Select the separation on the QUEUE tab
- Select FILE > OPEN to view previous separations.

If a sample had multiple injections, the run sequence is displayed within the FILE > OPEN dialog under a single name preceded by a "+" symbol. This symbol indicates there are multiple injections with the same base file name. Names are appended with "-I(#)" to signify the numerical order of the separation.

For example:

- If you have a single injection run, it can be named A01.
- For multiple injections, the first injection can be named A03-I1, the second will be A03-I2, and the third will be named A03-I3, etc.

AutoInjector

Sample queues can also be used with the AutoInjector. Although only one sample can be run in a single queue in a system without an AutoSampler, the queue is useful to test several columns without user intervention on systems equipped with column switchers. The columns can also be tested periodically to determine if they have deteriorated. Also, the NO INJECT/NO COLLECT toggle in the METHOD EDITOR can be used to wash all the columns at the end of the workday. This removes strongly retained compounds and washes buffers and solvent modifiers from the system.

Other queue operations

To reuse an existing run: On the QUEUE tab, open the run you want to reuse (FILE > OPEN). A RUN window opens. Select EXTRACT to add this column and method to the end of the queue on a new line.

To calculator a Focus Gradient: With the QUEUE tab selected, open the scouting run that you want to focus. Select FOCUS GRADIENT to create a focused gradient. The new gradient method appears on a new row in the queue.

3.3.6 The Queue Tab

Figure 3-7 Automation Queue tab

The queue is a list of samples to be run in the order listed. Create new entries on the QUEUE tab by touching to add a row, then by editing the fields under each column. Each row represents a sample.

To save a file for future use, select FILE > SAVE QUEUE AS.

To open a saved queue, select FILE > OPEN. The FILES window appears. Select QUEUE FILES (*.queue) as the FILE TYPE so that only queue files are displayed. Select the file you want, then select OPEN to load the queue to the QUEUE tab.

> ☑ ***Note***
>
> The number of rows in the queue is limited to 28. Additional samples can be added by deleting completed rows from the queue. If there are more rows than can be displayed on screen, up and down arrows that access hidden rows are displayed at the top and bottom of the screen.

≡ – A menu to edit a sample. Menu options only apply to the row from which they are chosen.

- START RUN or RUN NEXT starts the separation. RUN NEXT starts a separation regardless of its order in the queue.
- REMOVE ROW removes the row from the queue.
- ENABLE or DISABLE POST-SEPARATION PAUSE.
  - ENABLE causes the system to pause the run and allow detection parameters, gradient method, or injection volume to be changed to optimize the purification.
  - You can also DISABLE the pause for the remaining injections, or leave it enabled to allow a second condition scouting separation.
  - Rows with enabled post-separation pauses are highlighted yellow.
- MOVE to QUEUE FOR LATER from the QUEUE tab (Section 3.3.7) for later use.

Sample Name – The name of each sample. Naming is optional. If left blank, the sample is named after the date and time the separation was started.

Column (dropdown menu) – If a Column Selector Valve Module (CSV-4) is installed, this is list of separation columns and associated methods. If a CSV-4 is not installed, this lists different methods that may be associated with the configured and installed separation column.

Method – The methods that were defined for column configuration are displayed. After a column has been selected, or if there is only one column defined on the system, the default method created for that column is automatically loaded.

To change this method, select the method name. A list of methods associated with the column appears. These methods are listed in the order they were created. Alternatively, select CUSTOMIZE CURRENT METHOD or BROWSE to populate this field.

To modify a method, select a method on a row to modify and select CUSTOMIZE CURRENT METHOD to open the METHOD EDITOR. There, the gradient and detection parameters can be changed.

To use the modified method, exit the METHOD EDITOR and select SAVE when prompted. This creates a new method named “Temporary 1” that will be used for all injections of the current sample, then discarded after the sample is complete. All method parameters are saved with each separation.

To save the modified method for later use, select SAVE AS before you exit the METHOD EDITOR, then create a unique name for the new method.

Sample Position – The location of the sample for this separation. If the sample size is too large for a single vial, create a second line in the queue and access the second vial.

Sample Volume – The amount of sample to be separated. Typically this amount is slightly greater than the amount of sample to ensure all of the sample gets purified. This information is used in conjunction with the NUMBER OF INJECTIONS column to calculate the volume of each injection.

Number of Injections – Used in conjunction with the SAMPLE VOLUMES column to determine the size of each injection.

Start Tube – NEXT TUBE uses the next available tube in the current fraction collection rack, while NEXT RACK starts a new rack, if available. You can optimize rack usage by sharing racks for multiple samples or optimize the work flow by placing each sample's fraction into individual racks to support multiple users.

Export Column/Method List – Exports the samples list to a plain text file (.txt) for future use.

Import Sample List Into the Queue – Populates the QUEUE tab with a method list previously saved using EXPORT COLUMN/METHOD LIST.

3.3.7 The Queue for Later Tab

Figure 3-8 Queue for Later

This is similar to the QUEUE tab (Section 3.3.6), allowing you to add samples to the system. As in the QUEUE tab, these queues can be saved (FILE > SAVE QUEUE AS). But this tab differs from the QUEUE tab in important ways:

- Samples cannot be run from this tab directly; instead, you can get methods ready and run them when the sample is ready.
- You can MOVE TO QUEUE from the QUEUE FOR LATER tab. The row moves to the bottom of the list on the QUEUE tab.

3.3.8 AutoSampler Injection Techniques

This section describes operating protocols for the AutoSampler Module.

> ☑ ***Note***
>
> The sequence of operation described below is valid for software versions starting with 4.1.13.

The AutoSampler supports two different injection protocols. The default technique injects samples and washes the sample probe using the wash station solvent. The alternative bracketed sample

injection minimizes the risk of sample crash by placing a small amount of a user selected solvent on each end of the sample fluid. All sample injection steps are listed below for reference:

Default Injection Sequence

1. During column equilibrium, the sample loop is first placed into the run position. This position passes the column equilibration fluid through the loop to wash out any left-over fluid from a previous separation and fills the loop with the initial gradient conditions from the current separation. This prevents any strong solvent remaining in the loop from affecting the current separation.
2. The sample probe is placed in the wash station.
3. About 1.5 mL of the solvent is pulled from the wash station through the probe to remove air from the probe line.
4. The sample probe is lifted from the wash station and is moved up and down slightly. When the sample probe is dipped in the sample or solvent, some liquid sticks to the outside of the probe when it is raised. For that reason, the probe is moved up and down over the fluid source to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.
5. A small amount of air (0.05 mL) is drawn into the sample probe to minimize the mixing of the wash solvent and the soon to be loaded sample. This also minimizes the potential of the sample coming out of solution.
6. The sample probe is moved to the sample container.
7. A portion of the programmed injection amount is aspirated into the sample probe displacing most of the wash solvent in the sample probe. This compensates for the volume of the sample probe. Since the loop is still in the separation position, the wash solvent is sent to waste.
8. The loop is switched to the load position.
9. The remaining portion of the programmed injection volume is aspirated into the sample probe and loop. After the programmed amount of sample is aspirated into the probe, the probe is lifted out of the sample vial.
10. The probe is moved up and down over the sample tube to shake off any excess liquid to prevent the contamination of other samples or collection tubes.
11. The probe is dipped into the wash station to rinse the exterior of the probe to prevent sample residue from drying on the exterior of the probe.
12. Air is drawn into the sample probe to draw the remaining sample into the loop without leaving any sample in the probe.
13. The sample is now loaded and the separation begins.
14. The next injection of the sample is accomplished by repeating the process described above.

After the completion of the final injection of a sample, the system washes the probe in the following sequence:

1. The inject valve is moved to bypass to prevent contamination of the loop during the cleaning process.
2. Air is drawn into the probe to eliminate any remaining solvent in the probe.
3. The probe is placed into the wash station. The wash station pump flushes wash fluid over the exterior of the probe while 10 mL of wash fluid is drawn into the probe to wash the interior flow path. The probe syringe pump uses half strokes to improve the rinsing of any tiny amounts of compound that may be present due to the wash process. Once again, the probe is moved up and down over the wash station to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.
4. Air is drawn into the probe to eliminate the strong wash solvent from the sample flow path.

Bracketed Sample Injection Sequence

1. During column equilibrium, the sample loop is first placed into the run position. This position passes the column equilibration fluid through the loop to wash out any leftover fluid from a previous separation and fills the loop with the initial gradient conditions from the current separation. This prevents any strong solvent remaining in the loop from affecting the current separation.
2. The sample probe is placed into the wash station
3. About 1.5 mL of the solvent is pulled from the wash station through the probe to remove air from the probe line. This minimizes injection volume errors due to air in the probe. Since the loop is still in the column flow path, this aspirated fluid bypasses the loop and is sent to waste
4. The sample probe is lifted from the wash station and is moved up and down slightly. When the sample probe is dipped in the sample or solvent, some liquid sticks to the outside of the probe when it is raised. For that reason, the probe is moved up and down over the fluid source to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.
5. A small amount of air (0.05 mL) is drawn into the sample probe to minimize the mixing of the wash solvent and the bracket solvent.
6. The probe moves to the bracket solvent sample station (just to the left of the wash station). 0.05 mL of the bracket solvent is aspirated into the probe.
7. The probe is raised into the air and an additional 0.05 mL of air is aspirated into the probe.
8. The sample probe is moved to the sample container.
9. A portion of the programmed injection amount is aspirated into the sample probe displacing most of the wash solvent remaining in the sample probe. This compensates for the

volume of the sample probe. Since the loop is still in the separation position, the wash solvent is sent to waste.

10. The loop is switched to the load position.
11. The programmed injection volume is aspirated into the sample loop. After the programmed amount of sample is aspirated into the probe, the probe is lifted and a small amount of air is drawn into the sample probe. This minimizes mixing of the sample with the bracketing solvent, which is aspirated next
12. The probe is dipped in the wash solvent to rinse the exterior of the probe, then raised and shaken. This minimizes sample contamination of the bracket solvent.
13. The probe moves to the bracket solvent sample container. (0.05 mL of the bracket solvent is aspirated into the probe.)
14. The probe is raised into the air and the fluid in the loop is aspirated into the loop along with about 0.05 mL of air to ensure all of the sample and bracket solvent is loaded.
15. The loop is switched to the separate position.
16. The sample is now loaded and the separation begins.
17. The next injection of the same sample is accomplished by repeating the process described above.

After the completion of the final injection of a sample, the system washes the probe with the following sequence:

1. The inject valve is moved to bypass to prevent contamination of the loop during the cleaning process.
2. Air is drawn into the probe to eliminate any remaining solvent in the probe.
3. The probe is placed into the wash station. The wash station pump flushes wash fluid over the exterior of the probe while 10 mL of wash fluid is drawn into the probe to wash the interior flow path. The probe syringe pump uses half strokes to improve the rinsing of any tiny amounts of compound that may be present due to the wash process.
4. Once again, the probe is moved up and down over the wash station to shake off any excess liquid and to prevent the contamination of other samples or collection tubes.
5. Air is drawn into the probe to eliminate the strong wash solvent from the sample flow path.

3.3.9 Fraction Collection with the AutoSampler

The fraction collection racks in the AutoSampler double or triple the fraction collection ability of the ACCQ*Prep*. They are treated as an extension of the racks present in the ACCQ*Prep*. In general, if racks are placed in both the ACCQ*Prep* and AutoSampler 2x2, available racks are used in the following order:

1. ACCQ*Prep* left rack (A),
2. ACCQ*Prep* right rack (B),
3. AutoSampler left rack (C),
4. AutoSampler right rack (D).

If racks are placed in both the ACCQ*Prep* and AutoSampler 4x2, available racks are used in the following order:

1. ACCQ*Prep* left rack (A),
2. ACCQ*Prep* right rack (B),
3. AutoSampler center left rack (D),
4. AutoSampler center right rack (E),
5. AutoSampler right rack (F).

If any racks are missing or identified as containing fractions from a previous separation, the system moves to the next available rack in the order listed above.

When the system moves from the ACCQ*Prep* fraction racks to the AutoSampler fraction racks, the fluid uses an alternative path. This leaves a portion of tubing with fluid remaining for the fraction collector previously used. To prevent loss of any compound, the software monitors for a portion of the separation with no peaks while using the last available row of the fraction collection rack on that module. If a portion without a peak to collect is identified, the system moves to the next rack on the next module (the AutoSampler or ACCQ*Prep*). This may leave a few tubes unused in the previous rack. If the system is mid-peak or if the separation is set to Collect All, the fluid remaining in the tubing is placed into one of the tubes in the last row of the rack before the move to collect any undetected compound. Then the system advances to the next rack.

Funnel Racks - If a funnel rack is detected, you can set it up to collect into larger containers.

3.3.10 Stacked Injections

With PeakTrak v4.3.4 or greater, injections can be "stacked;" that is, multiple injections can be injected after each other during the same isocratic run. Stacked injections save time and solvent by reducing non-elution phases of the run and maximizing the column bed capacity. Methods suitable for stacked injection require a short isocratic method with all peaks eluting in close proximity. Although commonly applied to chiral compounds, this feature is useful for achiral purifications as well. Also, stacked injections allows the use of smaller, less expensive columns by maximizing the efficiency of the entire column bed by quickly performing multiple smaller injections versus larger single injection runs with larger capacity columns.

Procedure

The Focus Gradient Generator is used to calculate an isocratic method, which is used to determine the cycle time and the time windows for peak collection. These are used to run stacked injections.

1. Create an isocratic run, or use the Focus Gradient Generator to calculate an isocratic method from it. (See 2.4.4 *Creating Scouting and Focused Gradients*)

> ☑ ***Note***
>
> Verify that there are no impurities eluting after the desired compound(s).

a. Start with a scouting gradient run either from analytical HPLC data or from scouting data from an ACCQ*Prep* HPLC system.

b. Select FOCUS GRADIENT on a RUN VIEWER window to open the FOCUS GRADIENT GENERATOR.

c. Calculate the purification method:

· Select a column.

· Select the peak of interest.

· Set the FOCUS RANGE to +/- 0% ISOCRATIC.

d. Select FOCUS to close the window. A method is generated and displayed on the MAIN screen.

e. Delete the two step gradient points at the end of the run to create an isocratic method. (The software will not be able to create a stacked injection unless the gradient run is *completely* isocratic.)

f. Select START to start the run. The MINIMUM RUN REQUIREMENTS and SAMPLE OPTION windows open.

2. Create the collection time windows

a. Select EXTERNAL SAMPLE on the SAMPLE OPTIONS window.

· For systems equipped with an AutoSampler, disconnect the sample probe at the injection port and replace it with the AutoInjector probe (PN 60-5234-657).

· No probe change is needed for units with only an AutoInjector.

b. Select POST-SEPARATION PAUSE between injections so that adjustments to the gradient or injection volume can be made without configuring the system for an entirely new run.

c. Enter the TOTAL SAMPLE and an INJECTION VOLUME.

d. Select OK to close the SAMPLE OPTIONS window and reveal the MINIMUM RUN REQUIREMENTS window.

e. Check solvent amounts; if these are acceptable, select START EQUILIBRATION. A Run window opens after the run is finished.

f. Select PEAKS on the RUN VIEWER window. Detected peaks are used to determine the initial time windows.

g. If needed, EDIT INJECTION and EDIT METHOD to adjust the injection volume or the solvent composition.

h. Select STACK INJECTIONS on the RUN VIEWER window. The STACKED INJECTION window opens. The system displays a simulated stacked injection based on the isocratic run. The time windows displayed are based on the isocratic peak collection.

i. Adjust STACKED INJECTION settings as required. If no peaks were collected during the isocratic run, time win-

dows will not be displayed, but you can add them there manually. See *The Stacked Injection window* below for more information.

3. Prepare to run the stacked injections.
   a. Verify that there is enough solvent (as listed on the MINIMUM RUN REQUIREMENTS window).
   b. Verify that the waste container is empty.
   c. Confirm that the sample is in place.
   d. Run the stacked injections by selecting OK on the STACKED INJECTIONS window.

Exiting the STACKED INJECTIONS window brings you back to the MAIN screen. If you select the TERMINATE button at MAIN, PeakTrak offers you the option to continue or terminate the run, or to cancel:

- STOP INJECTING AND CONTINUE RUN stops any further injections from occurring but allows any injections that are still in the column to finish. The probe wash procedure is implemented to clean sample from the probe.
- TERMINATE RUN NOW immediately ends the run. Sample may be left in the injection loop and on the column; this will eventually need to be washed out of the column. As with STOP INJECTING, the probe wash procedure runs.
- CANCEL continues the current purification.

Figure 3-9 Stopping stacked injections

The Stacked Injection window

The chromatograph displays a simulated stacked injection chromatogram based on the isocratic run created previously. Each band's color corresponds to a different injection. Crosshatching denotes different time windows. As on the MAIN screen, you can ZOOM IN and MODIFY VIEW.

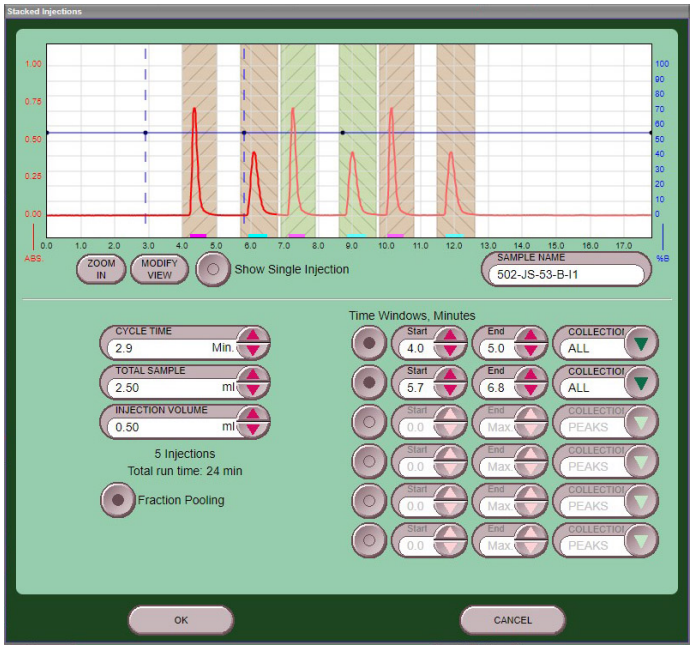


Figure 3-10 The Stacked Injections Window

Show Single Injection – Shows a single injection on the chromatogram. This view is useful for adjusting the time windows. For example, adjusting the time window to appear a bit larger than the collected peak can maximize sample recovery by collecting the peak's tail.

Cycle Time – A time in minutes. Displaying the set of three simulated injections allows a visual determination of the proper cycle time. When peak widths are adjusted, the Cycle Time will likely need to be changed also so that there is a small gap between the collection windows.

Total Sample – A sample volume in mL.

Injection Volume – By default, the value used from the last isocratic injection prior to entering the Stacked Injection window. Verify that this is the total volume of the sample before selecting OK.

Fraction Pooling – Collects corresponding peaks in the same container. This is the default. Use the largest containers practical to minimize the number of fractions. Fraction Pooling interacts with Collection to determine peak collection behavior, as summarized in Table 3-1

Time Window entry – Selecting a row enables that time window. Disabling a time window turns off peak collection during that time, whether a peak is present or not. This allows impurities to be ignored.

Start – The start of the time window in minutes.

End – The end of the time window in minutes.

Collection – Determines which peaks are collected, if any. Collection interacts with Fraction Pooling to determine peak collection behavior, as summarized in Table 3-1.

Table 3-1 Time window collection

	Fraction Pooling on	Fraction Pooling off
Peaks	Only the first detected peak is collected. Everything else diverted to waste.	Detected peaks collected in different containers. Non-peak eluant diverted to waste.
All	No peak detection. Everything within the time window collected into the same container.	Peak detection is enabled. Peaks in different containers. Non-peak eluant collected as fractions.

3.4 Operation Troubleshooting

Injection Valve Leak- refer to on rotor replacement if needed – (See Section 5.5.1 for more information)

- Failure to filter the sample can allow particles to scratch the valve sealing surface. Filter the sample with a 20 µm or finer filter.
- Dissolve sample in sufficient solvent so that evaporation of solvent doesn't cause formation of crystals
- Manual injection using a hypodermic needle with pointed tip can scratch the internal sealing surface resulting in leaks. When using a needle injection port only use a 22 gauge blunt tip needle. When using a Luer injection port, only use a Luer syringe tip.
- If performing manual injection, ensure that a check valve is mounted downstream of the injection valve to prevent solvent from dripping when the syringe is removed.
- If an automated injector is installed and the waste connected to other waste lines with a tee, ensure a check valve is installed in the injection pump waste line.
- Formic acid modifiers can result in reduced injection valve seal lifetime. If possible, consider other modifiers such as TFA.
- 100% water as a chromatographic solvent can also reduce seal lifetime.

Unexpected retention time or variable retention times for repeated injections –

- Temperature variation of only a few degrees can cause a visible retention time shift.
- Variable flow rates of the A or B solvent:
  - Check valves may require cleaning or replacement due to contamination from solvents or pump seal wear particles.

- Inadequate priming of the pump may cause the A or B solvent to flow at lower rates. This may be due to sticking check valves. Open the front panel and tap the check valves with a wrench while priming to assist in bubble passage through the pump.
- Solvent supply fitting may be loose, allowing small amounts of air into system, and causing minor flow rate errors of A or B solvent.
- Strong solvent used for injection (if the repeated injection is a larger volume).

- Solvent bottle refilled with incorrect solvent.

No peaks on chromatogram –

- No sample injected:
  - Sample vial empty.
  - Sample probe plugged, injection loop plugged, or probe or injection pump fitting loose.
    - Perform a dummy injection and watch the injector pump to determine if fluid is being aspirated. Disconnect tubing at different points and place it into fluid to determine the location of the problem.
    - If the fitting is loose, tighten or replace it as needed.
    - If the probe is plugged, replace that part or connect it to a high pressure pump to dislodge the plug.
- Incorrect wavelength used (assuming no other detectors than UV/UV-vis).
- Compound doesn't absorb light.
- B solvent not being delivered.
  - Check solvent level, connections, and check valves.
  - Confirm that solvent lines are in correct bottles.
  - Check for leaks.
- Solvent gradient method is too weak to elute the sample.
- Leak in system is preventing fluid from reaching the detector.
- UV or UV-Vis detector not functioning (the baseline is completely flat with no visible noise.
  - Liquid due to immiscible solvents could be trapped in the detection gap. When changing from normal to reverse phase, flush the system with an intermediate solvent miscible with both phases such as isopropanol, or flush the system when changing phases in order of polarity.
  - Flow cell detection gap obstructed. Remove the flow cell from the system and view through the liquid

path. The detection rods should be visible with a small gap between the rods.

- This is not a lamp or detector hardware problem. These failures have corresponding error messages.

Peaks elute too early –

- Solvent A not being delivered properly.
  - Check the solvent level.
  - Inspect or clean the pump A check valves.
  - Confirm that the solvent lines are in the correct solvents.
  - Look for leaks of air into the lines.
- Sample is injected in a strong solvent that carries the sample down the column.
- Column is not suitable for the compounds separated.
- Fungal or bacterial growth could be obstructing the inlet filters or effecting the check vales in pump A. Use an additive such as 5% B solvent in the A solvent to prevent any growth. Be sure to change the aqueous solvents frequently.
- Incorrect method used.

Peaks elute too late –

- Solvent B not being delivered properly.
  - Check the solvent level.
  - Inspect or clean the pump B check valves.
  - Confirm that the solvent lines in the correct solvents.
  - Look for leaks of air into the lines.
- Check for proper flow rate of both solvents. If flow of one of the solvents is only half of the expected flow, prime the system again since one of the pump heads isn't primed. If this is unsuccessful, open the front cover and tap on the heads of the problem pump.
- Column is not suitable for the compounds separated.
- Incorrect method used.

