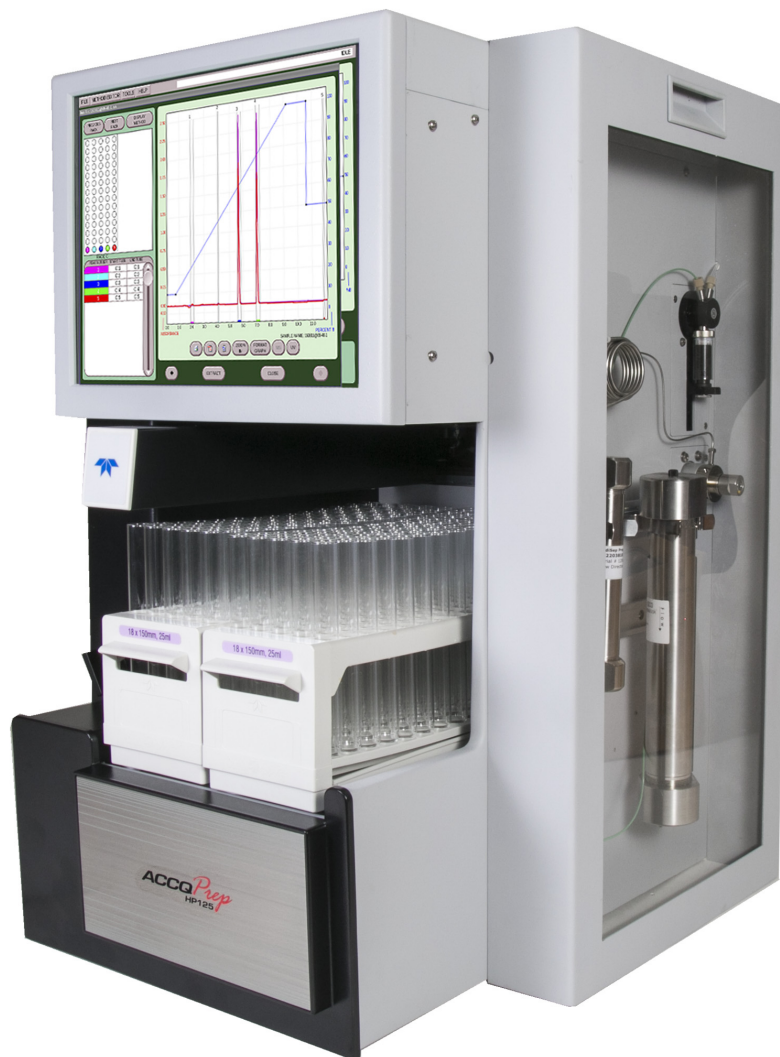


ACCQPrep HP125

Installation and Operation Guide



60-5233-812

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Rev. C, January 2019

Safety

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, take heed in 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 Sie 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 ACCQPrep HP125 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

ACCQPrep HP125

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ACCQPrep HP125

Section 1 Introduction

1.1 Overview

This Installation Guide provides:

- Safety information
- Unpacking instructions
- Installation instructions, including connections with Windows and iOS operating systems
- Certification and warranty information

The Operating section will guide you on basic system operation.

1.2 Product Overview

The Teledyne Isco ACCQPrep HP125 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 ACCQPrep HP125 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 for repeated injections of the same sample, an AutoSampler for single or repeated injections of multiple samples, or a column select module supporting up to 4 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).



Figure 1-1 ACCQPrep HP125

ACCQPrep HP125 – This Prep HPLC system has flow rates from 1-125 mL/min, with up to 6000 psi capability. A two component gradient can be formed from up to six solvents. The system includes active solvent level sensing and detection of a full waste container. The base system includes two fraction collection racks. With the optional AutoSampler up to four collection racks can be used. The RFID tagged racks can be replaced while in operation with new racks for practically limitless fraction collection.

ACCQPrep HP125 with optional ELSD – This system has the same high performance features as the ACCQPrep HP125 system, but 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.

ACCQPrep HP125 with optional PurIon Mass Spectrometer – This system has the same features as the ACCQPrep HP125, but 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-V (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 ACCQPrep HP125 system is equipped with a capacitive touch screen 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 ACCQPrep HP125 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 a Windows or Apple PC or Laptop, or an Apple iSO mobile device (iPod Touch, iPhone, and iPad).

Note

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

1.3.1 MultipleControl Possibilities

The system can be accessed from the built-in touch panel and 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 FileStorage

To support operation from a variety of direct and network connections, the software and all files are stored in the ACCQPrep HP125. 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

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

To remove power from the ACCQPrep 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 “1”. In addition, switching the breaker manually to the “0” position will remove power from the internal operating components.