Poor peak shape –

- Solvent modifier needed for sample.
- Sample injected in strong solvent.
- Column (or guard column) has voids (or other damage, such as loss of bonded phase).

"Phantom" or "Ghost" peaks – Sometimes peaks appear as a broad peak in an area of sharp peaks.

- Wash the column with strong solvent.

- May be caused by a compound left from a prior injection
  - Ensure that the gradient goes to 100% B in each separation to ensure all compounds are washed from the column.
- Impurities in solvent
  - Evaluate with runs with no sample injection to determine if impurity is from the sample. Make a run with a standard injection volume, then double the equilibration time. If the peak in question become larger, it is solvent related. Check mobile phase quality, check for fungal or bacterial growth, or ignore the peak.

Drifting baselines –

- Mobile phase absorbance, which may be caused by an impurity in the solvent or a solvent modifier.
- Column not equilibrated; increase the equilibration time to determine if this solves the problem.
- A compound from a previous run was not fully washed from the column and is slowing coming out of the column. Wash the column with strong solvent to eliminate compounds from prior separations.

ELSD detection issues –

- No peaks detected
  - If the compounds are volatile, they may not be detected by ELSD or may need a lower drift tube temperature. Refer to the discussion on ELSD operation in section 4.3.4 before adjusting the ELSD operating temperatures.
  - If the system detects peak shortly after the first separation of the day and then fails to detect, the drift tube temperature may be too low to vaporize the solvent aerosol or the spray chamber too warm to condense out excess higher boiling point solvents. Refer to the discussion on ELSD operation in Section 4.3.4 before adjusting the ELSD operating temperatures.
  - Verify that the P-trap contains fluid. If using reverse phase solvents with recommended conditions there should be a small amount of fluid draining from the P-trap. Fill the P-trap if needed.
  - Increase ELSD SENSITIVITY and SIGNAL GAIN in the METHOD EDITOR to see if the peaks are simply too small to be easily seen.
  - Flow splitter tube blocked. This 0.005" ID red colored tubing may be plugged by sample. Remove the tubing from the nebulizer during operation to verify flow

through the tubing. If it is plugged, replace both segments of tubing.

- Weak UV or ELSD peaks
  - Each detector has a different response to compounds and may be more or less sensitive to compounds than the other detector.
  - Semivolatile compounds may be difficult to detect or may benefit from modification of the ELSD sensitivity, gain, or operating temperatures. Refer to the discussion on ELSD operation in Section 4.3.4 before adjusting the ELSD operating temperatures.
  - Check the P-trap. (see discussion on "No Peaks Detected.")
- UV and ELSD signals aren't aligned in time.
  - The peak widths or shapes of the two signals may vary because of varying sensitivity of the two detectors causing the alignment to appear incorrect.
  - If the signals sometimes appear a few seconds out of alignment, it could be normal due to different peak shapes common with ELSD.
  - Partial blockage of the ELSD 0.005" red split tubing could reduce flow to the ELSD, causing the ELSD signal to lag the UV signal.
- UV or ELSD peaks are broader than the other detector.
  - This is normal due the detector response and a small increase in dispersion in the alignment compensation tubing.

PurIon detection problems –

- Little or no mass spectrum detected on method development screen, but compound should be visible.
  - Sample is dissolved in a solvent not suitable for MS injection such as DMSO or DMF. Dissolve in methanol or acetonitrile and try again.
  - Try other ionization parameters, such as switching to "Robust."
  - Verify that the gas supply is functioning. Small peaks may be visible without the gas. Nitrogen is typically used.
  - Make sure the proper gas is supplied. Argon causes arcing that results in noise.
  - Fluid interface carrier solvent bottle empty.
- MS peaks occur later than peaks from the other detector such as UV.
  - The MS carrier fluid flow rate is too low.
  - Be sure that the fluid interface priming port is fully closed.

- Check the carrier pump seals for leakage.
- Clean or replace the carrier pump check valves.

Excessive system pressure –

- If using a Column Select Valve Module (CSVM) make sure the column is connected properly. (If the column output goes to the wrong port, it is dead-ended.)
- Column getting clogged:
  a. Remove guard column (if installed) and check back pressure. If this corrects the problem, replace the guard column or following the washing procedure below.
  b. Remove preparative column and check back pressure.
  c. If the column is clogged, try flushing with stronger solvent than mobile phase. (This procedure may damage polymeric reverse phases.) A flush protocol could include 100% MeOH or ACN without buffers or modifiers followed by 100% DCM, then followed by 100% hexanes. Reverse the order of solvents to get back to 100% ACN or MeOH.
  d. Reverse the column connections and flush.
  e. Place the column outlet (which was originally the column inlet) over a beaker so any particles that are flushed out don't get into the system.
  f. Filter samples in the future to prevent problems.

Fractions not correct volume –

- Air trapped in pump head:
  - Reprime and test again.
  - COLLECT ALL with 100% A and 100% B. If only one solvent shows half-filled fractions, one of the heads is not priming. Remove the front panel and loosen the fitting of each individual head while running the pumps under manual control.
- Air leak in inlet lines:
  - Remove front panel and examine inlet lines while operating. If small air bubbles exist, follow tubing back to source, examining for source of air bubbles.

Injection valve dripping –

- Valve rotor is damaged by particulates in the flow stream or from injected sample.
  - Remove the injection valve stator using a 9/64" hex wrench. (The stator is the part with the tubing connections.) The rotor can be lifted out with a fingernail and replaced (PN 20-9009-917). Inspect the face of the stator for scratches. In rare cases, the stator must also be replaced (PN 20-9009-919).

ACCQ*Prep* HP150

Section 4 PeakTrak

4.1 Overview

PeakTrak is the software that controls the ACCQ*Prep*. This section discusses the basic functionality of PeakTrak when operated via the touchscreen or a remote browser window.

4.1.1 PeakTrak Window Elements

The MAIN screen displays the current METHOD FILE. From MAIN, you can access many of PeakTrak's features, view the system status, and view or edit the method file settings. These are the elements of the MAIN window:

Menu – The topmost item in the window is the PEAKTRAK MENU from which you can access all of PeakTrak's features. For more information, see Section 4.2 *PeakTrak Menu Options*.

System Status – Displayed to the right of the Menu. Status messages may include:

- system mode
- current position in a run, expressed in time or column volumes
- current %B
- flow rate

Main Region – Where method file settings can be viewed or modified. Frequently used method settings are displayed on the MAIN screen, while advanced method options are set on the METHOD EDITOR window. See Section 4.3.1 *The Main Screen*.

Many of the PEAKTRAK WINDOWS contain these main elements or a subset of them (Figure 4-1).

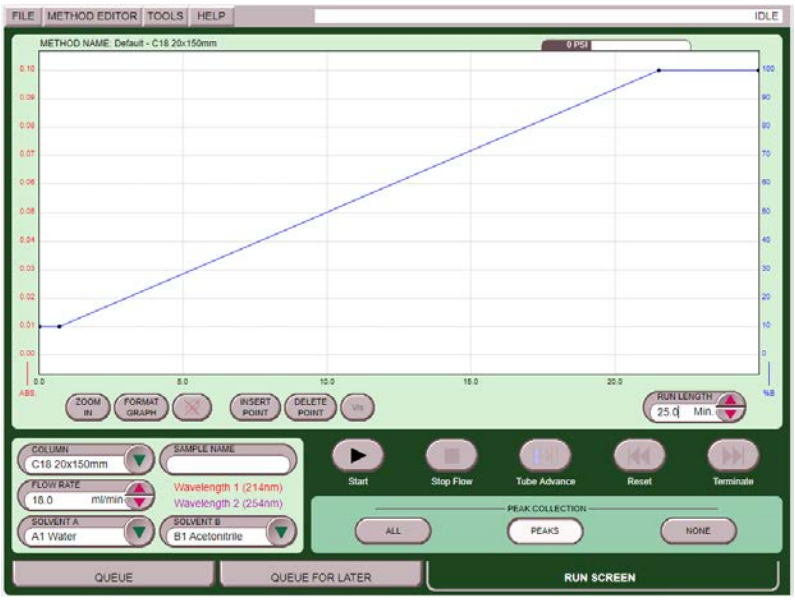


Figure 4-1 The Main screen

> **Note**
>
> The available commands and options will change according to the active window, the state of the system, and the current file.

4.1.2 Method Files

PeakTrak controls the separations performed by the system through a METHOD FILE. You do not have to create a complete method to perform a separation. When the system is turned on, it associates a default method in the system with the column selected as a default in the CONFIGURATION screen. In a single column system, this reflects the column mounted on the system. This default method is stored on the system hard drive, and a copy of it is automatically loaded into a temporary use area.

If the default method meets your needs, select START to start a separation. On the other hand, if you want to customize the method for use on this separation only, edit the method. (You can edit the gradient by dragging a gradient point is a commonly used modification.) This modified gradient will be used for the separation. This method is not saved for later reuse unless you select FILE > SAVE METHOD > and name the method. If you didn't save the method, but you later want to reuse it, open a data file that used the method and extract it for reuse. The system uses the method file in the temporary area to direct the system operation after you select START.

PeakTrak categorizes the method settings as Basic or Advanced. Basic settings are the frequently used controls accessed through the MAIN screen. Use the METHOD EDITOR window to access the advanced settings.

Method files are stored by the system and can be opened for review, reuse, or modification. To open a method file, select FILE > OPEN. Method files can be transferred to the system from an external storage device using FILE > IMPORT METHOD. Method files have an *.pmtd filename extension.

Default Methods

When PeakTrak is started, the system loads a default method. A default method contains Teledyne LAB's recommended basic and advanced settings for the use of a default column (as selected in the CONFIGURATION window). A default method for each size and type of column can be defined in the CONFIGURATION window. Default methods provide a starting point for your separation or purification. From these initial settings, you can perform a purification run, or you can modify the settings for your next run. Subsequent runs will use the settings in the active (temporary) window. If you have modified the settings, you can save the method file for future use.

If you find that the default methods are not a practical starting point for your applications, the methods can be changed to meet your specific requirements.

4.1.3 Run Files

When the system has finished a run, it saves the run data in a Run file. This includes the method parameters used during the separation (but not a reference to the method name, as that method could have subsequently been edited to different parameters), rack and tube information, a pressure trace of system pressure, spectral data, optionally mass spectrometer data and a chromatogram containing information from each of the detectors. You can open and review the RUN FILES stored by the system. To open a RUN FILE, select FILE > OPEN. Run files use an *.run file name extension.

4.2 PeakTrak Menu Options

PeakTrak menu options include

- FILE (Section 4.2.1)
- METHOD EDITOR (Section 4.2.2)
- MS (Section 4.2.3)
- TOOLS (Section 4.2.4)
- HELP (Section 4.2.5)

4.2.1 File

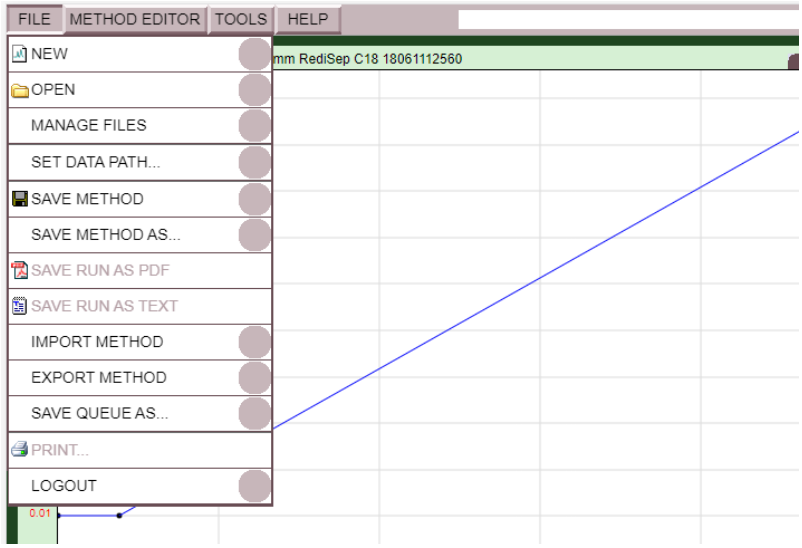


Figure 4-2 The File Menu

The FILE menu offers commands for managing and saving files:

New – Opens a new method file. PeakTrak opens the MAIN window using the default program settings for the default configured column size.

Open – Opens a METHOD FILE (.pmtd) or a RUN FILE (.run) stored on the system's internal hard drive.

Manage Files – Opens a window from which you can archive or delete method and run files from the current directory. You can archive files by connecting a USB Flash drive and selecting COPY or MOVE FILES TO FLASH DRIVE.

- To remove a file from the internal hard drive, highlight the file or enter the filename, then select DELETE.
- To remove multiple files, you can drag to highlight multiple files, or select DELETE BY AGE. Then specify the age limit in months and select OK.

> ☑ ***Note***
>
> Use the MOVE and DELETE options with caution, as the files cannot be recovered from the internal hard drive once the action is complete.

Set Data Path – Opens the SET DATA PATH window to specify your default METHOD FILE and RUN FILE storage folder location. Different users can have their own data paths.

Save Method – Saves the settings of the current method file to the system's internal storage. If the method is not named, SAVE METHOD prompts you to name the file.

> ☑ ***Note***
>
> Default methods are an exception and cannot be overwritten by SAVE METHOD. If a Default method is open and you attempt

to save any modifications to that method, the FILES window appears, so you can rename the method file. This preserves the default method. The Default method must be edited in the Prep HPLC tab.

Save Method As – Renames the current method with a name that you choose and saves it on the system's internal hard drive.

Save Run As PDF – Saves the displayed run. The default file name for the run is the same as for the Run file, except it has a .pdf file name extension. (PDF files can be opened and printed on the external computer with Adobe Reader, for example.) If you select SAVE RUN AS PDF from the touchscreen, a file download window opens on which you can select a location and enter a file name. If you select SAVE RUN AS PDF from a remote computer, the file is saved to the remote computer's hard drive.

Save Run As TXT – Saves the displayed run. The default file name for the run is the same as for the Run file, except it has a .txt file name extension. "TXT" files are actually XML code. These files can be opened with text editing software. They can also be imported into a spreadsheet for data manipulation. If you select SAVE RUN AS TXT from the touchscreen, a file download window opens on which you can select a location and enter a file name. If you select SAVE RUN AS TXT from a remote computer, the file is saved to the remote computer's hard drive.

Import Method – Loads a method file (.pmtd) from an external source onto the system's internal storage. If IMPORT METHOD is selected from the touchscreen, PeakTrak opens a FILES window to select the file on a USB flash storage device. If IMPORT METHOD is selected from a remote computer, you can select the method file from your local computer or network. After selecting the file, select OPEN to save the METHOD FILE on the system's internal hard drive.

Export Method – Saves a method file (.pmtd) in a location other than the system's internal hard drive. Use this to archive method files or to transfer the method to another system using IMPORT METHOD. From the touchscreen, EXPORT METHOD opens the FILES window so that you can save the file on a USB flash storage device. If EXPORT METHOD is selected from a remote computer, the file is exported and downloaded to that computer.

Save Queue As – Saves the queue on the system's internal hard drive for later use. The file name is given the .queue extension.

Print – Prints the completed run on the network printer if selected the touchscreen. If selected from a remotely

connected computer, PeakTrak displays the printer window. From this window, you can select and configure the printer of your choice. After configuring the settings, PRINT the completed run.

Logout – Logs you out of the system. Afterward, the system displays a login screen and waits for the next user to log in.

4.2.2 Method Editor

Selecting METHOD EDITOR from the menu opens the METHOD EDITOR window. Use METHOD EDITOR to view and modify basic and advanced method file settings.

METHOD EDITOR is described in detail in Section 4.3.4.

4.2.3 MS

The MS menu is only available when a PurIon Mass Spectrometer is added to the ACCQ*Prep* system. Furthermore, STANDBY and OPERATE are not available from a remotely connected computer via a web browser.

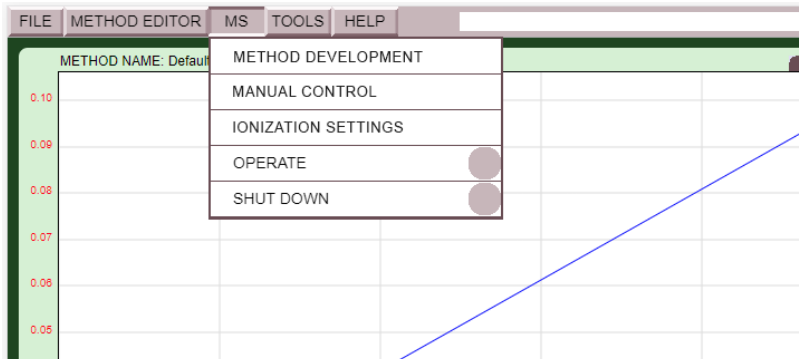


Figure 4-3 The MS menu

The following options are available:

Method Development – Opens the MS METHOD DEVELOPMENT window to verify ionization conditions for the compounds to be purified. The ION FINDER window may also be opened from the MS METHOD DEVELOPMENT window (Section 4.3.6).

Manual Control – Opens MS MANUAL CONTROL to prime and control the carrier solvent pump and switching valve on the mass spectrometer fluid interface. MANUAL CONTRO is not available from a remotely connected computer via a web browser.

Ionization settings – Opens IONIZATION SETTINGS to create custom ionization parameters that can be saved for various compound classes and projects. This command is not available from a remotely connected computer via a web browser.

Operate – Sets the status of the PurIon mass spectrometer from standby to an operational mode by applying power to the heaters and dynode detector. Ntrogen gas is also supplied to the nebulizer. When the mass spectrometer is

already in operate mode, this menu item is unavailable; STANDBY replaces it.

Standby – Turns off all high voltages within the PurIon, including the dynode detector, to prolong its life. In addition, it sets the inlet capillary heater temperature to 50 °C, turns the remaining heaters off, and lowers the gas flow rate to about 0.2 liters/minute. When in standby mode, OPERATE is used to return the PurIon to the operational state (high voltages on, heaters on, and gas flow at the proper rate of about 4 liters/min). When the mass spectrometer is already in standby mode, this menu item is unavailable; OPERATE replaces it.

Shutdown – Removes power to the heaters and removes power from the turbomolecular pump. A window appears to confirm that you want to initiate the shutdown procedure. SHUTDOWN is used to shut down the PurIon mass spectrometer for maintenance or to move the system.

⚠ WARNING

When a PurIon is installed, do not shut down or disconnect the vacuum line to the roughing pump until the PurIon is fully vented as indicated by PeakTrak.

4.2.4 Tools

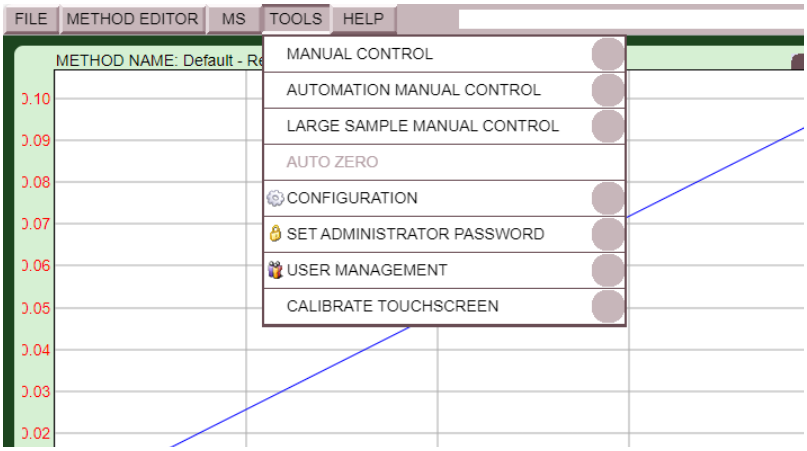


Figure 4-4 The Tools menu

The TOOLS menu provides options to configure and manually control your ACCQ*Prep* system.

Manual Control – Opens MANUAL CONTROL, from which you can operate the system manually. It can assist with priming or purging the internal plumbing and aid system troubleshooting. Options on this window are covered in detail in Section 4.3.9 *Manual Control*.

Automation Manual Control – Provides tools to prime the wash station and to manually wash the probe or injection loop.

Large Sample Manual Control – Runs the large sample pump. See Section 4.3.11 *Large Sample Manual Control*.

Auto Zero – Zeros the detector trace(s) during a run.

Configuration – Opens the CONFIGURATION window, from which you can specify the solvents used with the system, set default volumes for collection tubes, set the system date and time, configure network settings, etc. Options on the CONFIGURATION window are covered in detail in Section 2.1 *Configuration of the ACCQPrep*.

Set Administrator Password – Opens the SET ADMINISTRATOR PASSWORD window. You must enter a password to access this window. The default password set on the system is "accqprep". If you change the password, make a record of the new password in a safe place. Passwords prevent unauthorized modifications to:

- User management
- System date and time
- System password

See Section 4.3.13 for step-by-step instructions.

User Management – Opens the USER MANAGEMENT window, from which you can add or remove users from the system and change passwords. To prevent unauthorized changes to user management, this function is password protected. See Section 4.3.14 for step-by-step instructions.

4.2.5 Help

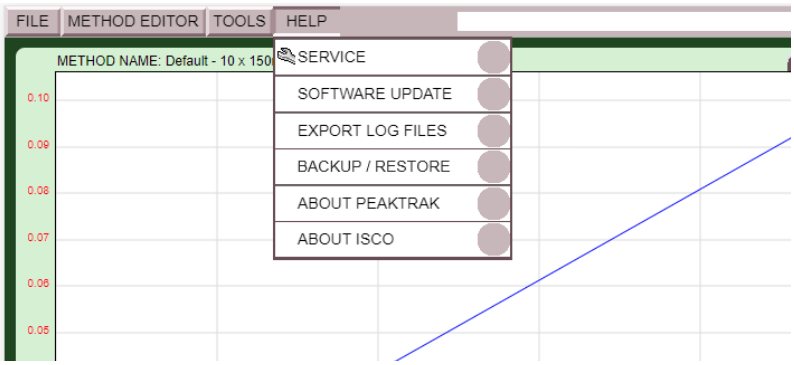


Figure 4-5 Help menu

The HELP menu provides information about the system:

Service – Provides access the system's service functions to qualified service personnel. These service functions are password protected to restrict their use to trained personnel. Access to SERVICE requires the administrator password.

Software Update – Opens a window used to install patch files to update your ACCQ*Prep* system software.

Export Log Files – During operation, the system writes operating data to a log file. If you are accessing the system over a network, the files are saved to your PC; otherwise, they are be saved to a connected USB drive. Teledyne ISCO service personnel can interpret this data to optimize system performance or to troubleshoot difficulties. Teledyne ISCO recommends that you use EXPORT LOG FILES only when advised by a qualified service technician.

Backup / Restore – Opens a window to back up a configuration, columns and methods, users, or user data to a ZIP file or restore it from a file. Access to these functions requires the administrator password.

About PeakTrak – Reports version information for PeakTrak and for firmware, and displays networking addresses.

About ISCO – Displays contact information for Teledyne ISCO. You may also contact Teledyne LABS at www.teledynelabs.com.

4.2.6 Backup / Restore Procedures

To back up instrument settings and data:

1. Plug in a USB drive.
2. Select HELP > BACKUP / RESTORE. The Backup / Restore window opens.
3. Select BACKUP. A backup file is saved to the USB drive.

To restore instrument settings and data from a backup:

1. Plug in a USB drive containing the backup file.
2. Select HELP > BACKUP / RESTORE. The BACKUP / RESTORE window opens.
3. Select items from the backup file that you want to restore.
4. Select RESTORE.