Figure 1-2 Location of power cord for the ACCQPrep

1.5 Specification

| Table 1-1 ACCQPrep HP125 | |
|---|---|
| Dimensions of ACCQPrep (Footprint without column or column cover installed) | 27.5 x 14.0 x 20.0 in (69.9 x 35.6 x 50.8 cm) |
| Dimensions of ACCQPrep 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 ACCQPrep 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 | 1-125 mL/min, ELSD peak collection limited to 4-125 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 4000 psi at 125 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 |
| Gradient Accuracy: | ±1% of full scale |
| Peak Detection Modes | Slope or threshold |
| Flow Cell Pathlength | 0.3 mm, ±25% (Standard, other pathlengths available) |
| UV Detection Wavelength | 200 to 400 nm, optional 200 to 800 nm UV-Vis |
| 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 |

Table 1-1 ACCQPrep HP125

| 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 ACCQPrep HP125 with Purlon System

| | |
|---|--|
| Dimensions (H x W x D) | ACCQPrep: 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 |
| Pressure Transducer | 316 and 17-4 PH stainless steels and perfluorelastomer (FFKM) |
| AutoSampler Wash | Pharm-A-Line™, polypropylene |

ACCQPrep HP125

Section 2 Installation

2.1 ACCQPrep Installation The following section will cover the installation, connection of the pump, and a review of the priming steps.

✓ Note

When the system ships, it will ship in two boxes to minimize the weight. One box will contain the unit and the other box will have the pump and assorted accessories.

Tools Required

- Hex wrench (included with the pump module)
- 1/4" open end wrench
- Fitting wrench (allows more secure connection of 'finger tighten' fittings) (Wrench is included in the ACCQPrep accessory kit)

2.2 Setting Up the System The ACCQPrep chassis including AutoInjector, Solvent Selector Valve Module, and Column Selector Valve Module, but without the pump assembly, weighs up to approximately 64 pounds (29 kg). Handles are provided on each side of the instrument to facilitate lifting.

⚠ CAUTION

A two person lift is recommended to prevent injury.

The pump assembly weighs 25 pounds (11.5 kg) resulting in a final weight of 93 pounds (42.3 kg) with the column cover installed, but not including an optional AutoSampler.

The fraction collector arm is secured to prevent damage during shipping. The arm securing bracket should be removed at this time.

1. Loosen the bracket's two thumbscrews along the sides of the arm (Figure 2-1).

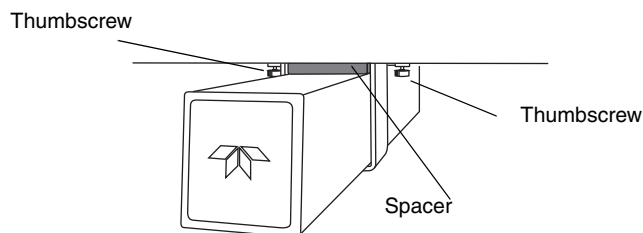


Figure 2-1 Remove arm stowing bracket and spacer

2. Push the arm to the left or right to remove the spacer.
3. Store the bracket and spacer. These parts should be reinstalled if the system must be shipped again.

When you are ready to place the system, be sure it is situated in such a way that the power cord is accessible to disconnect in case of malfunction.

2.2.1 Pump Installation

✓ Note

Be sure to move the two electrical connections and solvent tubing out of the way before sliding the pump assembly into the pump compartment.

To install the pump:

1. Remove the two pump retaining screws in the pump compartment.
2. Align the pump assembly horizontally with the two notches facing the opening of the pump compartment (Figure 2-2).

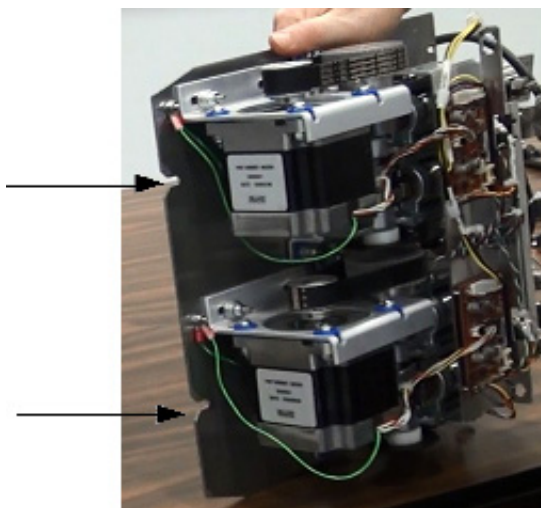


Figure 2-2 Location of notches on the pump sled

3. Slide the pump assembly into the pump compartment. It may require a little force since it is metal on metal sliding together. If the force seems excessive, ensure there is nothing obstructing the pump assembly. Be sure to keep the electrical connections and tubing out of the way so they do not get pushed inside of the compartment (Figure 2-3).