4.3 PeakTrak Windows

This section contains descriptions of the windows used for most PeakTrak tasks:

- MAIN window (Section 4.3.1)
- FILE menu windows (Section 4.3.2)
  - SET DATA PATH (Section 4.3.3)
- The METHOD EDITOR (Section 4.3.4)

- MS menu windows
  - MS Method Development (Section 4.3.5)
  - Icon Finder (Section 4.3.6)
  - MS Manual Control (Section 4.3.7)
  - MS Manual Ionization Settings (Section 4.3.8)
- TOOLS menu windows
  - Manual Control (Section 4.3.9)
  - Automation Manual Control (Section 4.3.10)
  - Large Sample Manual Control (Section 4.3.11)
  - Configuration (Section 4.3.12)
  - Set Administrator Password (Section 4.3.13)
  - User Management (Section 4.3.14)

4.3.1 The Main Screen

The MAIN screen gives you control of the frequently used functions before and during the run. After a run, or when viewing previous runs, MAIN displays peak collection data and the settings used for that run. MAIN dynamically changes to display the controls required for the current state of the system.

When RUN SCREEN is selected, the top region of the MAIN screen always displays a GRADIENT PLOT area (1). The bottom region of the window displays the RUN SETTINGS (2), RUN CONTROL buttons (3), and PEAK COLLECTION buttons (4). After a run, the left side of the window changes to show the collection rack map or the method parameters in a PEAK COLLECTION DATA pane (5).

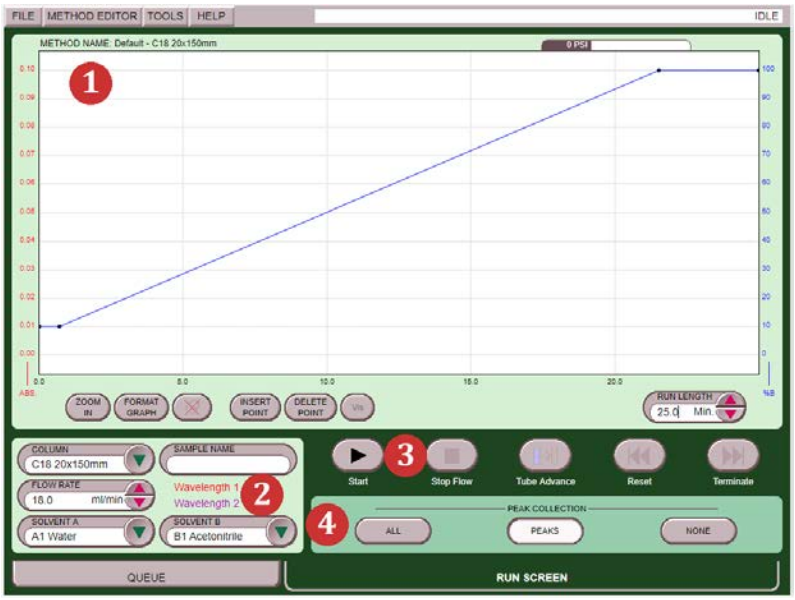


Figure 4-6 The Main screen, annotated

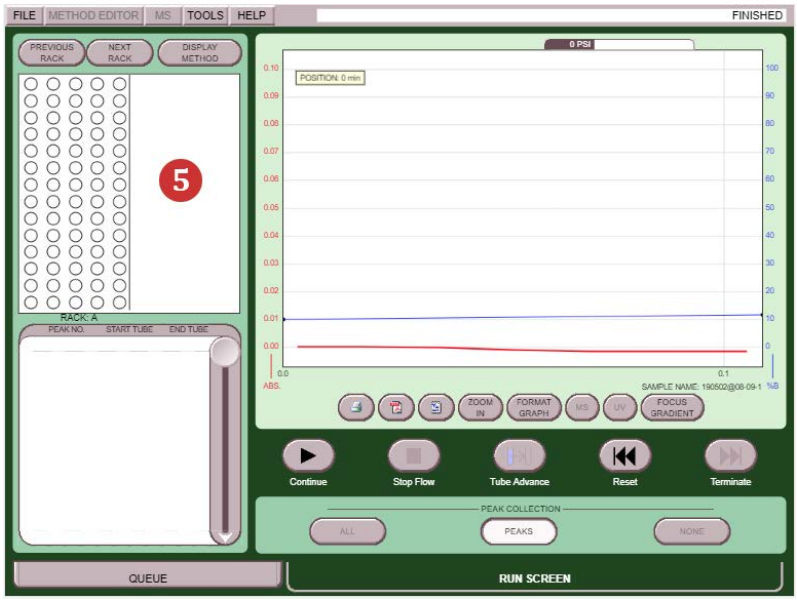


Figure 4-7 The Run Screen at the end of a run

Gradient Plot – The gradient plot area depicts the current gradient that will be used for the run. Depending on the setting to the CV TO TIME/CV TO TIME/TIME TO CV toggle (see *Button bar*), the X-axis of the plot displays the run time in minutes or in column volumes. The X-axis scale can be adjusted by typing or selecting the RUN LENGTH. The left Y-axis displays detector(s) units of measure, and the right Y-axis indicates the percentage of solvent B. By default, PeakTrak automatically scales the left Y-axis to best display the detector trace. PeakTrak also sets the right Y-axis to 100%. You can override the Y-axes scales by selecting the FORMAT GRAPH button and setting the values you want.

To zoom into a portion of the graphic area, "pinch zoom" and "swipe." To pinch zoom, place two fingers on the touchscreen in the graphics area, then move them apart to zoom in. Moving both fingers together (swiping) pans the display area. (A single finger touch is interpreted as a selection rather than a pan.)

The gradient plot area also provides a convenient method to edit the gradient curve. You can select and drag any of the points to change the shape of the curve and use the buttons at the bottom of the plot area to insert and delete points. For complete instructions on defining gradient curves, see Section 4.4.3 *Defining a Gradient*.

MS – Displays the current mass spectrum on PurIon systems. This spectrum hides the flow rate and solvent selection controls. Selecting the MS control again causes the spectrum to disappear.

When the spectrum is displayed, touching the chromatogram at any point prior to the current elution time displays a spectrum at that point in time. The elution time corresponding to the spectrum is displayed under the spectrum.

- LIVE DATA shows the current spectrum.
- ADD SPECTRUM is only displayed after completion of the separation and saves the spectrum at a point for inclusion in the run report screen. Up to 4 spectra can be saved in a run report. The button with a triangle pointing down cycles through saved spectra.
- The ± button displays the positive or negative ion spectrum (PurIon S and PurIon L systems only). A dot in the button indicates whether the positive or negative ionization spectrum is displayed.

UV – UV (UV-Vis on ACCQ*Prep* systems with a UV-Vis detector) toggles the display of the UV spectrum. This spectrum hides the flow rate and solvent selection controls. Selecting UV again causes the spectrum to disappear. When the spectrum is displayed, touching the chromatogram at any point prior to the current elution time displays a spectrum at that point in time. The elution time corresponding to the spectrum is displayed under the spectrum. LIVE DATA shows the current spectrum. ADD SPECTRUM saves the spectrum at a point for inclusion in a run report screen. Up to four spectra can be saved in a run report. The button with a triangle pointing down cycles through saved spectra.

Run Settings –

- COLUMN — The column used on the system. If the system has a Column Selector Valve Module, other installed columns to use for separation can be selected from the list. A second level menu provides methods already associated with the selected column.
- SAMPLE NAME —A label for the run. Type a unique descriptor. PeakTrak saves the run information under this name. Therefore, PeakTrak cannot accept any characters that are reserved by the operating system (* ? / \, etc.). Spaces are not allowed in the name. If you do not type a Sample Name, PeakTrak generates a date/time stamp for the name when you select START to begin the run.
- FLOW RATE — A flow rate for the run. Type it or select a rate from the list.
- DETECTOR — Controls the current peak detection mode. To change the peak detection settings, open the METHOD EDITOR window.
- SOLVENTS A AND B— If the system doesn't have the Solvent Selector Valve Module, you can select a solvent name from the list. The solvents in the list are those defined by the CONFIGURATION window. If the Solvent Selector Valve Module is installed, this control selects a solvent for use in the current separation.

Run Control Buttons – From left to right, the control buttons:

- START (the "play" symbol) — Starts or resumes the run. This is only present before the run has started; afterward, this is replaced by ISOCRATIC HOLD.
- ISOCRATIC HOLD (the "pause" symbol) — Holds the %B at the current value while the system continues to operate (sometimes called an *isocratic hold*). This is only present after the run has started; otherwise, START replaces it.

> **Note**
>
> Pausing the run extends the run length. While in the paused state, you can resume the gradient by selecting START, or stop the run by selecting STOP FLOW. If you resume the run, the system continues the gradient curve from the %B when the system was paused.

- STOP FLOW— Suspends the entire run. Unlike in the paused state, the pump, peak detection, and fraction collection will not operate. While stopped, you can abort the run by selecting RESET or TERMINATE, or resume the run by selecting START. In either case, the data is always saved. Using TERMINATE is the recommended way to abort a run because this runs sample probe wash and cone cleaning routines (PurIon systems only) to clean the system for another run.
- TUBE ADVANCE — Advances the fraction collector to the next tube position. This allows you to conveniently collect the eluent of interest in a new tube.
- RESET (the "rewind" symbol) — Available when the system has completed the run or when the run was stopped by selecting STOP. RESET returns you to the MAIN screen.

  If you want to terminate the separation prematurely, selecting TERMINATE is recommended. Unlike REWIND, TERMINATE does not modify the current method's run length setting.
- TERMINATE (the "fast forward" symbol) — Jumps to the next step of a run. After you've started a run, the system performs several steps. The first step is to deliver solvents using the programmed gradient for the entire run length. When this step is complete, the system enters the sample probe cleaning step.
  Selecting TERMINATE causes the system to skip any remaining time in the current step and advance to the beginning of the next step.
  If you have stopped the run before its programmed run length has elapsed, the current method is modified using the new run length. This modified method is ready for the next run, or can be saved for future runs

Peak Collection Buttons – Three peak collection buttons are located at the bottom of the MAIN window.

- ALL — Collects all eluent in the fraction collection tubes. Detected peaks advance to the next tube automatically to maximum peak concentration and purity
- PEAKS — Collects only eluted peaks in the fraction collection tubes.
- NONE — Diverts all eluent to the waste port. This is sometimes used to divert all peaks except the peak of interest to waste. Diversion can also be accomplished with the INITIAL WASTE & TIME WINDOWS functions.
- NO INJECTION/NO COLLECT— Runs a method without injection and without collecting peaks. Useful for setting up column conditioning or washing methods for using in the queue.

Peak Collection Data – Peak Collection data is displayed in the MAIN window after a run. You may also open a run file for viewing data from previous runs. Selecting on a test tube with the mass spectrum window displayed displays the mass spectrum for compounds collected into that tube (PurIon systems only). Selecting on adjacent tubes allows you to determine which fractions may potentially contain impurities.

The peak collection data is displayed on the left side of the MAIN window:

- Rack and tube information — Collected peaks are color coded in the rack diagram so that you can easily locate the peaks of interest. The tube colors correspond to the color bars under the peaks displayed on the chromatogram. If more than one set of tube racks was filled during the run, select NEXT and PREVIOUS RACK to view the additional racks. The table below the rack diagram displays the peak data in tabular form.
- DISPLAY METHOD — Toggles a summary display of the method settings for the run. You can return to the rack and tube display by selecting DISPLAY RACK again.

4.3.2 Files

The Files window is modal. That is, its function and features change according to the command used to open the window. Commands such as FILE > OPEN and SAVE METHOD AS or the OPEN and SAVE AS buttons open this window. Use this window to browse the system's files and folders using the following controls:

- Current Path — The top-left corner of the window displays the path (current folder). As you browse through the files, the path updates as you go. You can select the folder names to return to upper folder levels.
- File and Folder operation buttons:

- COPY — Copies a highlighted file to the system's clipboard.
- PASTE — Pastes a file from the clipboard. If the file already exists in the current folder, the system ignores PASTE to prevent the original file from being overwritten.
- DELETE — Deletes the highlighted file or a folder and its contents.
- NEW FOLDER — Creates and names a new folder.
- UP — Navigates to the next-higher folder level for browsing.
- SEARCH — Finds file names matching a KEYWORD or words. Select CLEAR SEARCH to clear the results.
- FILE MANAGEMENT options — Select COPY FILES TO FLASH DRIVE or MOVE FILES TO FLASH DRIVE to create archive copies (PDF or Text) of the files on a connected flash drive. Select DELETE BY AGE to specify an age limit beyond which to delete files from the internal hard drive.
- FILE/FOLDER info scroll box — Lists the contents of the current folder. The contents can be sorted by selecting the column headings.
- FILE TYPE — Limits the file list to certain types of files. Select a file type from the list.
- FILE NAME — Identifies the currently selected (highlighted) file or folder when browsing and opening files. When using SAVE AS, enter this to name the file.
- OPEN/SAVE/DELETE — Performs the listed action (e.g., OPEN a file).
- LOAD PREVIOUS RUN FROM DETECTED RACK — (Appears when selecting FILE > OPEN.) Instructs the system to read the RFID tag on a single rack and displays the last RUN FILE collected on the detected rack on this instrument. This feature is useful you are unsure of the rack's contents.

> ☑ ***Note***
>
> The rack must be placed in the left position in the instrument. If the AutoSampler is present, the rack must still be placed inside the ACCQ*Prep*.

- CANCEL — Closes the window without saving or opening the file.

4.3.3 Set Data Path

Use the SET DATA window to select a default folder for the current user. After selecting a folder, file operations such as saving or opening files will use this selected directory.

File > Set Data Path

SET DATA PATH is primarily used with the USER MANAGEMENT feature. USER MANAGEMENT automatically creates a folder for each user. When using the system, set the data path to your folder or a subfolder within. Each user has a different data path. The window contains the following controls:

- Current Data Path — Displays the path (current folder) at the top-left corner of the window. As you browse through the files, the path updates as you go.
- NEW — Adds and names a subfolder within the currently selected folder.
- DELETE — Deletes the selected folder.
- Folder selection box — Lists available folders and buttons that select them.
- OK — Saves the selected folder as the data path and closes the window.
- CANCEL — Closes the window without changing the data path.

4.3.4 Method Editor

Each section of the METHOD EDITOR groups its options by function. The BUTTON BAR (1) at the top of the window gives you quick access to file operations, and column data. Below the BUTTON BAR are the RUN SETTINGS (2) and the RUN NOTES (3). The lower part of the window contains the GRADIENT PLOT AREA (4) and options for PEAK COLLECTION (5), PEAK DETECTION (6), and to EDIT [the] GRADIENT (7).

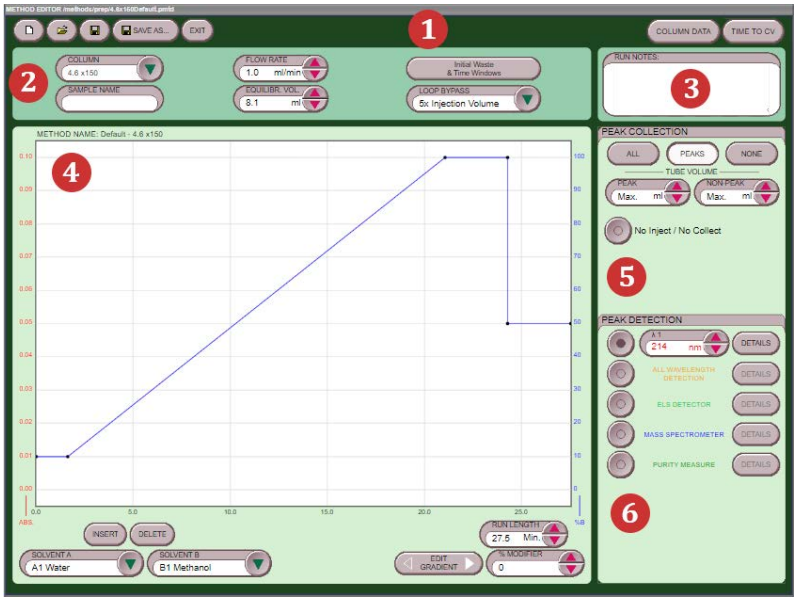


Figure 4-8 Method Editor screen with Peak Collector and Peak Detector panels

Selecting the Edit Gradient button at the bottom of the METHOD EDITOR toggles either the PEAK COLLECTOR and PEAK DETECTOR panels (shown above) or the EDIT GRADIENT panel (shown below).

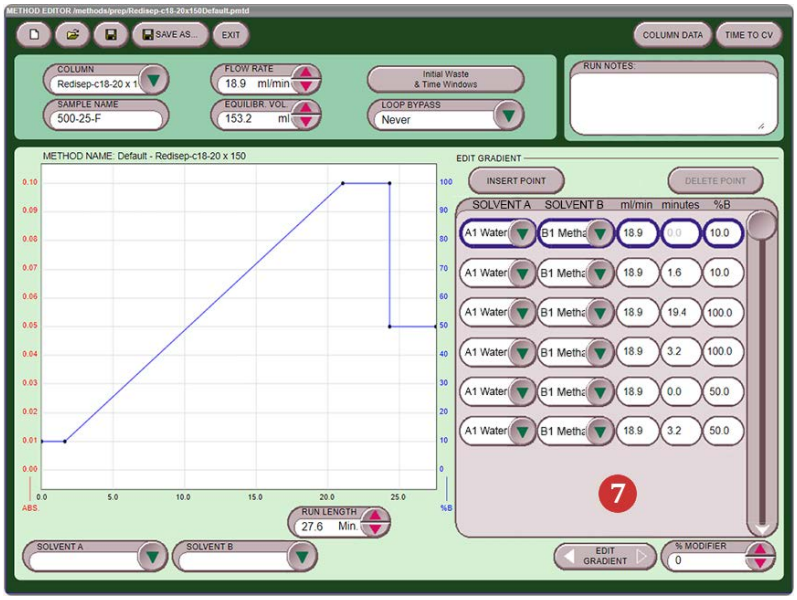


Figure 4-9 Method Editor screen with Edit Gradient panel

Button bar – Provides commands for opening, editing, and saving methods.s. Saved methods can be retrieved for further editing or use.

Figure 4-10 Method Editor Button Bar

The METHOD EDITOR Button Bar contains the following options from left to right:

- NEW (a blank document icon) — Opens a new method file using the default method settings for the selected column.
- OPEN (an open folder icon) — Opens a method file stored on the system's internal hard drive.
- SAVE (a diskette icon) — Saves any modifications to the current method file. If you attempt to save modifications to a default method file, the FILES window opens instead and allows you to rename the file to preserve the default method.
- SAVE AS — Opens the FILES window. From this window you can rename the current method and save it on the system's internal storage.
- EXIT — Closes the METHOD EDITOR and returns to the MAIN WINDOW.
- COLUMN DATA — Opens the Column Data window, which reports information about the Redi*Sep* ACCQ*Prep* column installed in the system. This information includes. The "Number of times used," "First used on," and "Last used on" information can help you determine when the column should be replaced. The "Last fluid

used" helps you determine if any solvent remaining in the column will be miscible with the solvent currently used in the system. To view the Column Data window, open the METHOD EDITOR window, then select COLUMN DATA.

- CV TO TIME/TIME TO CV— Toggles the units used to enter and/or report EQUILIBR. VOL., RUN LENGTH, and the Gradient Plot X-axis units. Setting units to CV may make method scale-up or other calculations easier.

Setting	Time toggle	CV (colume volume) toggle
Equilibration Vol.	mL	CV
Run Length	minutes	CV [Min.]
X-axis	minutes	CV

Run Settings – Sets options for the run.

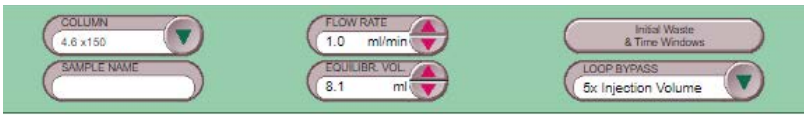


Figure 4-11 Method Editor Run Settings

- COLUMN —Displays the name of the currently selected column. If a Column Selector Valve Module is installed, this selects the column used for the current separation.
- SAMPLE NAME — The sample name is a text entry box in which you can label the run. Type a unique descriptor. PeakTrak saves the run information under this name. Therefore, PeakTrak cannot accept any characters that are reserved by the operating system (* ? / \, etc.). A space can't be used in the sample name. If you do not type a SAMPLE NAME, PeakTrak generates a date/time stamp for the name when you select START to begin the run.
- FLOW RATE — Type or select a flow rate for the run. When using the default column methods, the system sets the flow rate to the configured flow rate of the column.
- EQUILIBRATION VOLUME — Type or select the volume of solvent that is pumped through the column and out the waste port before the sample is introduced. The volume can be entered in minutes or in column volumes, depending on the setting to the CV TO TIME/CV TO TIME/TIME TO CV toggle. (See *Button bar*.) Data is not collected while this volume is being pumped.
- INITIAL WASTE & TIME WINDOWS — Opens a window from which you can view and modify these settings.

For the INITIAL WASTE, type or select the volume of eluent that is allowed to pass through the column to the waste port after the sample is injected. Use this feature to conserve collection tubes by diverting all fluids during this time period, even if a peak is detected.

TIME WINDOWS can limit the fraction collection to specific time durations of the run. To use time windows,

1. Enter a START time and an END time. All times are relative to start of the run, just following the sample injection.
2. Then choose a PEAK COLLECTION mode. If you select ALL, the module collects all fluid during the time window regardless of the peak state. PEAKS collects fluid only when the time window is active and a peak is present.
3. Enter more time windows as needed.

Detected peaks will still trigger tube advances.

- LOOP BYPASS— Automatically switches loop out of fluid path after selected times of sample volume has passed through. Useful for analytical scale, as it simulates a loop size equivalent to the value you choose without physically changing to a smaller loop, minimizing delay time for the gradient to reach the head of the column.

Run Notes – Use this text entry box to enter comments or notes for the run. These comments are be saved with the run and appear in TXT and PDF reports. Column identification information is automatically placed in this box.

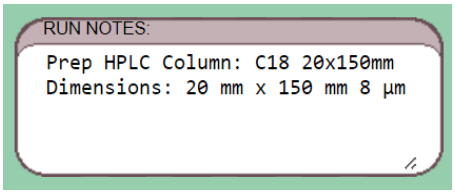


Figure 4-12 Method Editor Run Notes

Gradient Plot Area – Depicts the current gradient profile that will be used for the run. The X-axis of the plot displays the run time in minutes or in column volumes, depending on the setting to the CV TO TIME/CV TO TIME/TIME TO CV toggle. (See *Button bar*). The X-axis scale can be adjusted by typing or selecting the RUN LENGTH in minutes (or column volumes). The left Y-axis displays absorbance units, and the right Y-axis indicates the percentage of solvent B.

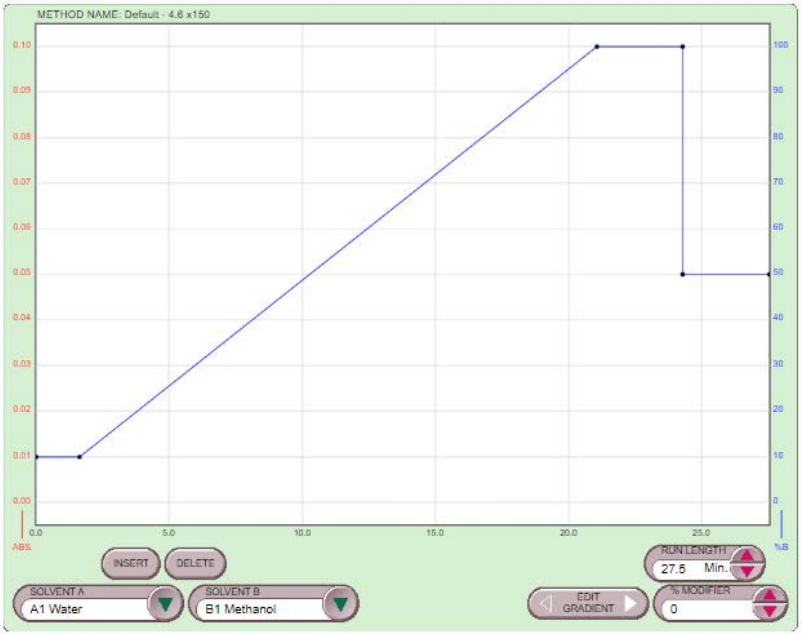


Figure 4-13 Method Editor gradient plot area

The gradient plot area also provides a convenient method to edit the gradient curve. You can select and drag any of the points to change the shape of the curve, or use the buttons at the bottom of the plot area to insert and delete points. To zoom into a portion of the graphic area, use “pinch zoom” and “swipe.” Pinch zoom is accomplished by placing two fingers on the graphics area and moving them apart to zoom. Once zoomed in, moving both fingers pans the display area. (A single finger touch is interpreted as a selection rather than a pan.)

The following controls appear in the Gradient Plot Area:

- INSERT — Activates the gradient point insert mode. When active, select the gradient curve to add a single point. You can then drag the new point to any desired position. Select INSERT once for each gradient point that must be added to the plot area.
- DELETE — Activates the gradient point delete mode. When active, the point nearest to the next point selected on the gradient curve is deleted. Select DELETE once for each point that must be deleted from the plot area.
- RUN LENGTH — Type or select the length of the run. The length is expressed in minutes or in column volumes,

depending on the setting to the CV TO TIME/CV TO TIME/TIME TO CV toggle. (See *Button bar*.)

Note

Run Length changes the scale of the X-axis on the gradient. Points that define the gradient will be automatically scaled to fit the new run length.

- Solvents A and B— Select a solvent from the list. The available solvents are those defined by the CONFIGURATION settings. You can program mid-run solvent B changes by selecting EDIT GRADIENT to open the GRADIENT TABLE.
- EDIT GRADIENT — Opens the gradient table. Although the gradient may be edited directly within the plot area, you can also edit the gradient in tabular form. The gradient table also lets you program solvent changes in the gradient. See the *Edit Gradient* section below.
- For complete instructions on defining gradient curves, see Section 4.4.3 *Defining a Gradient*. The gradient table depicts the points that define the gradient curve in a tabular format. From this table you can change Solvent, the duration, and %B concentration of any point on the curve. To do so, select the new value for a gradient point. Changes that you make to the table are reflected in the gradient plot area. You can also change the number of points on the curve by inserting or deleting rows in the table. To change the number of points, first highlight a table cell or row. Then select the appropriate GRADIENT button for the action you desire.
- INSERT POINT — Inserts a row below the selected point on the gradient table.
- DELETE POINT — Deletes the selected row on the gradient table. You cannot delete the initial point. There must be at least two points to define a gradient. The system automatically updates the RUN LENGTH setting or scales the other points when you change the number of points and their duration on the curve. To close the gradient table, select the EDIT GRADIENT toggle. See Section 4.4.3 *Defining a Gradient*.
- 3RD SOLVENT MODIFIER (IF EQUIPPED) — Allows you to configure a third solvent modifier. Requires the additional 3rd solvent modifier pump module.

Peak Collection – Sets the collection mode.

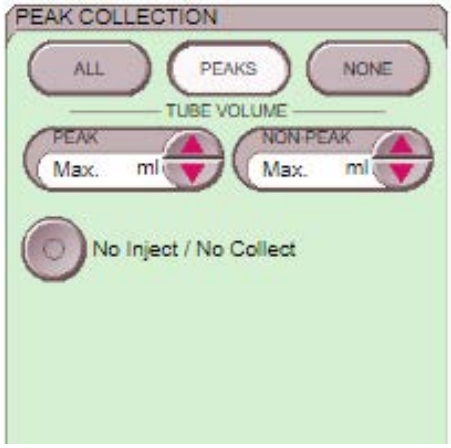


Figure 4-14 Method Editor Peak Collection

- ALL — Collects all fluids in the fraction collection tubes during a run.
- PEAKS — Collects only eluted peaks in the fraction collection tubes during a run.
- NONE — Diverts all fluids during a run to the waste port.
- Tube Volume — Specifies the tube volume for collected fluids. This volume can be the default maximum volume for that tube size (Max option) as entered in the CONFIGURATION window, or a method-specific volume that is less than the capacity defined by the CONFIGURATION window.

Note

The actual fraction size may be less if a newly detected peak causes a tube change or if you select TUBE ADVANCE.

- Peak — Type or select a volume to be collected in each tube when the system detects a peak.
- Non-peak — Type or select a volume to be collected in each tube when peaks are not detected. This setting allows you to conserve tubes without diverting non-peak elute to waste. This setting is ignored when the peak collection mode is set to Peaks or None.
- NO INJECT/ NO COLLECT —Runs a method without injection and without collecting peaks. Useful for setting up column conditioning or washing methods for using in the queue.

Peak Detection – This section of the METHOD EDITOR provides peak detection options.

Note

You can select up to four peak detection options on the ACCQ*Prep* HP150 system. If more than one option is selected, such as λ_1 with λ_2, the system considers a peak to be present when an option is true (a logical OR).

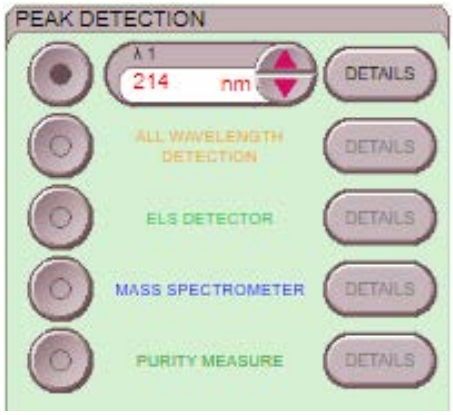


Figure 4-15 Method Editor Peak Detection

When a detection method is enabled, selecting DETAILS opens a DETECTION OPTIONS window that modifies that method's settings.

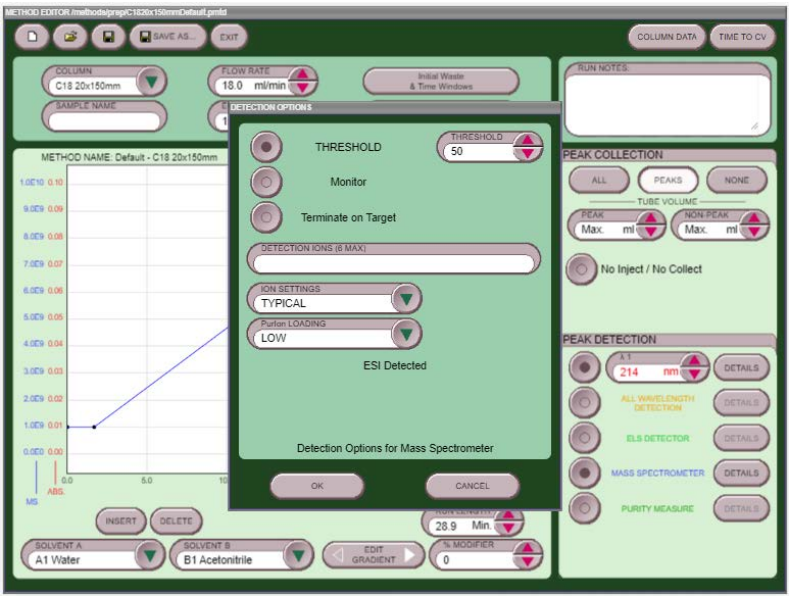


Figure 4-16 A Detection Options window

All enabled options are displayed on the chromatogram. Options and settings for ACCQ*Prep* systems are described below.

Wavelength Peak Detection

- λ_1 and λ_2 (wavelength 1 and 2) — Select these to use and configure primary and secondary wavelength detection. λ_2 is set to monitor (displayed but not used for peak detection and cutting) by default.

  When selected, type or select the peak detection wavelength in nanometers, then select DETAILS to configure additional settings described below.

Wavelength Peak Detection Details

- Signal Gain – Modifies the gain to scale the detector signal.
- Slope Based — Enables slope-based peak detection. Peaks are recorded when the slope algorithm detects a peak similar to the Peak Width setting.
- Peak Width — Select the average peak width setting.

  Peak widths are measured at the baseline. The slope detector typically detects peak widths ranging from about 0.2 to 2 times the peak width setting. For example, if you entered a peak width of 1 minute, the range would be 12 seconds to 2 minutes. For best operation, the peak width should be set to just over the average peak width being separated. For instance, if the average peak width is 45 seconds, enter a peak width of 1 minute. For most flash chromatography, 1 minute is a good starting point for a peak width. Sometimes very small peaks need a larger peak width setting since their small height results in a smaller slope than larger peaks of the same width.

  Detection ends when the detector signal drops to 0.01 AU below the value entered here. This reduces the potential of multiple peaks being detected if a noisy signal oscillates around the threshold value.
- Threshold — Enables threshold peak detection. When enabled, peaks are recorded if the Absorbance Units (AU) value is exceeded. Type or select the Absorbance Units value to be used for Threshold detection.

☑ Note

If both Slope and Threshold peak detection methods are checked, the system considers a peak to be present when any one condition is met. This logical OR operator means that the system will cut a peak when either the Slope condition is true, or when the Threshold condition is true.

- Monitor — Uses the detection source as a monitor. When enabled, the detection source is displayed as a trace on the gradient plot area but is not be used to cut peaks.

All Wavelength Peak Detection

- All Wavelength Detection — Detects peaks within a user-selected range of wavelengths. When checked, select DETAILS to configure additional settings. These settings include the slope-based and peak width options

described for λ_1 and λ_2 above. Here, PEAK WIDTH is used to determine solvent absorbance suppression. Peaks that are twice the peak width are deemed to be solvent or other baseline drift, and their absorbance spectrum are subtracted as an assumed baseline spectrum.

All Wavelength Details

- MINIMUM λ and MAXIMUM λ sets a limit in nanometers. This limit eliminates areas of little spectral information from the all wavelength detection signal, resulting in a stronger signal.

ELS Peak Detector

- ELS Detector — Enables the evaporative light scattering (ELS) detector on an ACCQ*Prep* system. When enabled, select DETAILS to configure additional settings:

ELS Detector Details

- Sensitivity - Can be selected as NORMAL to keep largest peaks on scale or HIGH to detect small peaks. The default setting is HIGH to ensure most compounds are detected.
- Signal Gain – Modifies the gain to scale the detector signal.
- Slope Based and Threshold – Refer to these features described above for λ_1 and λ_2 .
- Spray Chamber Temperature – The default settings are 30 °C (normal phase default methods) and 20 °C (reverse phase). This setting may be adjusted from 10 to 60 °C so that detection can be optimized for the solvent system in use. However, operation at temperatures greater than 5 °C below the ambient temperature is not guaranteed. Lower temperatures cause more of the solvent to condense before entering the drift tube. This is useful to limit the amount of solvent aerosol entering the drift tube allowing the drift tube temperature to be set lower. This setting is limited to a minimum of 5 °C below the ambient temperature.
- Drift Tube Temperature – The default settings are 60 °C (normal phase default methods) and 60 °C (reverse phase). This setting may be adjusted from 30 to 90 °C but is limited to a range of 5 °C below and 60 °C above the ambient temperature.

  The drift tube temperature must be high enough to evaporate the solvent aerosol entering the drift tube. If the temperature is not high enough, unevaporated solvent may form a fog that condenses on the detection system preventing detection of the compounds. This can be corrected by selecting ELSD ON in the MANUAL CONTROL window to turn on the flow of gas through the drift tube without liquid. Temperatures that are too high can cause semivolatile compounds to vaporize and become undetectable.

Mass Spectrometer Peak Detection

- Mass Spectrometer – Monitors or detect compounds with a PurIon mass spectrometer system (PurIon

systems only). Mass-directed peak detection can be set for up to 6 masses or 5 masses and 1 range.

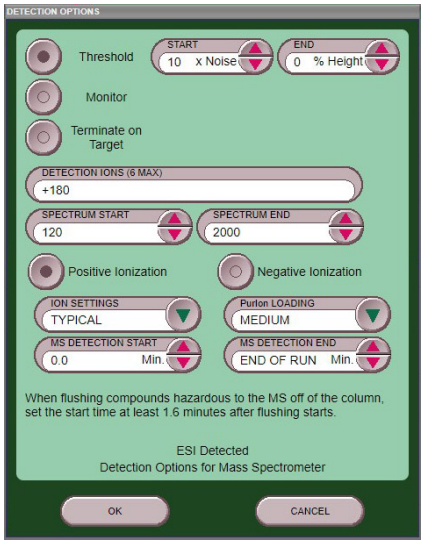


Figure 4-17 MS Detection Options window

MS Detection Details

- Threshold – Selects the START and END signal intensity ratios for directing fraction collection.

  This limits fraction collection for peaks that have tailing tendencies. Such peak tails may cause collection of many fractions that contain a very small amount of compound. Consequently, MS detection can lead to compound tailing that causes excessive fraction collection.

  Usually, the default THRESHOLD settings control compound tailing adequately. However, adjusting the START or END thresholds to define an asymmetric threshold may be required to improve peak cutting to minimize collection of such fractions and speed the collection process:

- Start Threshold – The multiple of the mass spectrometer signal's noise baseline level at which peak collection starts. Setting it lower can allow the software to start collecting compound more quickly, and it may slightly improve recovery while minimizing the number of collected fractions. The noise level is measured at the first column volume.

- End Threshold – The percentage of the maximum peak height of the current peak at which detection stops and collection ends. For example, entering 20% ends collection at 20% of the peak height. Setting a percentage of peak height can allow proper collection of both large and small peaks. Setting this to 0% instructs peak collection to use the START noise threshold.

  Selecting THRESHOLD deselects MONITOR.

- Monitor – When selected, prevents fractionation based on the mass spectrometer signal. Selecting MONITOR deselects THRESHOLD.
- Terminate On Target – When selected, stops the run after all mass spectrometer detection ions have been detected.
- Detection Ions – Sets ions for detection or ions to be monitored. Up to four single ions may be chosen, or a range of ions and up to three single ions may be selected. On PurIon S and PurIon L systems, detected ions may be a mixture of positive and negative ions.
- Spectrum Start/End – (PurIon S and PurIon L systems only)
- Positive Ionization – Selects positive ion spectrum (PurIon S and PurIon L systems only). Selecting POSITIVE IONIZATION deselects NEGATIVE IONIZATION.
- Negative Ionization – Selects positive ion spectrum (PurIon S and PurIon L systems only). Selecting NEGATIVE IONIZATION deselects POSITIVE IONIZATION.
- Ion Settings – Sets ionization parameters to enhance detection of molecular ion peaks.
- PurIon Loading – Sets the mass load (High, Medium, or Low); that is, the quantity of the carrier flow rate that is sent to the mass spectrometer (MS).
  - High: 20 µL/min
  - Medium: 6 µL/min
  - Low: 1.467 µL/min

  Setting the mass load too low results in significant signal delays, while setting it too high results in poor signal and clog the MS with excess sample.

The PurIon MS doesn't have a defined carrier fluid split ratio; instead, it uses an active splitter valve assembly that portions off a specific, programmable volume of the system flow. Portion volume is set independently of the carrier fluid flow rate (always 0.2 mL/min).

The effective split ratio is *system flow rate ÷ split flow rate*. Some examples:

- At a system flow rate of 20 mL/min, the split ratios are 1000:1, 3333:1, and 13633:1. (That is, 20 mL ÷ 20, 6, and 1.467 µL/min, respectively.)
- At a system flow rate of 100 mL/min, the split ratios are 5000:1, 16666:1, and 68166:1 (That is, 1000 mL ÷ 20, 6, and 1.467 µL/min, respectively.)

- MS Detection Start/End – Sets the start and end times at which the MRA valve is active during the method. This avoids salts and other contaminates at

the start of a run and prevents unwanted compound from being sent to the mass spectrometer during a column wash.

> **☑ Note**
>
> If the MS is not active during the start of the run, the MRA valve will not be active during equilibration or any pre-run sample loading when using the sample loading pump.

Purity Measure Peak Detection

- Purity Measure — When the DETAILS button is available, select it to configure additional settings:
  - Show Ratio – Displays a ratio of the selected wavelengths when two absorbance wavelengths are used. The ratio trace is often a useful indicator of purity.

Purity Measure Details

  - Show Spectral Purity – Measures purity by using a comparison of the entire UV-spectra measured at differing times as a purity measurement.
  - Spectral Purity Detection – Bases fractionation on a spectral purity threshold that you enter.

External Detector

- External Detector — Enable to use a 0 to 1 volt analog signal from an external detector. When enabled, select DETAILS to configure additional settings. These settings include the slope-based and peak width options described above for λ_1 and λ_2. The system uses its internal algorithms to cut detect and cut peaks on the basis of the analog input signal. Refer to the External Detector instruction sheet for cable and plumbing requirements.

  The external detector option is not available on ACCQ*Prep* systems with ELSD or PurIon detectors.

Edit Gradient – This table changes the FLOW RATE at the time points on the gradient plot area. Select the EDIT GRADIENT button to toggle the gradient table open or closed. After the table is opened, add or delete time points, or select a field to highlight it and make the row editable.

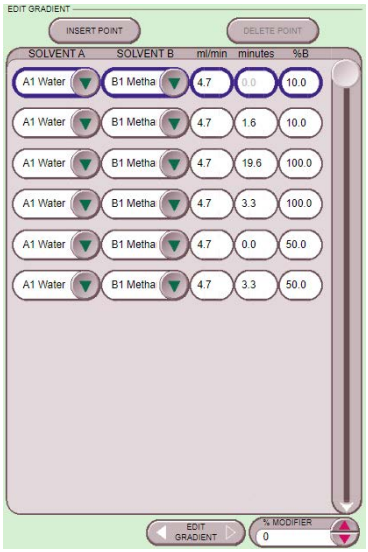


Figure 4-18 Gradient Table

INSERT POINT – Add a time point before the point whose row is highlighted on the table. The new point appears at the same location as the highlighted row's point. You can move it along the X-axis by changing the MINUTES or CV entry.

DELETE POINT – Remove the highlighted row and its associated point from the table.

SOLVENT A or B – The solvents that have been entered on the CONFIGURATION window > INSTRUMENT CONFIGURATION tab.

ML/MIN – The flow rate in mL per minute. A change in flow rate at a point is shown in the gradient plot area as a vertical line through and a callout at the previous point.

> **Note**
>
> Any changes to the flow rate in the gradient table occur almost immediately at that point. There is not a gradual change to the new set flow rate over the time period, unlike the gradual change of %B composition in the gradient table.

MINUTES/CVS – The number of minutes or column volumes from the previous time point, as viewed along the X-axis of the gradient plot area. The label of this control, the units used here, and units on the X-axis depend on the EDIT GRADIENT control toggle. (See *Method Editor*: *Button bar*.)

%B – The percentage of solvent B. Adjusting a value on a row also moves its point along the Y-axis.

4.3.5 MS Method Development

(The MS METHOD DEVELOPMENT window is available on PurIon equipped systems only).

MS > Method Development

MS METHOD DEVELOPMENT provides a means to test and verify ionization conditions for their compound. This is not available from a remote connection.

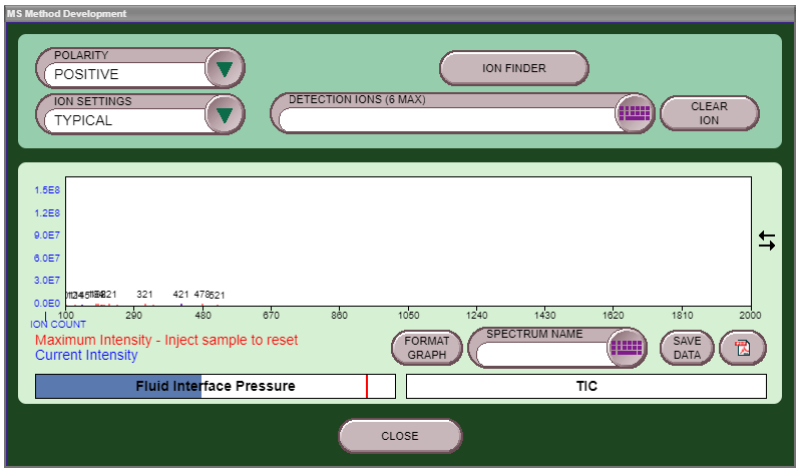


Figure 4-19 Mass Spectrometer Method Development window

MS METHOD DEVELOPMENT contains the following controls:

- **Polarity —** On the PurIon system: changes the probe polarity between positive and negative ionization. On PurIon S and PurIon L systems: toggles the displayed mass spectrum between positive and negative ionization.
- **Ion Settings —** Allows selection of different ion settings to maximize the intensity of the molecular ion. The factory selections include “Robust” for compounds that do not easily ionize. “Typical” works well for most compounds. The “Fragile” setting is used for those compounds that are delicate or easily fragment. Ion settings that you define may also be loaded.
- **Ion Finder —** Opens the ION FINDER window. The Ion Finder identifies potential adducts, fragments, and multiply charged species of the compound of interest when the compound of interest does not show up in the spectrum graph. Such ions can then be used to detect the compound of interest. See Section 4.3.6 *Ion Finder*.
- **Detection Ions—**After a spectrum is collected, selecting a peak adds that ion to this list. Alternately, masses can be entered using a keypad control.
- **Clear Ions —** Clears the last value entered into the DETECTION IONS list.
- **Mass spectrum graph —** Displays the mass spectrum. There are two mass spectra displayed. The spectrum depicted in blue shows what the PurIon is currently detecting. The red spectrum depicts the largest peaks detected. The red spectrum is reset after injecting a sample.

To inject a sample, move the PurIon injection valve to the "Inject Sample (>20 µg/mL)" position first. Inject a sample (>20 mL), then move the valve to the "Scan Mass" position.

> ☑ ***Note***
>
> Only use 22 gauge square tip needles (PN 29-9001-911) to avoid scratching the internal surfaces of the injection valve! Filter the sample with a 0.45 µ filter to avoid clogging the PurIon tubing and probe capillary. Sample concentration should be less than 20 µg/mL.

- **Modify View—**Sets the displayed range of the mass spectrum. The PurIon system still collects the entire mass range even when the range is set within than the minimum and maximum values allowed for the spectrometer. Values can range from 10 to 1200 Da (PurIon or PurIon S) or 2000 Da (PurIon L). Changing the mass range defined by these controls may cause the Y-axis (ion count) scaling to change on the basis of the tallest peak within the range. The display only labels the m/z for the 10 most prevalent ions *currently displayed*. Formatting the graph to a narrower range causes the system to relabel the ions to identify ions that may not have been intense enough to be labeled on the broader range graph.
- **Spectrum Name—** A file name for the mass spectrum is needed only if you want to save the mass spectrum to a file for later viewing.
- **Save Data—** Saves the mass spectral data to the internal hard drive.
- **Save as PDF—** Saves the displayed spectrum on either a USB drive or a remote computer's hard drive. The file name is the same as the spectrum name except it has a PDF extension. PeakTrak displays a file download window so that you can select a location and change the file name.
- **Fluid Interface Pressure —** The carrier solvent pressure, displayed in a ribbon gauge. If the pressure approaches the level of the red line, the system is clogged and should be cleaned to allow proper operation.

4.3.6 Ion Finder

(The ION FINDER window is available on PurIon equipped systems only).

MS > MS Method Development > Ion Finder

The Ion Finder identifies potential adducts and fragments of the compound of interest when the compound of interest does not show up in the spectrum graph. It is useful when working with large molecules.

After entering a molecular weight, the Ion Finder algorithm looks for masses in the spectrum that match loss of various fragments, adducts, and multiply charged ion species. It can detect

and report multimers, solvent adducts, and charge carriers that replace protons such as sodium and potassium. It can detect positive, negative, and multiply charged ions.

After ions are detected, you can select any such ions that correspond to the mass of interest and use them for detection.

> **☑ Note**
>
> The Ion Finder has no knowledge of the compound, so some items in the Detection Ion list may not be associated with or even possible for the compound to be purified. Or, they may only be part of the injection or carrier solvent.

The Ion Finder only acts on the ten most intense peaks in the display. When impurities are present, some of the desired compounds' mass peaks may not be noted by the finder. In that case, rescaling the mass can eliminate unwanted intense peaks (possibly due to impurities) and allow detection of the desired compound.

To use the Ion Finder:

1. Use the METHOD DEVELOPMENT window (MS > Method Development) to inject a sample.
2. Select POLARITY and ION SETTINGS. Changing the ION SETTINGS changes the ions seen. Remember that Ion Settings choices do not transfer to the detection options for the mass spectrometer in the METHOD EDITOR—the ion settings that gave a good result in the MS METHOD DEVELOPMENT window should be chosen.
3. Select ION FINDER. The ION FINDER window opens to display a list potential adducts and fragments detected in the compound of interest. Because of isotopes, multiple peaks near one another may be found. (For example, 546 and 547, also 818 and 819; Figure 4-20.)
4. Select the radio button next to a listed ion to add it to the ions to be detected.

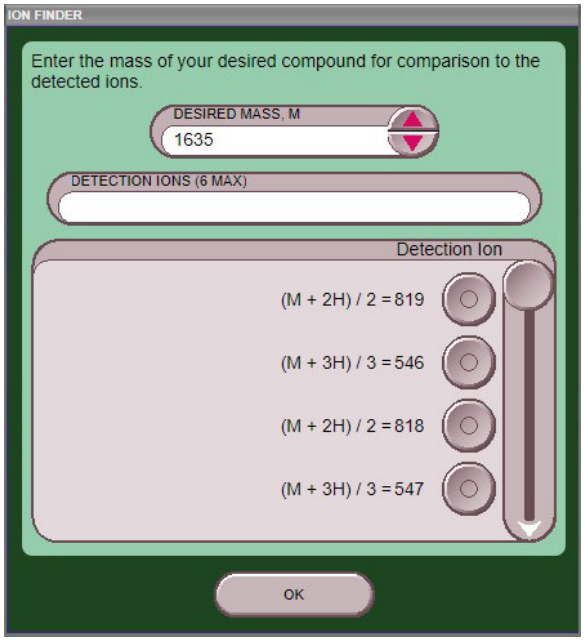


Figure 4-20 The Ion Finder window

- DESIRED MASS – The molecular weight of your compound, rounded to the nearest integer.
- DETECTION IONS – The list of possible adducts, fragments, or multiply charged ion species detected.
- DETECTION ION – The list of possible adducts or fragments detected based on the value entered in the DESIRED MASS and detected by the system. Checking an item adds it to the DETECTION IONS list.

4.3.7 MS Manual Control

(The MS MANUAL CONTROL window is available on PurIon equipped systems only).

MS > Manual Control

MS MANUAL CONTROL allows the fluid interface carrier solvent pump to be run for priming and to purge the carrier solvent. It can also be used for troubleshooting.

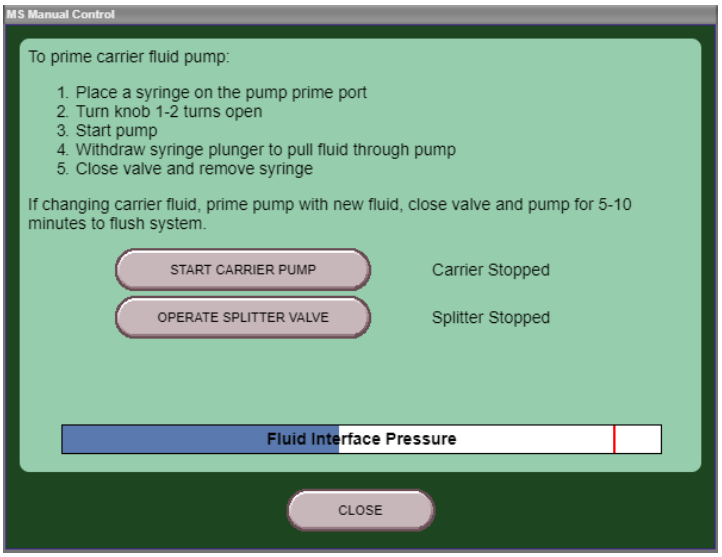


Figure 4-21 Mass Spectrometer Manual Control window

- **Start Carrier Pump-** Turns the carrier pump on for priming or to replace one carrier solvent with another one. A purge is complete in about 5 minutes. The button is replaced by STOP CARRIER PUMP when the pump is running.

  To prime the pump, open the priming valve on the fluid interface counterclockwise. Use the 5 mL Luer lock syringe provided with the accessory kit to draw liquid into the pump. Repeat this procedure once more to completely fill or purge the line from the carrier solvent reservoir to the priming valve. Make sure the priming valve is tightly shut after priming the system.
- **Stop Carrier Pump-** Stops the carrier pump. This is replaced by START CARRIER PUMP after the pump is stopped.
- **Operate Splitter Valve-** Runs the splitter valve to verify operation.
- **Fluid Interface Pressure -** The carrier solvent pressure is displayed in a ribbon gauge. If the pressure approaches the level of the red line, the system is clogged and should be cleaned to allow good response.
- **Close -** Closes the window and stops the carrier pump if it is running.

4.3.8 MS Ionization Settings

(The MS IONIZATION SETTINGS window is available on PurIon equipped systems only).

MS > Ionization Settings

IONIZATION SETTINGS provides settings to improve the ionization of a particular compound by reducing fragmentation or adduct formation.

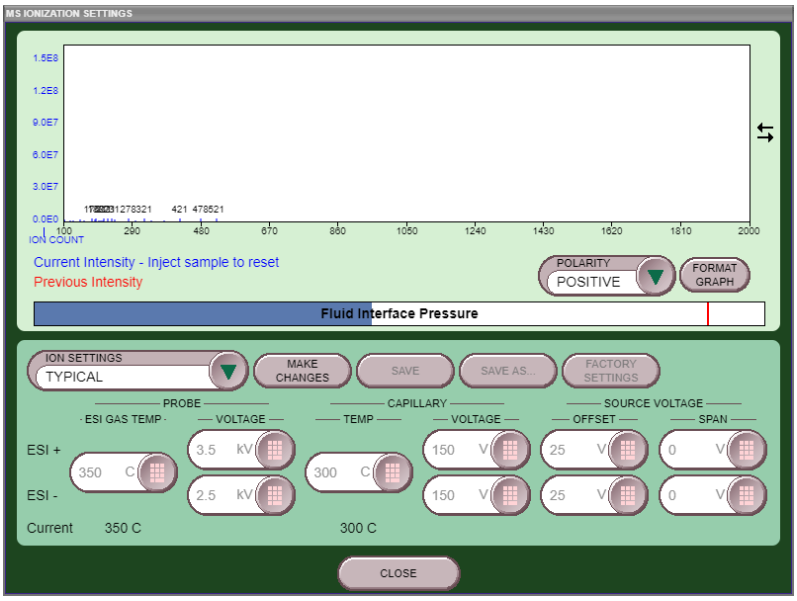


Figure 4-22 Mass Spectrometer Ionization Settings window

- **Mass Spectrum Graph -** Displays the mass spectrum. There are two mass spectra displayed. Blue displays the maximum of the current injection. Red displays the maximum of the previous injection for comparison. When a new injection is performed, the blue spectrum replaces the red spectrum, and a new blue spectrum is started. To inject a sample, move the PurIon injection valve to the “Inject Sample” position.

  Inject the sample (20 µL) and move the valve to the “Scan Mass” position.

☑ Note

Only use 22 gauge square tip needles (PN 29-9001-911) to avoid scratching the internal surfaces of the injection valve. Filter the sample with a 0.45 µ filter to avoid clogging the PurIon tubing and probe capillary. Sample concentration should be less than 20 µg/mL.

- **Polarity -** Switches the mass spectrometer between positive and negative polarity.

☑ Note

The PurIon S and L systems capture both polarities. The setting changes which polarity is displayed.

- **Format Graph-** Sets the displayed range of the mass spectrum. The PurIon system still collects the entire mass range even when the range is set within than the minimum and maximum values allowed for the spectrometer. Values can range from 50 to 1200 Da

(PurIon and PurIon S) or 2000 Da (PurIon L). Changing the mass range defined by these controls may cause the Y-axis (ion count) scaling to change on the basis of the tallest peak within the range.

- **Fluid Interface Pressure -** The carrier solvent pressure is displayed in a ribbon gauge. If the pressure approaches the level of the red line, the system is clogged and should be cleaned to allow proper operation.
- **Ion Settings -** Allows selection of different ion settings to maximize the intensity of a molecular ion. The factory selections include “Robust” for compounds that do not easily ionize. “Typical” works well for most compounds. The “Fragile” setting is used for those compounds that are delicate or easily fragment. Settings that you define may also be loaded.
- **Make Changes -** Allows changes to settings to improve the intensity of an ion.
- **Save -** Saves changes to an existing ion settings file.
- **Save As -** Saves changes and create a new ion settings file. This new file can be selected in ionization settings or method development. Additionally, this file can also be selected when setting up a separation run.
- **Factory Settings -** Restores the “Typical”, “Robust”, and Fragile” ion settings to the factory default values.
- **Probe -** The probe nebulizes and ionizes the sample. Choose between ESI (electrospray interface) or APCI (atmospheric pressure chemical ionization) probes. The software changes the labels on the control to reflect the probe installed in the mass spectrometer.
- **Gas Temp -** The temperature of the nebulization gas for the probe. Lower temperatures are used for more delicate, heat-labile compounds. The temperature is set to quickly evaporate the carrier solvent. (Note that PurIon S and PurIon L systems display a single temperature for both positive and negative ionization).
- **Voltage (Current) -** Displays a voltage setting (ESI probes) or current value (APCI probes). Lower values are used for more delicate compounds.

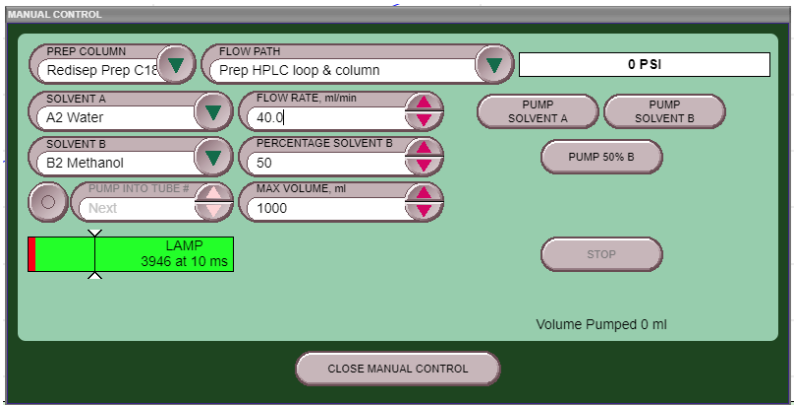
  The capillary is heated to complete the evaporation of solvent. It also carries a voltage; lower voltages are used for more delicate compounds.
- **Source Voltage -** Source voltage settings have the greatest effect on fragmentation. Higher values induce more fragmentation but also reduce adduct formation.
- **Offset -** The voltage applied to all masses. Large values tend to increase fragmentation but reduce adduct formation.

- **Span -** Span voltage defines an increased voltage applied as the mass increases. As with the offset, larger values increase fragmentation.
- **Close -** Closes the window.

4.3.9 Manual Control

MANUAL CONTROL can assist with method development, maintenance of the system plumbing, and system troubleshooting. For examples, see 5.4.2 *Quick Cleaning when Recommended* and 5.4.3 *Monthly Flow Cell Cleaning*.

Tools > Manual Control



- **Solvent Selections –** (Solvent Selector Valve module only.) Selects the solvent to pump during manual pump operation.
- **Percentage Solvent B –** Type or select the mixture percentage.
- **Flow Rate –** Type or select the pump's flow rate in mL/min.
- **Pump into Tube # –** Collects solvent in a tube that you choose. This can be useful when trying to recover a compound that has precipitated or "crashed" somewhere in the fluid path. By default, solvent is pumped to the waste port during manual control. If you want to collect the solvent in a tube, select the button next to PUMP INTO TUBE to enable it, then enter the tube number.
- **Prime A –** Pumps 100% of solvent A at the selected flow rate.
- **Prime B –** Pumps 100% of solvent B at the selected flow rate.
- **PRIME XX% B –** Pumps a mix of Solvent A and B.
- **STOP –** Stops the pump. This button is only available while the pump is running.

The raw lamp energy display can be used to verify flow cell cleanliness. If the energy is low, it can be monitored while pumping a solvent to clean the flow cell. Select CLOSE MANUAL CONTROL to close the window.

4.3.10 Automation Manual Control

This opens the Automation Manual Control window, which can be useful in flushing different portions of the AutoInjector/AutoSampler flow paths by choosing the flow path, fluid source, and the amount of fluid to be pumped by the AutoInjector. It can also be used to prime the wash station.

Tools> Automation Wash Control

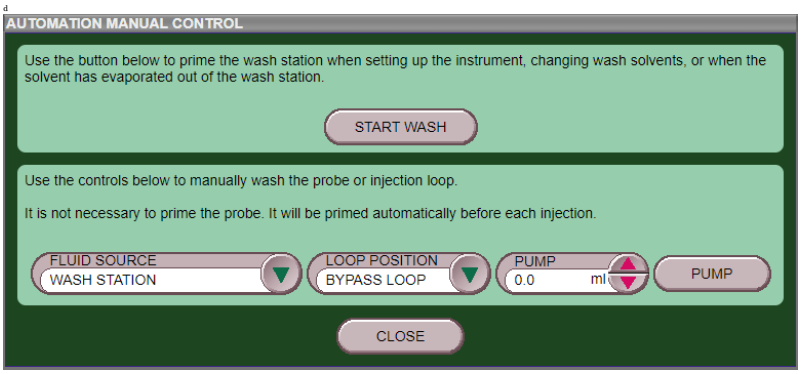


Figure 4-23 The Automation Manual Control window

To prime the wash station:

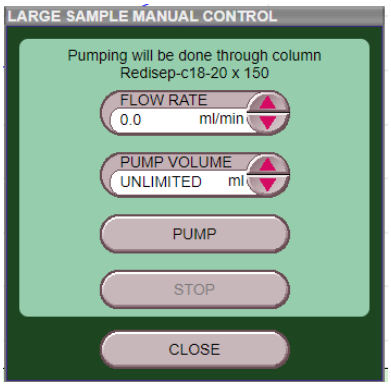
1. Verify that the wash station supply line is placed into a suitable solvent (typically the strong chromatographic solvent) and that the waste line is routed to a suitable container.
2. Select START WASH to begin the priming process. Be sure that the wash solvent is pumped into the center portion of the wash station and that the fluid drains into the overflow drain when the station is full.
3. After the wash station is primed, select STOP WASH.

4.3.11 Large Sample Manual Control

The large sample load pump provides a convenient way to load and concentrate dilute samples.

Its window can be opened from the TOOLS menu. It is also available when SAMPLE OPTIONS > SAMPLE TYPE is “Large Sample”. The option is even present when the pump is turned off—no reboot is required after the pump is powered back on. Removal of the loop is unnecessary.

Tools> Large Sample Manual Control



- **Flow Rate –** Type or select the pump's flow rate in mL/min.
- **Pump Volume –** Type or select the pump's volume in mL, or choose UNLIMITED.
  - UNLIMITED – The pump runs until you stop it; you could run the pump until all of the sample is collected. Afterward, you can CLOSE and continue with the injection.

4.3.12 Network Configuration Options

Network configuration, while useful, is not required for basic configuration.

Network configurations settings require assistance from your network administrator. Contact your Information Technology department before changing the settings. For more information about networking, see *TN28 Networking Guidelines for Combi-Flash Products* available at www.teledynelabs.com.

Tools > Configuration

Network Configuration – The CONFIGURATION window groups its settings under tabs. The NETWORK tab is described below. (The other tabs are covered in Section 2.1 *Configuration of the ACCQPrep*.)

Instrument IP Address Configuration. Use these settings to specify the instrument's network address and its parameters:

- **Network Type -** STATIC IP or DHCP.
  - STATIC IP requires an IP address, netmask, and gateway provided by your network administrator.
  - DHCP retrieves the IP address, netmask, and gateway from a DHCP server.
- **IP Address -** The static IP address for the system. (Available when NETWORK TYPE is STATIC IP.)
- **Netmask -** The netmask for the system. (Available when NETWORK TYPE is STATIC IP.)
- **Gateway -** The gateway for the system. (Available when NETWORK TYPE is STATIC IP.)
- **DNS Server -** The domain name server for your network. Enter an address if you use a domain name server to translate domain names to IP addresses. (Available when NETWORK TYPE is STATIC IP.)

Network Printing. The system can print to a network printer, and it supports JetDirect and line printer (LPR) queues when configured.

Consult with your network administrator to determine these settings:

- The IP address of selected printer.
- The queue name, if the printer uses an LPR print queue.
- The port number, if the printer uses a JetDirect print queue.
- The printer type: either PostScript or PCL.

When this information is known, you can proceed with configuring system for network printing.

Follow the on-screen instructions for entering the address, queue, and printer type information. After successfully printing a test page, the network printer will be available for printing by choosing FILE > PRINT from the menu and the AUTOMATICALLY PRINT AT END OF RUN feature.

Network File Save Configuration. When configured, this feature allows the system to access a network directory from which it can save run files as PDF, text, or run monitor. To enable this feature, select a file type to be saved and enter the remaining settings necessary for network access. Your network administrator can provide the required settings.

> ☑ ***Note***
>
> All server and domain names must be fully qualified. That is, entries must include the full name (server name.domain.domain…). Use forward slashes (/), not back slashes, when entering the network share path.

4.3.13 Set Administrator Password

This window, opened by selecting TOOLS > SET ADMINISTRATOR PASSWORD from the menu, is used to enter and change the system password.

Tools > Set Administrator Password

To enter or change a password:

1. Type the password. The factory default password is "accqprep".
2. Type the new password.
3. Type the password again to verify your entry.
4. Select OK to save your settings and close the window.

The following commands require a password before displaying the requested window:

- TOOLS > USER MANAGEMENT
- HELP > SERVICE
- TOOLS > SET ADMINISTRATOR PASSWORD. This protects the current password.

4.3.14 User Management

User Management – Opens the USER MANAGEMENT window, from which you can add or remove users from the system and to change passwords. To prevent unauthorized changes to user management, this function is password protected.

Tools > User Management

- To add a user, select ADD NEW and enter the user name in the window that appears. Another window will appear into which a password for the new user can be entered. Leave the field empty if no password is needed. When you add a user, a working folder for that user is automatically created. When they log in, their data files will automatically be stored in their user folder. It will be reset when logging out. Use FILE > SET DATA PATH to

select your folder. To prevent unauthorized changes to user management, this function is password protected.

- To delete a user, select the DELETE button next to the user's name and confirm the action.
- To change a password for a user, select CHANGE PASSWORD.
- To delete a password, leave the text box empty.

After users are created, they can be assigned access levels for the system (Figure 4-24).

Function	Administrator	Standard User	Limited User	Restricted User
Administrative Functions: User Management, Service Screens, etc.	X			
Configuration Screen	X	X		
File Management: Move, Copy, Rename, Delete, etc. Runs and Methods	X	X	X	
Edit every gradient point	X	X		
Modify run length	X	X		
Edit gradient points before wash stage	X	X	X	
Select solvents for the run	X	X	X	X
Modify detection parameters for the run	X	X	X	X
Modify collection parameters for the run	X	X	X	X
Prime / Manual Control	X	X	X	X

OK

Figure 4-24 Table of user level access

4.4 Examples of PeakTrak actions

In this section you will find instructions for typical PeakTrak tasks, such as:

- editing a default method (Section 4.4.1),
- creating a method file in other ways (Section 4.4.2),
- defining a gradient (Section 4.4.3),
- real-time gradient editing (Section 4.4.4),
- bypassing the injection loop (Section 4.4.5),
- monitoring the purity measure (Section 4.4.6),
- viewing runs (Section 4.4.7),
- controlling the ACCQ*Prep* manually (4.4.8).

4.4.1 Editing a method

Editing a method allows you to tailor the operation of the ACCQ*Prep* system to best separate or purify the compounds of interest.

PeakTrak has two types of method settings: basic and advanced.

Basic Method Settings

Basic settings allow you to control the %B gradient mix, flow rate, solvents, and run length. These settings are part of the MAIN window and are the most frequently changed settings when developing or improving purification methods.

The %B gradient mix and run length are shown on the plot area. Refer to Section 4.4.3 *Defining a Gradient* for details.

Other settings:

- Flow Rate — This defaults to the value created when setting up the HPLC column but can be changed for each separation if desired.
- Solvent A and B — Select solvents from the lists. Listed solvents are those defined by the CONFIGURATION window.

Advanced Method Settings

The METHOD EDITOR window gives you access to advanced settings such as EQUILIBRATION volume, INITIAL WASTE, air purge, PEAK DETECTION, and the PEAK COLLECTION mode.

See Section 4.3.4 for detailed information about METHOD EDITOR window settings. Some highlights:

- EQUILIBRATION VOLUME COLUMN — equilibration is optional, but you may find that separations are more effective if the column is equilibrated before injecting the sample.
- INITIAL WASTE & TIME WINDOWS — The initial waste diverts a user-determined volume to the waste port until the eluent is about to be collected. At the start of a run, the internal solvent lines hold some solvent from the previous run. This volume can be diverted to waste, along with the anticipated volume of fluid that will pass through the column before a compound of interest will elute.

  Time Windows can limit the fraction collection to specific time durations of the run.
- PEAK COLLECTION — There are three peak collection settings: All, Peaks, or None.
  - ALL collects peak and non-peak fluid in tubes.
  - PEAKS collects only peak fluid in tubes and divert all other fluids to the waste port. NONE diverts all fluids—peak and non-peak—to the waste port.
  - NONE is useful for developing custom methods that perform a column wash, system cleaning, or similar function where solvents and elute do not need to be collected.

When using the ALL or PEAKS options, you can specify the maximum peak and non-peak volumes to be collected in the tubes. Be sure to enter volumes less than the maximum tube

capacity. Different peak and non-peak tube volumes can be used to conserve tubes when using the All peak collection mode by collecting large volumes of non-peak fluid while creating more, smaller fraction volumes of fluids of interest.

The fraction collector advances to the next tube whenever a peak is detected. Also, the system advances to the next tube if a new peak is detected before completing the last one (sometimes called a double advance). Tubes may also advance when using multiple detectors, depending on impurities or sensitivity to a given compound by each detector. Keep in mind that the slope and threshold detection methods use algorithms which optimize the peak detection. For example, peak detection includes a hysteresis to reduce the likelihood of multiple tube advances that may occur if there is noise at the beginning or tail end of a peak.

You can also set

- TUBE VOLUME for collected fluids. This volume can be the default maximum volume for that tube size (Max option) as entered in the CONFIGURATION window, or a method-specific volume that is less than the capacity defined by the CONFIGURATION window.
- PEAK DETECTION options. When an option is selected, the window also allows you to modify the setting details for that peak detection option. All enabled options are displayed on the chromatogram.

☑ Note

You can select up to four peak detection options (excluding the External option) on the ACCQ*Prep* systems. If using more than one option, such as λ1 with λ 2, the system considers a peak to be present when either option is true (a logical OR).

Peak detection options include:

- λ_1 and λ_2 (wavelength 1 and 2): Provides primary and secondary wavelength detection. When enabled, type or select the peak detection wavelength in nanometers, then select DETAILS to configure additional settings, including SLOPE and THRESHOLD peak detection.

☑ Note

If both Slope and Threshold peak detection methods are checked, the system considers a peak to be present when any one condition is met. This logical OR operator means that the system cuts a peak when either the Slope condition is true or when the Threshold condition is true.

- All Wavelength Detection: Detects peaks within a user-selected range of wavelengths. When enabled, select DETAILS to configure additional settings. These settings include the slope-based and peak width options described in λ1 and λ2 above. Additionally, you can type or select the minimum and maximum wavelengths

limits in nanometers. PEAK WIDTH for All Wavelength Detection also determines the length of time before an automatic auto-zero of baseline occurs. This period is twice the peak width setting.

- External Detector: Uses a 0 to 1 volt analog input signal from an external detector. When enabled, select DETAILS to configure additional settings. These settings include the slope-based and peak width options described in λ1 and λ2 above. The system uses its internal algorithms to cut detect and cut peaks on the basis of the analog input signal. Refer to the External Detector instruction sheet for cable and plumbing requirements.
- ELS Detector: The evaporative light scattering (ELS) detector on an ACCQ*Prep* system.
- Mass Spectrometer (PurIon systems only): Monitors or detects compounds with a PurIon mass spectrometer system.
- Purity Measure: Displays a ratio of the selected wavelengths if two absorbance wavelengths are used. The ratio trace is often a useful indicator of purity. Select SHOW SPECTRAL PURITY to measure purity by using a comparison of UV-spectra measured at differing times as a purity measurement. Select SPECTRAL PURITY DETECTION for fractionation based on spectral purity.

Selecting PEAK DETECTION DETAILS opens additional detector options for λ_1 and λ_2, ALL WAVELENGTH DETECTION, MASS SPECTROMETER, ELS DETECTION, EXTERNAL DETECTION, and PURITY MEASURE peak detection. See Section 4.3.4 for detailed information about peak detection settings.

Saving Changes to the Method File – After you have edited the method file, you can save the changes for future use. Select SAVE AS, give the method file a descriptive name, then select SAVE. The method file is stored by the ACCQ*Prep* system and will be available for future runs.

4.4.2 Alternative Ways to Create Method Files

There are other ways to create method files besides editing them on the ACCQ*Prep* system:

Importing Method Files from another System – If a method file is located on another ACCQ*Prep* system, you can use FILE > EXPORT METHOD to save the method file on an external storage device. From the touchscreen, this storage device is a USB flash drive connected to the USB port below the display. If you are accessing system via an Internet browser, the storage device may be any device that can be accessed by the local computer.

To move the method file onto the system, insert the USB flash drive into the USB port or connect to the system from the remote computer, then use FILE > IMPORT METHOD to

locate and import the method file.

Extracting a Method File from a Previous Run – After the run, you can extract the run parameters as a method file so it can be used on future runs. To do so, open the Run file, then select EXTRACT. The system loads a new method with identical run parameters. You can then save the method using FILE > SAVE METHOD AS.

4.4.3 Defining a Gradient

The simplest way to change the gradient is to select and drag the inflection points that define the shape of the gradient. Add a point by selecting the INSERT button, then select the gradient curve to add a point. You can then drag the new point to the desired location. Delete a point by selecting the DELETE button and then selecting the undesired inflection point.

The methods described above work in the MAIN window and METHOD EDITOR WINDOW. Alternatively, you can modify, add, insert, and delete points using a tabular view on the METHOD EDITOR window. Select the EDIT GRADIENT button to toggle the gradient table, then use the controls to modify the settings. Selecting EDIT GRADIENT again closes the table view. (See Section *Method Editor*: *Edit Gradient*)

4.4.4 Real-time Gradient Editing

The gradient shape can be changed during the run. Any time after the fraction collector has positioned the drop former over the first tube, select and drag the points on the gradient plot area of the MAIN window. You can also add or remove points by selecting the INSERT or DELETE point buttons. INSERT allows you to add a single point where you select it on the gradient profile. Repeat this action to add more points. DELETE allows you to remove a single point when you select it.

> ☑ ***Note***
>
> Only the portion of the gradient that has not yet occurred during the run can be modified.

4.4.5 Bypassing the Injection Loop

BYPASS LOOP button overrides the position of the Prep injection loop. During normal operation the loop is placed into the flow path ahead of the column after the equilibration is completed. It remains in this position for the remainder of the separation. This feature allows you to remove the loop from the flow path and is useful for manually stacking injections when doing an isocratic separation. It requires you to perform sufficient method development to know when all of the compounds elute from the column.

For example, assume that a separation is developed with two compounds that elute at 5 and 10 minutes. At the 5 minute point of the separation, the sample loop can be removed from the flow path and loaded with another sample of the same materials. The loop can then be placed into the flow path at the 6 minute point. In this scenario, the first compound will elute at 5 minutes, the second compound will elute from the column at 10 minutes. The

first compound in the second injection will elute at 11 minutes (6 minute injection time plus 5 minutes to elute), and the second compound from the second injection will elute at 16 minutes.

In this example, the two injections can be separated in 16 minutes instead of 20 minutes. The process can be repeated as needed in increase system throughput. While isocratic separations may typically be slower than a gradient separation, you are also able to eliminate the time required for column equilibration after a gradient separation.

4.4.6 Monitoring the Purity Measure

When using two wavelength detection options, a ratio of the two wavelengths can also be displayed which at times can provide the best indication of compound purity. Consider the following:

If a pure compound is eluting, the absorbance is linearly related to the concentration of the compound in the solvent. If the compound absorbs differently at different wavelengths, the absorbance at each wavelength may be different but still linearly related. For example, assume that a compound eluting from the system has an absorbance equal to 2 times the concentration at 254 nm. This same compound at 220 nm has an absorbance of 1.5 times the concentration. The ratio of these signals is 1.33. Since the relationship of absorbance to concentration is not variable, the ratio remains steady while the concentration changes from the beginning to the end of the peak. During the duration of the peak, the ratio will be 1.33, and this constant value is displayed as a horizontal line.

Now assume that there is a second compound eluting, only slightly shifted in time from the original compound. It is possible that the detection absorbance trace alone would indicate a single, valid chromatographic peak. In reality, it is a combination of two peaks. By monitoring a second wavelength, it may be possible to reveal the second compound. Because of the slight shift in time and the different absorbance properties of the two compounds, the changing ratio during the detected peak would reveal the impurity. Therefore, you can assume that if the ratio is not constant for the entire duration of the peak, the compound eluting may not be pure.

The following controls can be found on the PURITY MEASURE window (METHOD EDITOR > ALL WAVELENGTH DETECTION > DETAILS >).

- SHOW SPECTRAL PURITY measures purity by using a comparison of UV-spectra measured at differing times. The algorithm used is the “similarity index.”
- SPECTRAL PURITY DETECTION allows fractionation based on spectral purity. However, the spectral purity algorithm doesn’t work on saturated peaks (flat on top due to detector saturation).
- SHOW RATIO displays the ratio when using two absorbance wavelengths.

4.4.7 Viewing runs

After completing a run, the PeakTrak MAIN window is used to display all collected run data. You can also open previous runs to view the chromatogram and the peak/tube locations. To open a previous run:

1. From the MAIN window, select FILE > OPEN, or select the OPEN button. The FILES window is displayed.
2. Choose a Run file and select OPEN. PeakTrak displays the run in the RUN VIEWER window, which includes the following:

- Rack and tube information — The left pane of the window lists the current rack, a map for that rack, and a table that lists the peaks and their corresponding tube numbers. If the window is currently displaying the collection parameters, select DISPLAY RACK to view this information. On PurIon equipped systems, the mass spectrum of the fraction contents can be displayed by selecting MS button and selecting on a tube.
- Method parameters — DISPLAY METHOD displays summary of the peak detection and collection settings for the run.
- Chromatogram — Displayed on the right side and identified by the sample name in the window's title bar.
- OPTION buttons — Quickly access frequently used commands while viewing a run:

  
  PRINT — Prints a run summary. When viewing the run from a remote personal computer, the summary can be printed on any installed printer. If you are attempting to print from the systems touch panel display, you must first set up NETWORK PRINTING in the NETWORK CONFIGURATION settings.

  
  Save As PDF — Saves the run summary as a PDF file. When viewing the run from a remote personal computer, the summary can be saved to any connected storage device. If you are attempting to save a PDF file from the touch panel display, insert a USB Flash drive in the USB port below the display panel.

  
  SAVE AS TXT — Saves the run summary as an ASCII text file. When viewing the run from a remote personal computer, the summary can be saved to any connected storage device. If you are attempting to save a TXT file from the touch panel display, insert a USB Flash drive in the USB port below the display panel.
- Use pinch zoom or pan to view the results screen in greater detail. Panning requires the use of two fingers to differentiate it from selecting.
- FORMAT GRAPH — Opens a window from which you can set the left and right Y-axis scales. These scales are controlled by the absorbance and %B upper limits. Selecting the SHOW THRESHOLD LEVEL button shows the

threshold level setting of all detectors except the mass spectrometer. The threshold levels are color-coded to the detector trace.

- REFERENCE CHROMATOGRAM — Pulls up a previous run to compare to current run. Time scale matches the longest run. If runs are of different lengths, then the shorter run will be missing that part of the chromatogram. Also, zooming on one spectrum scales the other spectrum similarly.
- MS — Displays a mass spectrum (PurIon systems only). Selecting in the Chromatogram window displays a mass spectrum at that point during the run. Selecting on a tube in the rack map shows the mass spectrum of the contents of that fraction.
- STACK INJECTIONS — Opens the STACK INJECTIONS window, from which time windows can be adjusted. See Section 3.3.10 *Stacked Injections*.
- UV — Displays a UV spectrum. Selecting in the chromatogram window displays a mass spectrum at that point during the run. On UV-Vis equipped systems, this is displayed as "UV-Vis" and displays UV-visible spectrum.
- EXTRACT — Loads a new method file on the basis of the parameters for the run you are viewing.
- CLOSE — Closes the Run File window.

General instructions for reading the data – Most elements on the window are color-keyed to help you locate the tubes containing the peaks of interest. A color bar below each collected peak matches a tube in the map on the left. The tube map provides a visual representation of the tubes that contain the peaks of interest. If you want to identify the tube by number, refer to the table below the rack diagram.

If the run used multiple racks, PREVIOUS and NEXT RACK buttons are available. Select them to scroll through the available racks. The currently displayed rack is identified by letter which is shown below the rack.

Reading the Chromatogram – The plot area displays the following:

- The red absorbance trace produced by the system's peak detector. Absorbance units (also shown in red) that correspond to this trace are shown on the left Y-axis.
- Purple absorbance and green purity measurement traces that may be visible if you are monitoring a second wavelength.
- Green traces from ELSD or external detectors.
- PurIon traces color coded with the selected masses below the graph Y-axis.
- The blue gradient curve that was used during the run. The % Solvent B scale is shown in blue on the right Y-axis.
- The X-axis depicts the run time, shown as minutes.
- Vertical lines that appear at intervals along the X-axis. These lines indicate collection tube changes. To prevent the plot area from being obscured by tube change marks, PeakTrak may limit the number of marks.

4.4.8 Manual Control of the ACCQ*Prep*

MANUAL CONTROL assists with method development, maintenance of the system plumbing, and system troubleshooting. To manually control the ACCQ*Prep* system, first go to TOOLS > MANUAL CONTROL.

Pumping Solvents

To pump either solvent, select the solvent from the Solvent A or Solvent B lists. Then, select PUMP A or PUMP B to pump the selected solvent. To pump a mixture or Solvent A and B, adjust the Percentage Solvent B setting and then select PUMP% B. When finished, select STOP.

You can control the flow rate by adjusting the FLOW RATE mL/MIN.

By default, the system pumps the solvent directly to the waste port, you can also pump the solvent into a collection tube. Select PUMP INTO TUBE #, then select which tube ("Next" or a specific tube number).

Raw Lamp Energy

The left side of the MANUAL CONTROL window shows a RAW LAMP ENERGY gauge. This provides an indication of the UV light measured by the optical detection system at 254 nm. High lamp energy (green) means that the flow cell easily passes through a sufficient UV light source. Lower lamp energy (yellow or red) means that either the light source is weak or that the flow cell is obstructed.

- Green – lamp energy is sufficient to detect peaks up to 2.4 Absorbance Units.
- Red – lamp energy is obstructed to a degree that the system might not reliably detect peaks. If you attempt to operate the system, peak collection will be forced to collect all. This prevents diverting desired compounds to waste.

Depending on what you are doing with the MANUAL CONTROL functions, low lamp energy could be a normal indication. For example, a UV-absorbing compound could be present in the flow cell as you are pumping solvent. Or, the selected solvent absorbs UV light at 254 nm. Abnormal indications could be a flow cell blocked by a compound that has precipitated, or there is a film built up on the flow cell. See 5.4.2 *Quick Cleaning when Recommended* for more information about the Raw Lamp Energy gauge and flow cell cleaning.

ACCQ*Prep* HP150

Section 5 Maintenance

5.1 Introduction

This section covers some common maintenance routines for the ACCQ*Prep* HP150.

5.1.1 Cleaning

To clean the exterior surfaces, use a cleaning cloth dampened with a mixture of distilled water and a mild detergent. Use isopropyl alcohol for tougher stains.

On printed areas such as labels, avoid rubbing vigorously or using aggressive solvents like acetone. These will ruin the printed text.

⚠ CAUTION

Do not immerse the instrument in a water bath or subject it to a liquid spray. The instrument is not watertight, and these actions could damage the internal electronics.

5.1.2 Collection Rack and Tray Cleaning

⚠ WARNING

Risk of fire or equipment damage. Unclean collection racks and tray might have inhibited conductive properties. The racks and tray must be kept clean to dissipate static electricity.

The collection tube racks and tray are made of conductive plastic. Dirt, film, or coatings might prevent their ability to dissipate static electricity. To avoid problems that could result from an electrostatic discharge, clean the racks and tray monthly. Use distilled water with a mild detergent. For tougher stains, use isopropyl alcohol.

5.2 System Standby and Shut Down

During extended periods of inactivity, you can place the system in STANDBY to conserve power. To do so, log off the system (FILE > LOG OUT) and place the ⏻ On/Standby switch in STANDBY.

When in the STANDBY state, normal system operation is no longer available from the touchscreen or remotely. However, some internal components are still powered.

⚠ WARNING

As long as the AC mains power cord is connected, power is inside the unit. The mains power cord is the disconnect device. Position the ACCQPrep system so that the power

cord can be unplugged, or use a power strip where the plug can quickly be removed from the outlet in the event of an emergency.

When you first place the system in STANDBY, internal components continue to operate for almost one minute while performing file maintenance and preparing the system for possible power removal.

⚠ CAUTION

Removing the AC mains power cord before the file maintenance is complete might corrupt files on the internal hard drive. These corrupted files can cause abnormal operation or a complete system failure that requires service. Unless power must be removed due to an emergency, always wait at least one minute after placing the system in STANDBY before removing the AC mains power cord.

5.2.1 Tubing Inspection

⚠ WARNING

Risk of fire or equipment damage. Faulty tubing, fittings, and drains may allow organic solvents to pool in unsafe areas, creating a potential for dangerous levels of flammable vapors. Improper draining may damage the instrument's internal components.

Perform a tubing inspection monthly:

1. Visually inspect the solvent, waste, and drain tubing. The tubing must be free of any damage, kinks, or deterioration. Fittings must show no signs of leaks.
2. Test the collection tray drain by connecting a vacuum or air supply source to the outlet end of the drain tubes. Then, verify the presence of such vacuum or air supply source on the drain hole (Figures 5-1).

Correct any deficiencies before returning the instrument to operation.

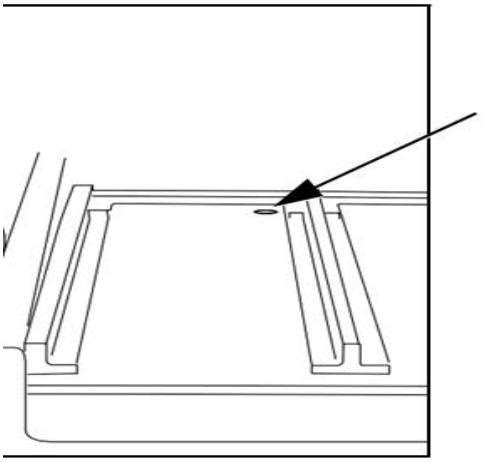


Figure 5-1 Fraction collection tray drain hole

5.3 Preventive Maintenance

The system requires preventive maintenance for safe and reliable operation. Refer to the schedule below for the minimum periodic maintenance requirements.

As Needed – Perform these tasks as conditions require:

- Cleaning (Section 5.1.1).
- Quick flow cell cleaning when recommended by a system alert message (Section 5.4).
- Wipe the cone on PurIon system with a wipe soaked with methanol or water to remove visible residue near the cone inlet. (PurIon systems only).

Every Run – Perform these tasks at the end of each run:

- Allow the separation run to finish with a high percentage of solvent B to flush residual compounds from the column, internal tubing, and flow cell. Refer to section 5.4.
- Allow the Cone wash to run to completion (PurIon systems only) to wash residual compounds from the fluid interface, probe, and to clean the cone area.
- Allow the Valve Wash sequence to run to completion to wash residual compounds from the injection valve and ELSD (if installed) flow path.

Monthly – Perform these tasks at least monthly, more frequently if conditions warrant:

- Tubing Inspection (Section 5.2.1).
- Collection rack and tray cleaning (Section 5.1.2).
- Monthly flow cell cleaning (Section 5.4.3).

Annually – Perform these tasks at least annually, more frequently if conditions warrant:

- Change roughing pump oil (PurIon systems only).

5.4 Flow Cell Cleaning

5.4.1 Post Separation

As a preventive measure, all default column methods finish the separation run with a high percentage of solvent B (Figure 5-2). This brief time (one to six column volumes) of strong solvent flushes residual compounds from the column, flow cell, and internal tubing.

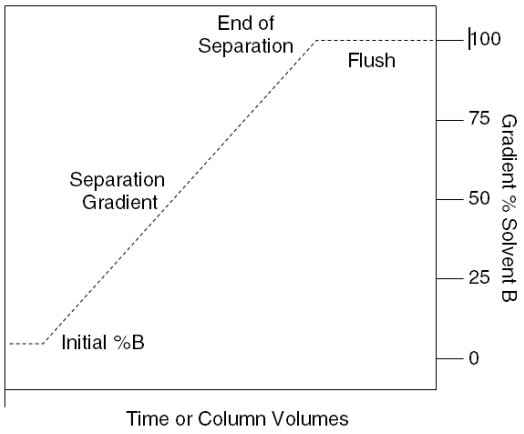


Figure 5-2 Default post-separation column and flow cell flush

Skipping the post-separation flush may cause residual compounds to build up and crystallize, which might result in

- cross contaminating later separation runs,
- higher operating pressures,
- reduced flow cell lamp energy,
- a noisy baseline on the absorbance trace, and
- frequent messages recommending flow cell cleaning (Figure 5-3).

Typically, chemists STOP and then TERMINATE the run after the last compound elutes. This action skips the post-separation flush. If any of the conditions listed above appear, consider allowing some of the runs to continue through the flush, or run a high percentage of %B solvent through the system for a few minutes at the end of each day.

If the separation runs always continue through the flush and the conditions still occur frequently, edit the DEFAULT COLUMN METHODs to extend the flush duration.

⚠ CAUTION

Do not use polar, basic solvent systems with silica column media. These solvent systems may break down the silica structure, possibly causing obstructions in the flow path. Examples of such solvent systems include, but are not limited to, those containing more than 20% methanol with ammonia.

5.4.2 Quick Cleaning when Recommended

When the lamp energy is lower than normal, the system will recommend flow cell cleaning (Figure 5-3) before starting a separation run. You can then choose one of the following:

- **Cancel Run** (recommended) – Perform a quick cleaning described in the following steps.
- **Continue Collect All** – Ignore the message. Because the peak detection operation might be impaired, the system automatically collects all fluids to avoid diverting compounds of interest to waste.
- **Help** – Display the flow cell cleaning on-line help topic.

To perform a quick cleaning:

1. After CANCEL RUN, select the TOOLS > MANUAL CONTROL. The MANUAL CONTROL window opens.
2. Note the Raw Lamp Energy level at 254 nm. The Raw Lamp Energy gauge has two ranges: red and green (Figure 5-3).

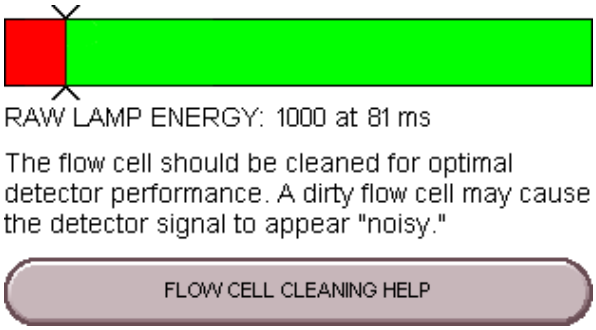


Figure 5-3 Raw lamp energy gauge

- **Red** – Lamp energy is obstructed to a degree that the system might not reliably detect peaks. If you attempt to operate the system, peak collection will be forced to collect all. This prevents diverting desired compounds to waste.
- **Green** – Lamp energy is sufficient to detect peaks within typical system limits.

3. Remove the column and insert a union between the tubing.
4. Set the Flow Rate to 40 mL/min (Figure 5-4).

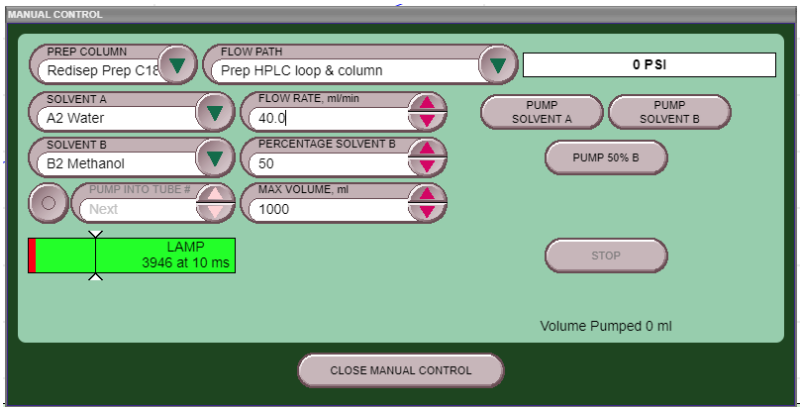


Figure 5-4 Manual Control Settings - Quick Cleaning

5. Select THROUGH HPLC COLUMN for the FLOW PATH option.
6. Select NEXT for the Pump into Tube # option. By pumping into a collection tube, the diverter valve is also cleaned of any residue during this operation.
7. Select PUMP SOLVENT B to pump 100% Solvent B through the bypass tube and into the collection tubes.

☑ Note

Pumping solvent B at a moderate flow rate (25 to 45 mL/min) overtime usually solubilizes obstructions. Generally, the recommended solvent is the highest polarity solvent you have recently used (solvent B).

8. Monitor the Raw Lamp Energy gauge. As the system pumps solvent, the raw lamp energy should gradually improve. Pump solvent for two to five minutes or until the indicator reaches the far-right of the green range.

If after five minutes the indicator is still in the red range, repeat the cleaning steps using an alternative solvent. Or complete the steps in the Monthly Flow Cell Cleaning procedure (section 5.4.3).

☑ Note

If the numerical values of the Raw Lamp Energy (Figure 5-3) do not change, or if the first number remains at zero, contact Teledyne ISCO's Technical Service department.

5.4.3 Monthly Flow Cell Cleaning

Perform this procedure as part of your scheduled preventive maintenance, or when QUICK CLEANING AS REQUIRED (section 5.4.2) does not improve the lamp energy.

1. Remove the column and insert a union between the tubing.
2. From the menu, select TOOLS>MANUAL CONTROL. This opens the MANUAL CONTROL window.

3. Set the Flow Rate to 40 mL/min.
4. Select THROUGH HPLC COLUMN FOR THE FLOW PATH option.
5. Select NEXT for the Pump into Tube # option.
6. Place the B1 Solvent inlet line into a reservoir of methanol, acetone, or a strong solvent that readily dissolves residual sample material.
7. Select PUMP SOLVENT B to pump 100% Solvent B through the bypass tube and into the collection tubes.
8. After three minutes, select STOP. Allow the system to stand for at least six hours. Overnight is recommended.
9. Return the B1 solvent line to the original solvent container.
10. Perform the QUICK CLEANING AS REQUIRED (Section 5.4.2 and monitor the Raw Lamp Energy. (Figure 5-3)

    If the lamp energy is in the green range return the system to operation. If the lamp energy is red, contact Teledyne ISCO's Technical Service department for assistance.

5.5 ACCQ*Prep* HP150 Maintenance

5.5.1 Injection Valve Rotor Replacement

The following instructions are for the removal and replacement of the injection valve rotor. (PN: 20-9009-917).

ACCQPrep systems with a serial number starting with 218H or higher may have spare rotors included with the system. For these units, access the pump compartment by gripping the edges of the front panel and pulling forward on both sides (Figure 5-8). Inside the front cover an envelope contains spare rotors, a hex key needed for removal and a copy of the replacement instructions.

> ☑ ***Note***
>
> The injection valve rotor is a common wear part and is therefore not covered under warranty. Since it is a sealing surface, it can be damaged by particulates in the sample. To prolong the life of the rotor, ensure your sample is properly filtered (<20 µm) to prevent premature failure. Failure is evident by leakage from an unexpected port or location such as the sample load port after injection.

Removing the Injection Valve Rotor

1. Use the supplied $^{9}/_{64}$" hex key to remove the 2 screws that retain the stator (the portion of the inject valve with the tubing connections). Loosen these in quarter turn increments until the tension is removed and a gap starts to appear (Figure 5-5).

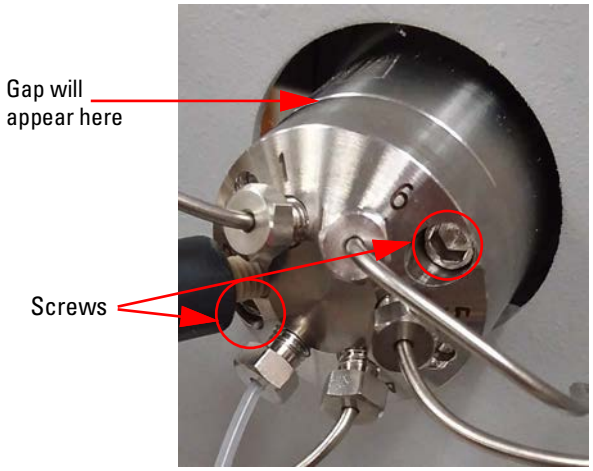


Figure 5-5 Location of the stator retaining screws

2. Remove the screws and pull the stator outward. This may require a side-to-side motion until removed.
3. When the stator is removed, use your finger or a small tool to pry the black plastic rotor out of its recess (Figure 5-6).

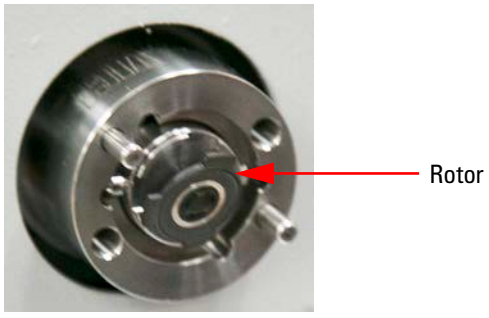


Figure 5-6 Removing the rotor

4. Usually the failure mode is a visible scratch between the normal passages on the rotor.

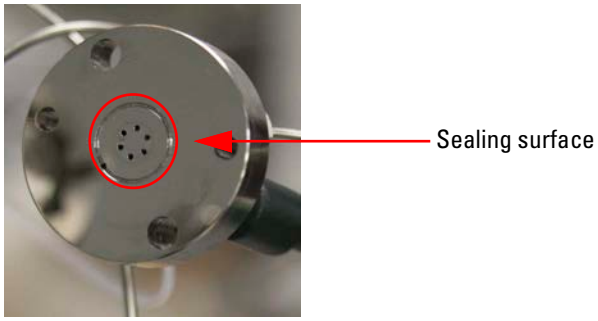


Figure 5-7 Sealing surface of the stator

5. Clean and inspect the stator sealing surface (which contacts the rotor) for scratches. If this part is scratched, it may need replacement also.

Installing the New Injection Valve Rotor

1. Place the new rotor into the recess with the passages facing outward. The tabs on the rotor are not symmetric, so the rotor only fits in one orientation.
2. Install the stator onto the locating pins and use a side-to-side motion until secure.
3. Install the screws evenly until spring force is felt. Then tighten the screws in quarter turn increments on each side until the stator contacts the valve body evenly. The rotor seal is accomplished by a spring so excessive screw torque is not necessary.

5.5.2 Pump Seal Replacement

1. Turn off the power to the system.
2. Access the pump compartment by gripping the edges of the front panel and pulling forward on both sides (Figure 5-8).

Figure 5-8 Front panel of the ACCQPrep

3. Remove the two screws securing the pump assembly in place using the included 3/16" hex wrench.
4. Disconnect the solvent inlet lines from the pump inlet tees and disconnect the high pressure lines from the bulkhead fittings.
5. Disconnect the cables from the pump assembly before pulling it forward to avoid damage to the cables. Then, pull the pump assembly forward.
6. Remove the inlet fittings.
7. Remove the outlet fittings.
8. Remove the two pump head fasteners. Use the supplied 3/16" hex wrench (Figure 5-9).

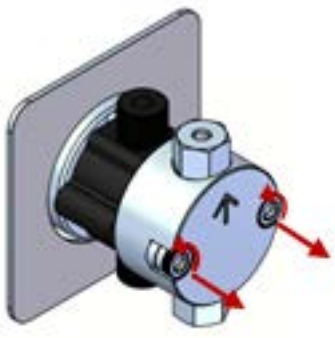


Figure 5-9 Removing the fasteners

9. Carefully pull the pump head forward and off the guide pins. Pull straight and slowly to prevent damage to the piston (Figure 5-10).

> **Note**
> The seal back-up washer may remain on the piston. Remove the washer from the piston if it did not stay in the pump head.

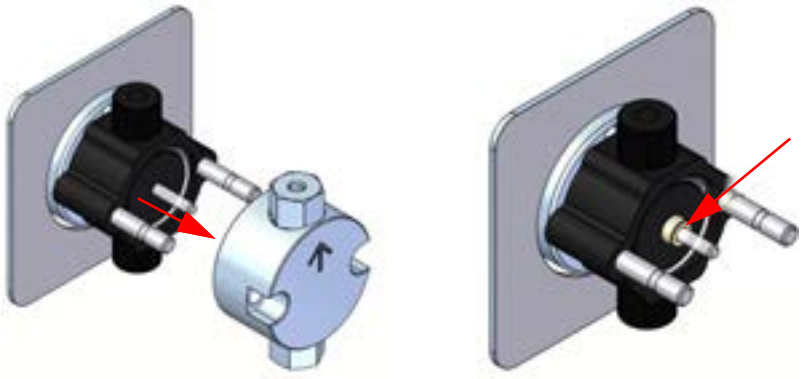


Figure 5-10 Removing the pump head

10. Carefully pull the pump spacer block forward and off the guide pins. Pull straight and slowly to prevent damage to the piston. Also, remove the guide bushing from the piston if it did not stay in the pump spacer block (Figure 5-11).

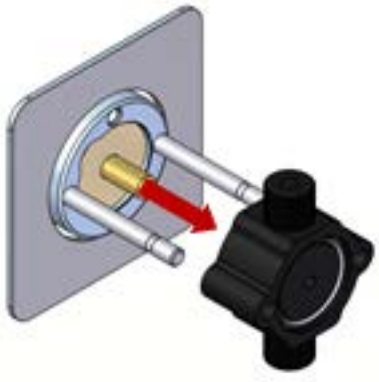


Figure 5-11 Removing the pump spacer block

11. Insert the flanged end of the seal insertion/removal tool into the seal cavity on the pump head. Tilt it slightly so that the flange is under the seal, then pull out the seal (Figure 5-12).

⚠ CAUTION

Using any other tool will scratch the finish of the sealing surface and create a leak.

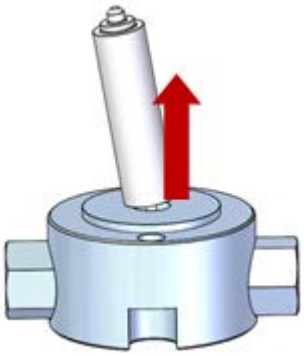


Figure 5-12 Removing the seals

12. 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. Rotate the piston frequently to assure the entire surface is scrubbed. After scouring, use a lint-free cloth, dampened with alcohol, to wipe the piston clean (Figure 5-13).

☑ *Note*

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

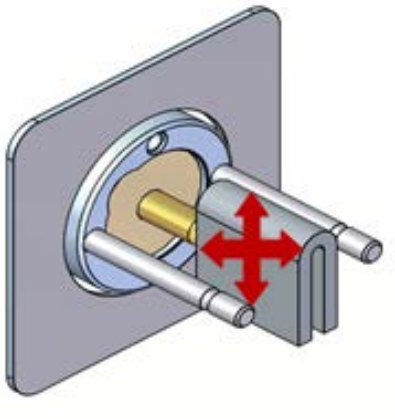


Figure 5-13 Cleaning the piston

13. Place a replacement seal on the rod-shaped end of the seal insertion/removal tool so that the spring (energizer) is visible when the seal is fully seated on the tool. Insert the tool into the pump head (Figure 5-14).

☑ Note

Be careful to line up the seal with the cavity while inserting.

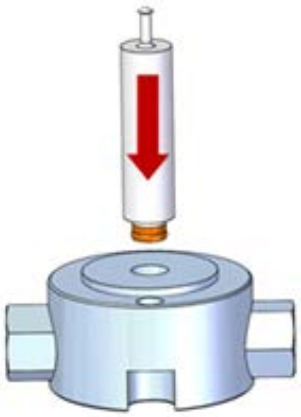


Figure 5-14 Replacing the seal

14. Carefully replace the pump spacer block, making sure the O-ring is properly installed (Figure 5-15).

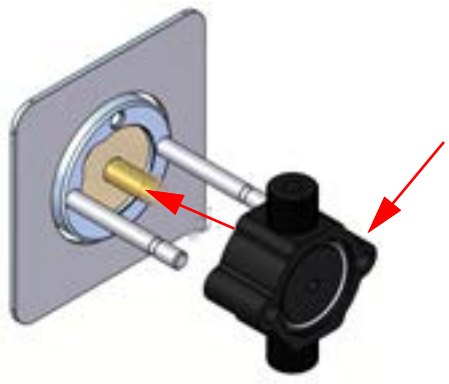


Figure 5-15 Reinstalling the pump spacer block

15. Place the seal back-up washer on the piston. Replace the pump head. Make sure that the inlet check valves are on the bottom and the outlet check valves are on top (Figure 5-16).

Note

Push onto guide pins straight and slowly to prevent damage to the piston. Do not force the self-pump spacer block or pump head into place.

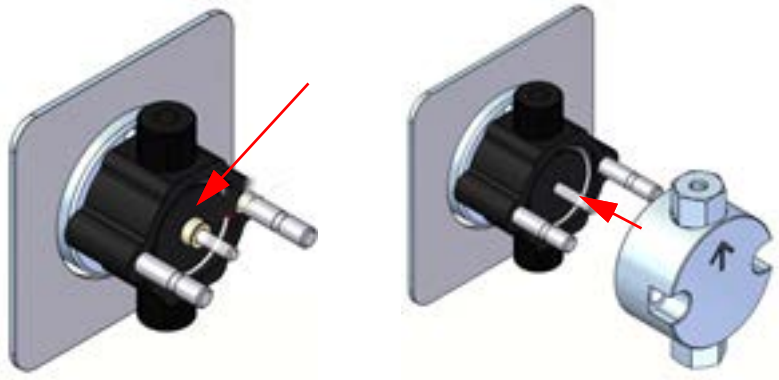


Figure 5-16 Reinstalling the pump head

16. Reinstall fasteners. As you tighten, alternate side-to-side until snug. Turn 1 flat past snug using a $^{3}/_{16}$" hex key (Figure 5-17).

Note

Be sure the Inlet Check Valve Housing is on the bottom and the Outlet Check Valve Housing is on the top.

Figure 5-17 Reinstalling the fasteners

5.5.3 Seal Conditioning

New seals must be conditioned prior to use. Conditioning is the process of running the seals wet under controlled conditions to allow surfaces to seat and break-in for proper function of the seal.

> **Note**
>
> Buffer solutions and salt solutions should never be used to condition new seals. Recommended solvents are HPLC-grade methanol or acetonitrile and water

Suggested Conditioning Parameters

Using a suitable column, run the pump with a 50:50 solution of methanol/water for 30 minutes at a flow rate that results in a pressure of about 1000 psi. Then run the pump for another 15 minutes at a flow rate that results in 3000 psi.

5.5.4 Check Valve Replacement

The following instructions are for the removal and replacement of the pump check valves for the ACCQ*Prep* HP150 system.

1. Turn the power OFF.
2. Access the pump compartment by grabbing the edges of front panel and pulling forward on both sides (Figure 5-18).

Figure 5-18 Front panel of the ACCQPrep

3. The solvent supply lines to the pumps have fluid in them that are drained during this procedure. If draining this fluid back into the supply bottles is acceptable, follow step 3a below; if not, go to step 3b.
   a. Place the solvent supply bottles below the instrument. Next, loosen the solvent supply fitting that enters the 'Y' fitting below each pair of pump heads. This allows the fluid to drain back into the supply vessel.
   b. Remove the solvent supply lines from the solvent bottles. Place a tray or absorbent pad beneath the 'Y' fitting below each pair of pump heads. Loosen the fitting entering the 'Y' fitting to allow the solvent to drain from the supply lines.
4. Remove the two screws securing the pump assembly in place shown in Figure 5-19 using the included $^{3}/_{16}$" hex key.

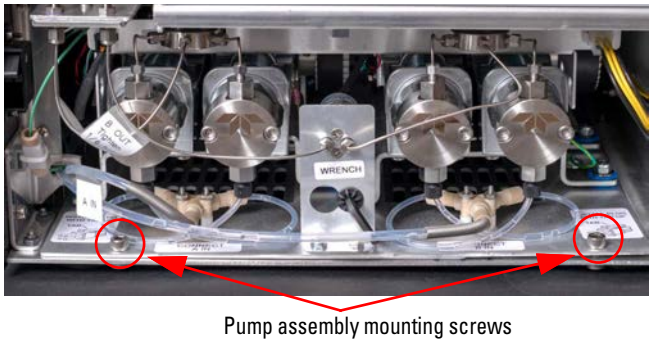


Figure 5-19 Location of the pump assembly mounting screws

5. Remove the two stainless steel outlet lines connected to the bulkhead fittings using a $^{1}/_{4}$" wrench. Remove the two bottom inlet lines going to the bottom 'Y' fittings and disconnect the pump drive connector (Figure 5-20).
6. Remove the Pump Communication Connection located behind the right side arrow pointing to the outlet lines (Figure 5-20).

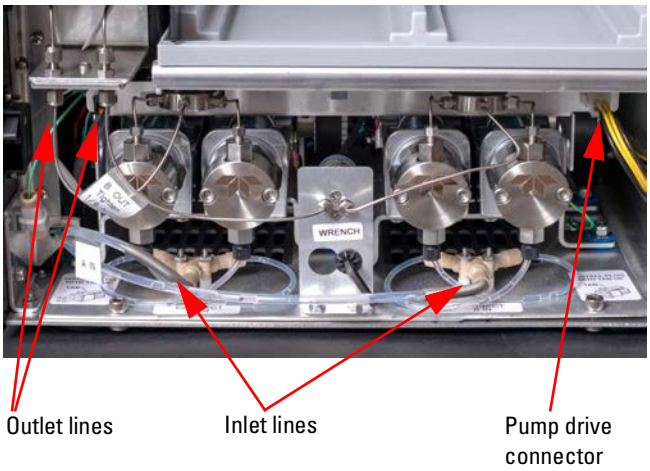


Figure 5-20 Pump assembly

7. Pull the pump assembly forward to the position shown in Figure 5-21.

Figure 5-21 Pump assembly

8. With the pump assembly pulled out, remove the inlet fittings connected to the bottom of the pump head. Using a $^{1}/_{4}$" wrench, remove the stainless steel outlet lines on the top of the pump head (Figure 5-22).

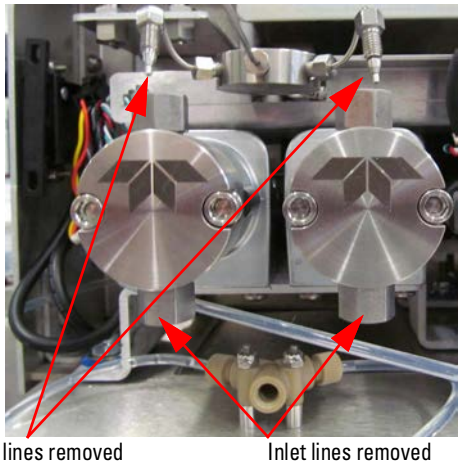


Figure 5-22 Location of fittings

9. Using a Phillips screwdriver, remove the 'Y' fitting located above pump heads (Figure 5-23).

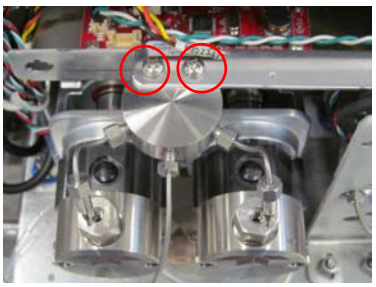


Figure 5-23 Location of tee mounting screws

10. Using a $^{1}/_{2}$" wrench, remove the check valve housings from the top and bottom of the pump heads (Figure 5-24). With the check valve housings removed, remove the existing check valve cartridge and install the new check valve cartridge into the housing. Make sure the arrow on the body of the check valve cartridge is pointing upwards in the direction of the flow (Figure 5-24).

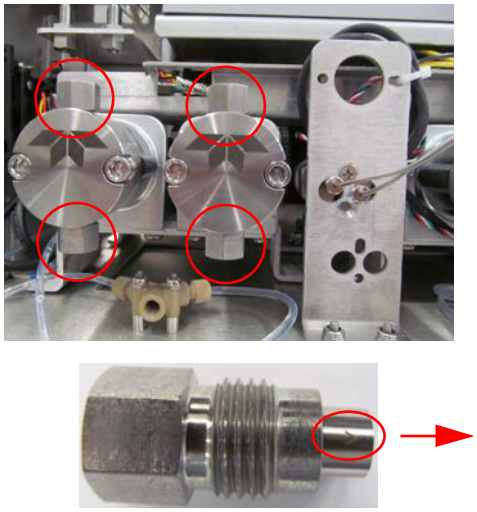


Figure 5-24 Check valve housings (top) and inlet check valve and housing (bottom)

☑ Note

On an outlet check valve, the arrow is reversed.

11. Reinstall the check valve housings into the pump heads and tighten. Reconnect the 'Y' fittings removed in step 5 and reconnect the inlet and outlet lines. Reassemble the instrument in the reverse order of disassembly.
12. Place the solvent supply lines into the proper connectors and reprime the system.

5.6 AutoSampler Maintenance

Peristaltic Tubing Replacement

The Peristaltic pump on the back of the AutoSampler may need replacement.

Peristaltic tubing part number: SP7477.

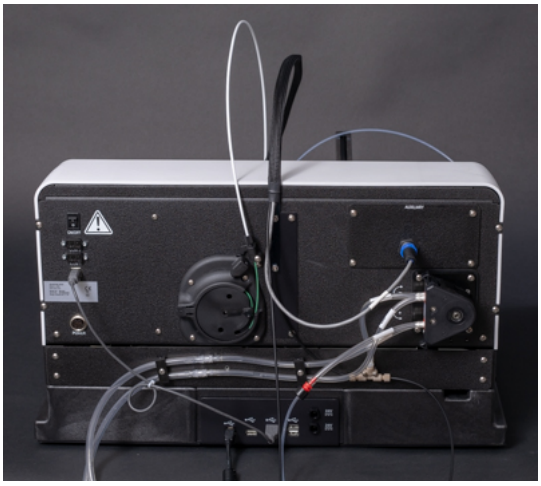


Figure 5-25 Back of the AutoSampler

1. Remove the outer band of the peristaltic pump (Figure 5-26).

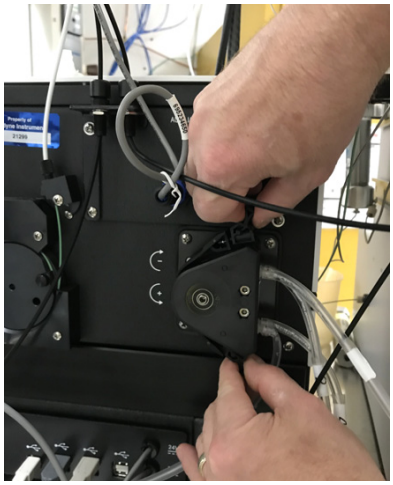


Figure 5-26 Removing the outer band

2. Lift up to remove the peristaltic tubing from the pump, repeat on the bottom (Figure 5-27).

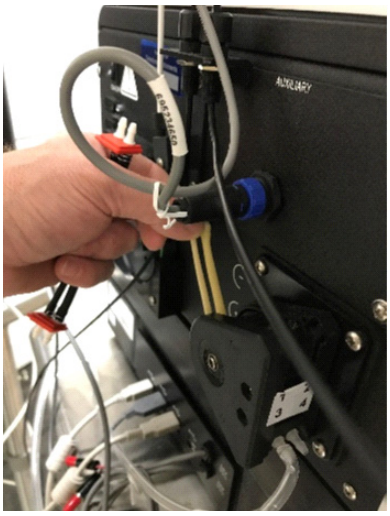


Figure 5-27 Removing the tubing

3. Slide the new peristaltic tubing with the thicker tubing towards the AutoSampler (Figure 5-28).

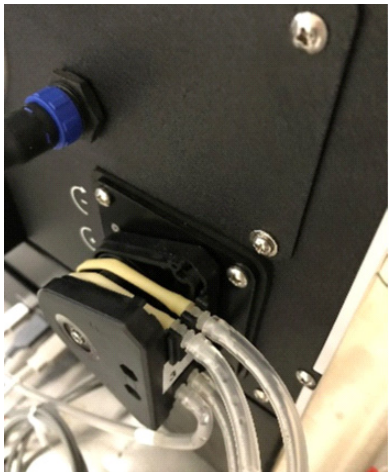


Figure 5-28 Attaching the new tubing

4. Replace the outer band of the peristaltic pump (Figure 5-29).

Figure 5-29 Replace the outer band

5.7 ELSD Maintenance

Periodic cleaning of the spray chamber will keep the ACCQPrep operating at maximum performance. Follow the steps below if spray chamber cleaning is recommended:

5.7.1 Cleaning the ELSD Detector

1. From the RUN SCREEN, open the METHOD EDITOR.
2. At the Peak Detection section, select ELS DETECTOR, then select DETAILS. The DETECTION OPTIONS window opens.
3. Set the spray chamber temperature to 40 °C.
4. Exit the METHOD EDITOR and accept the changes. Allow approximately 5 minutes for the chamber to reach the set temperature.

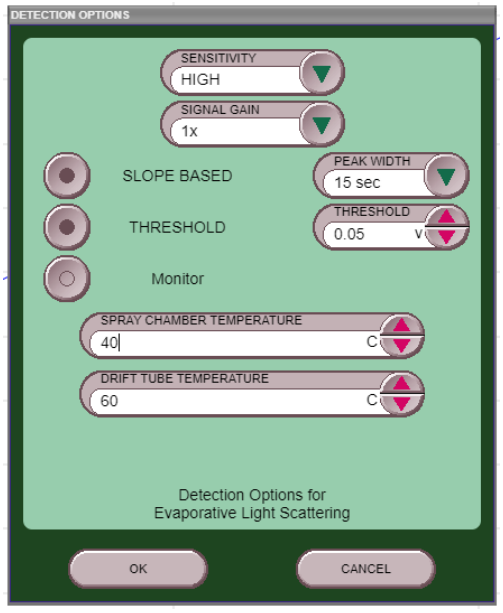


Figure 5-30 ELSD Detection Options window

5. Lift the P-trap drain tubing up to approximately case top level. Using a wash bottle, syringe, or suitable measuring device, slowly fill the drain line with up to 40 mL of ace-

tone. Lift the end of the tubing as needed to transfer most of the liquid into the spray chamber. Make sure the fluid level in the tubing doesn't exceed the level of the instrument case top. If the tubing is raised too fast, fluid may flow out the top of the vent tube causing a spill. Hold the tubing up for at least 1 minute after the fluid has been transferred to the spray chamber.

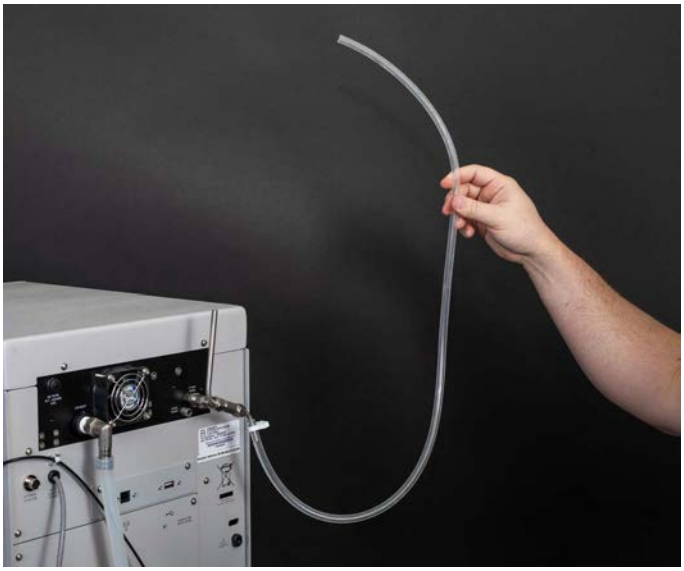


Figure 5-31 Line filled with 40 mL of acetone

6. Place the open end of the P-trap drain line in a 100 mL (or greater) beaker.

Figure 5-32 Draining the acetone into a 100 mL beaker

7. Allow the acetone to drain out of the unit by dropping the end of the P-trap drain line, while it is in the 100 mL beaker, below the P-trap drain line level.
8. Fill the P-trap pump drain line with up to 40 mL of isopropyl alcohol using the same procedure as step 5.
9. Place the open end of the P-trap drain line in a 100 mL (or greater) beaker.

10. Allow the isopropyl alcohol to drain out of the unit by dropping the end of the P-trap drain line, while it is in the 100 mL beaker, below the P-trap drain line level.
11. From METHOD EDITOR > ELSD DETECTOR DETAILS > DETECTOR OPTIONS, set the spray chamber to 60 °C.
12. Fill the P-trap with up to 40 mL of isopropyl alcohol as before and drape the P-trap drain line of the top of the unit and let it set for at least 20 minutes.
13. Allow the isopropyl alcohol to drain out of the unit by dropping the end of the P-trap drain line, while it is in the 100 mL beaker, below the P-trap drain line level.
14. To ensure that there is no fluid remaining in the drift tube, set the drift tube to 90 °C. Open MANUAL CONTROL to turn the gas on and let run 10 minutes. Turn off the gas.
15. Select FILE and then NEW to reset the method temperatures. Use the instrument normally.

☑ *Note*

During the rinse steps, it is normal to have flakes or particles in the wash liquid.

5.8 PurIon Maintenance

5.8.1 ESI and APCI Removal from PurIon S and PurIon L

Refer to Figure 5-33.

1. Place the mass spectrometer in STANDBY mode.
2. Unscrew the ¼-28 PEEK fitting at the top of the ion source housing.
3. Loosen the two clamps at both sides.
4. Gently lift and pull out the source housing.

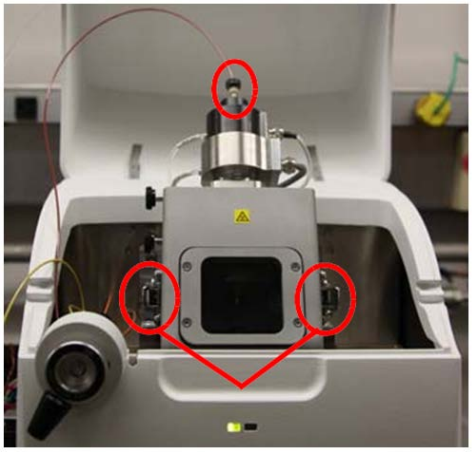


Figure 5-33 ESI and APCI removal from PurIon S and PurIon L

5.8.2 ESI and APCI Replacement PurIon S and PurIon L

1. Carefully place the ion source housing on top of the base plate and line up with the rear electrical connection. Push down until source chamber is seated evenly across the base plate.
2. Lock down two housing clamps at both sides.
3. Finger-tighten the ¼-28 PEEK fitting at the top of the source housing.

5.8.3 Cleaning the Ionization Source Capillary

Plugging of the capillary (either in the ESI or APCI probe) causes the pressure of the carrier fluid from the fluid interface to exceed the maximum operating pressure indicated by Error 310 or Error 316. This error can be avoided by filtering directly injected samples through a 0.45 µ syringe filter. To unplug the capillary, complete the following:

1. Using adapters as needed, connect a 1 mL syringe to the ESI or APCI capillary, then push fluid through to remove plug. If this procedure does not resolve the issue, an HPLC pump can be connected to the inlet fitting.
2. If this procedure fails to unplug the capillary, refer the instructions for the rebuild kit provided with your PurIon system.
3. Reinstall the probe following the appropriate procedures for your system.

5.8.4 Replacement of Ion Source Housing

1. Place the ion source housing on top of the base plate and line up with the rear electrical connection. Push down until source chamber is seated evenly across the base plate.
2. Finger tighten the two thumb screws and finger tighten the ¼-28 PTFE tube fitting labeled as 'heated desolvation' on the side of the housing.

5.8.5 Overpressure Error

The fluid interface has a pressure transducer to monitor pressure of the carrier fluid. Since the sample is introduced at the splitter valve, plugs usually occur between the valve and the PurIon source sprayer. The most common location for a plug is within the probe capillary. The occurrence of plugs can be reduced by using a 0.45 µ syringe filter when injecting samples for Method Development and Ionization Settings. To trouble-shoot an overpressure error, complete the following:

- If the error occurs during a run, select CONTINUE WITHOUT PURION, The purification can continue without the PurIon signal or peak detection, but it will rely on any other detector selected such as UV or ELSD. This allows the run to be completed before trouble-shooting the plug.
- Select MS > MANUAL CONTROL.
- Select START CARRIER PUMP.
- Watch the pressure on the ribbon gauge.
- Loosen the fitting at the source inlet. If the pressure drops, then the source capillary is plugged.
- If there is still an error or the pressure remains high, then the plug is between that point and the splitter valve (or the valve itself). Continue to loosen fittings going back the to the splitter valve until the error is corrected.

5.8.6 Check Valve Cleaning

If the check valves are allowed to dry out after using volatile salts (e.g., ammonium acetate or ammonium formate), they may stick and fail to function. Complete the following to clean the check valves:

1. Remove the inlet and outlet lines from the check valve holders (Figure 5-34), then remove the check valve holders. Pliers may be needed to remove the holders.

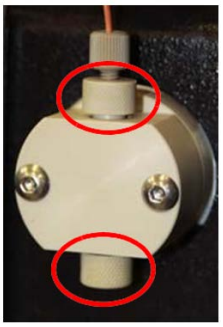


Figure 5-34 Check valve holder

2. Remove check valves from pump head and place in a beaker of methanol. Sonicate check valves for 15 to 20 minutes.
3. Reinstall check valves into the pump head, making sure that the ends of the check valves (with multiple holes) are facing up towards the outlet of the pump.
4. Reinstall check valve holders and tighten finger tight. Then using pliers tighten an additional ¼ turn.
5. Check the flow rate delivery. If the flow rate delivery is still incorrect, replace the check valves. The correct flow rate depends on the back pressure of the carrier solvent. The flow will be 0.5 mL/minute until the fluid interface reaches operating pressure, then it reduces the flow rate to 0.2 mL/minute under typical operating conditions for the fluid interface. The operating mode can be determined by listening to the pump operate. While running at 50 mL/min, the pump motor speed is continuous. After operating pressure is reached, the pump performs a rapid refill stroke approximately every 8 seconds.

5.8.7 Replacing Check Valves

1. Install new check valve cartridges (PN 250-0001-17) into pump head housing making sure the ends of the cartridges, with three small holes, are facing upwards towards the outlet (Figure 5-35).

Figure 5-35 Correct orientation of cartridge

2. Tighten check valve holders finger tight, then with pliers tighten an additional ¼ turn.

5.8.8 PurIon Cone Cleaning

This error occurs if the capillary inlet to the PurIon vacuum region is restricted causing a higher vacuum reading than normal. PurIon S & L models compatible with the NextGen

systems have an internal valve that allows removal of the capillary inlet without venting the vacuum system to allow easy cleaning. Even with the easy cleaning capability, Teledyne LABS recommends keeping a spare capillary inlet cone assembly (PN 25-0000-085) to allow rapid replacement to minimize downtime while cleaning the plugged capillary.

5.8.9 Capillary Inlet Cone Removal

1. Set the PurIon to standby.
2. Wait ~15 minutes for the cone to cool.
3. Remove the ion source assembly. See section 5.8.3.
4. Wearing gloves (typical lab gloves are usually sufficient), place your fingers on the top surface of the cone and turn counterclockwise to unscrew the capillary inlet cone. Often this is sufficient to remove the capillary. If you are unable to unscrew the part manually, use an adjustable wrench on the flats of the cone to unscrew them. The flats are not large, and they may be difficult to see while a wrench is seated on them.
5. Remove the O-rings from the capillary inlet cone.

5.8.10 Capillary Inlet Cone Cleaning

1. Remove the O-ring under the capillary base, then sonicate the capillary in a methonal:water (50:50) mixture for 30minutes (Figure 5-26).

Figure 5-36 Location of O-Ring

2. If heavily contaminated, sonicate in methanol:water + 1% formic acid (50:50) mixture for an hour.
3. Rinse the capillary thoroughly with acetone, isopropanol, methanol then dry the capillary using nitrogen air.

5.8.11 Capillary Inlet Cone Installation

1. Ensure that the O-ring seals are in position.
2. Place the capillary inlet cone into the opening and press down until the threads are able to engage. There may be slight restriction in a downward motion as the part is almost completely inserted. This is the capillary opening the valve to the vacuum region.
3. Screw the capillary inlet cone into the inlet. Finger tight is sufficient as long as the part is fully seated.
4. Replace the ion source.
5. Place the PurIon in the operate mode.

5.8.12 PurIon Troubleshooting

If your instrument stops working and the touch panel display is off, check the line cord connection.

If the line cord is connected properly, check circuit breaker on the system's rear panel to ensure it is switched to the ON position.

Table 5-1 Common PurIon Error Codes and Resolutions

PurIon temperatures are stabilizing. [r] seconds remain. (where [r] is a number)	The PurIon has several areas with heaters. The software has a set timer to allow temperatures to come up to operating conditions. After that time, a separate error is thrown if the heaters are not within an acceptable band. The default time is 300 seconds after entering the operate condition. During the standby condition most heaters are set to OFF except the inlet capillary with is set to 50 °C during standby.
The PurIon vacuum level is too low to operate. Verify that the roughing pump is on and operating correctly. Pirani Pressure: [s1] mbar. (where s1 is the vacuum reading).	The Pirani pressure must be below 5.5E-3 mbar before the PurIon turbo pump will operate. If trying to place the PurIon in operate without turning on the roughing pump, this message will appear. This error generally occurs if turning on the roughing pump back is forgotten after cleaning the capillary or changing the pump oil.
The PurIon has been shut down. It will be unavailable for use until the NextGen has been rebooted. Ensure the PurIon and fluid interface are both turned on before rebooting the NextGen.	This message is displayed after the PurIon has been successfully shut down through the SHUTDOWN command. It serves as a reminder that an NextGen reboot will be necessary for the PurIon to be enabled again by the NextGen.
The splitter valve seals have exceeded their recommended life. The PurIon will continue to operate, but there is an increased possibility of leakage at the splitter valve and loss of PurIon detection during a separation.	The splitter valve supplier has stated that typical valve life exceeds 1,000,000 actuations. After that time, there is no method of determining when it will leak. The valve can be rebuilt using the valve rebuild kit (PN 60-5234-629) or continue to use and monitor for leakage. Leakage should not drip out the bottom of the fluid interface front cover.
No ion source is detected on the PurIon. Verify that the ion source is properly installed and connected. If the source is still not detected, contact a qualified service technician. Error 309	The PurIon has reported that the ion source high voltage cable is not properly plugged into its socket. This is the cable with the round connector and is to the right and behind the ion source. This generally occurs upon changing or cleaning probes.
A plug has occurred in the PurIon fluid lines. The separation can be continued without PurIon detection or continued if the plug is corrected. Error 310	This error message is displayed if a plug is detected during a separation.
The PurIon inlet cone may be plugged, which could prevent detection. Continued operation will not cause damage. Contact a qualified service technician to clean the cone; Pirani Pressure: [s1] mbar. Error 315	During normal operation, the Pirani pressure should be >1.5E-3 mbar. Anything less is an indication of either partial or complete plugging of the capillary cone entrance to the vacuum area. See cleaning the cone capillary (5.8.3).
A plug has occurred in the PurIon fluid lines. The plug must be corrected to allow continued operation. Error 316.	This error occurs if the tubing is plugged while the method development screen is in use. See cleaning the probe capillary (5.8.3).
The ionization probe (ESI or APCI) isn't fully seated into the ion source housing. Verify that the probe is fully seated, then select OK to continue. Error 317.	On PurIon systems (not PurIon S or PurIon L), the probe isn't seated properly. Loosen the thumbscrew on the front of the ion source housing, push down on the probe, then tighten the thumbscrew.
The fluid interface pressure is too low. Error 325	This could be due to lack of carrier fluid, loss of pump prime, or leakage.

ACCQ*Prep* HP150

Appendix A

A.1 Diagrams for the AutoSampler and ACCQ*Prep*

The following diagrams are for connecting the AutoSampler to the ACCQ*Prep* system. These diagrams include:

- A tubing diagram of the connections from the AutoSampler to the ACCQ*Prep* *(Figure* A-1*)*
- A wiring diagram of the USB connections from the AutoSampler to the ACCQ*Prep* *(Figure* A-2*)*

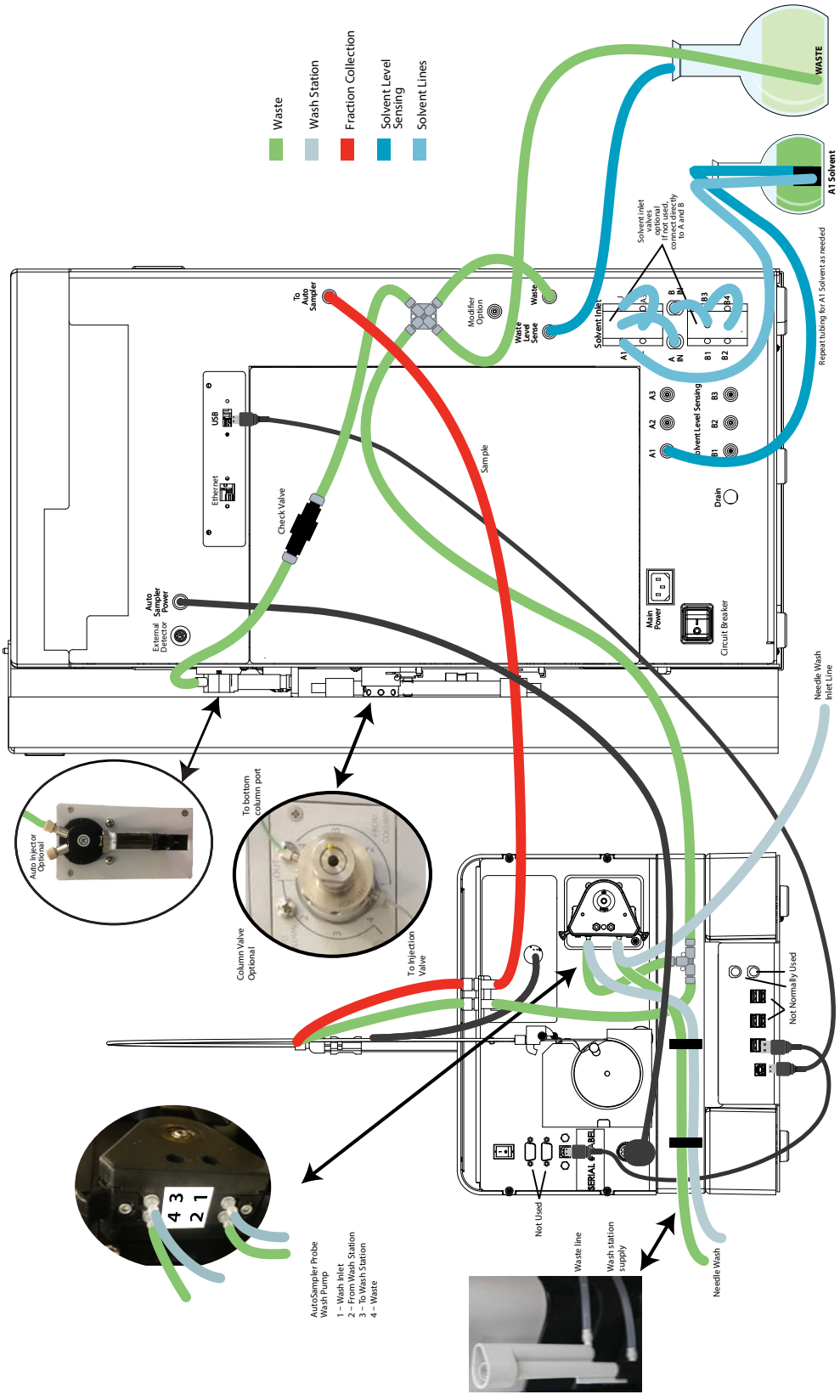


Figure A-1 Tubing diagram

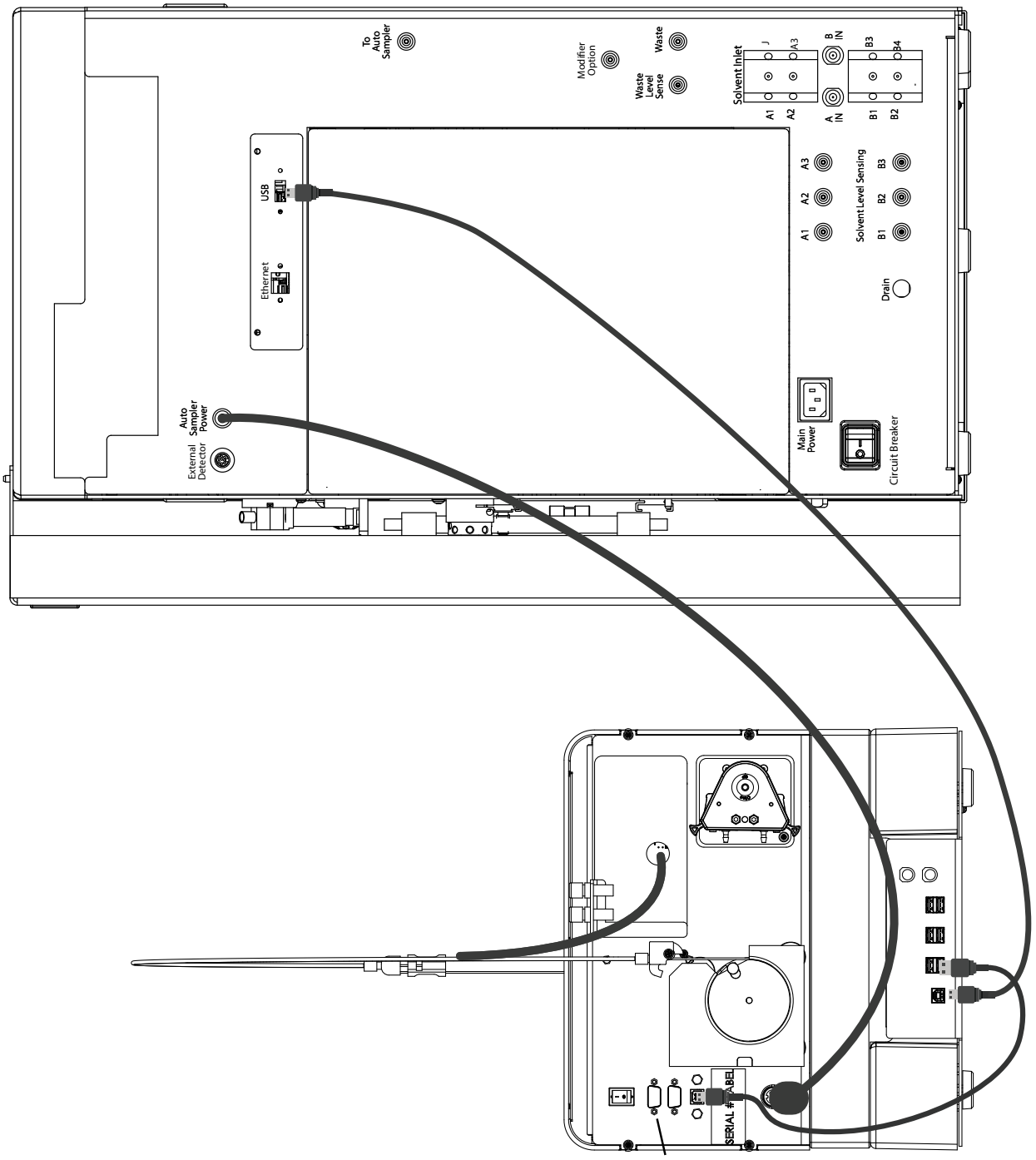


Figure A-2 Wiring diagram