✓ Note

Be sure to observe the top of the pump assembly as it is being inserted into the system. Make sure no wires from the case or pump assembly are being snagging.

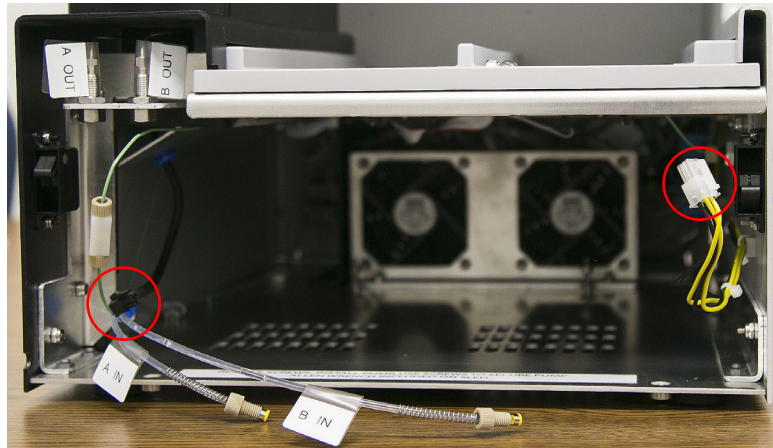


Figure 2-3 Pump compartment with electrical connection shown

4. When the pump assembly is most of the way in the pump compartment, connect the electrical connections on each side of the pump. First, connect the black connection on the left side. Ensure the tab on top of the connector is facing up before pushing it into the mating connector (Figure 2-4).

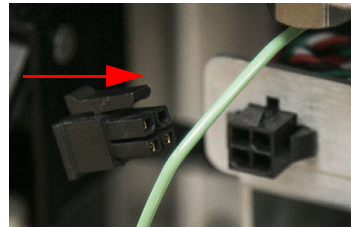


Figure 2-4 Black connection with tab facing up

Then, connect the white connection on the right side. Ensure the tab on top of the connector is facing up before pushing it into the mating connector (Figure 2-5).

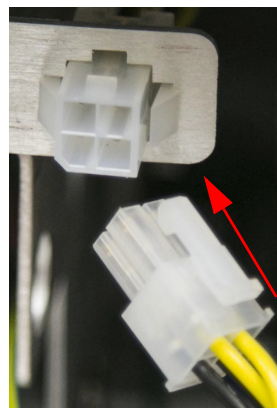


Figure 2-5 White connection with tab facing up

5. Once the electrical connections are complete, push the pump assembly the rest of the way into the pump compartment until the holes in the front of the pump assembly are aligned with the corresponding holes in the case bottom.

✓ Note

Hold downs at the back of the pump may cause extra resistance for the last $\frac{1}{2}$ " of travel.

6. To hold the pump in place, use the two hex screws, removed previously, and the hex wrench to secure the pump assembly (Figure 2-6).

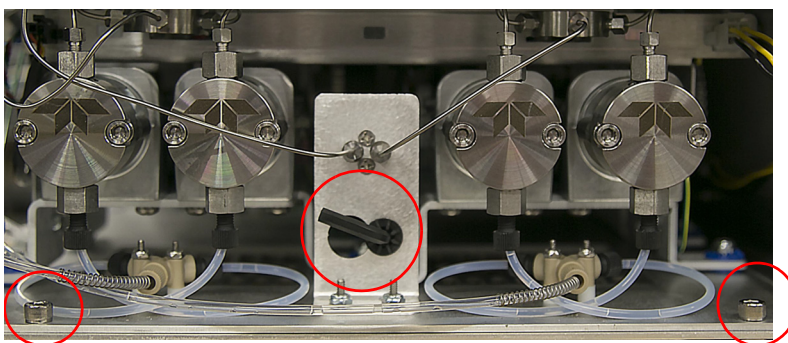


Figure 2-6 Location of threaded holes, in the base of the unit, and the hex wrench

2.2.2 Solvent Line Installation

1. The left two pump heads form the A solvent pump and the outlets are connected to a tee. The outlet of this tee connects to the left bulkhead union. Tighten with the $\frac{1}{4}$ " open end wrench.
2. The right two pump heads form the B solvent pump and the outlets are connected to a tee. The outlet tee on this side is connected to the pressure transducer in the middle of the assembly. The remaining connector on the pressure transducer must be connected to the right bulkhead union. Tighten this fitting with the $\frac{1}{4}$ " open end wrench (Figure 2-7).

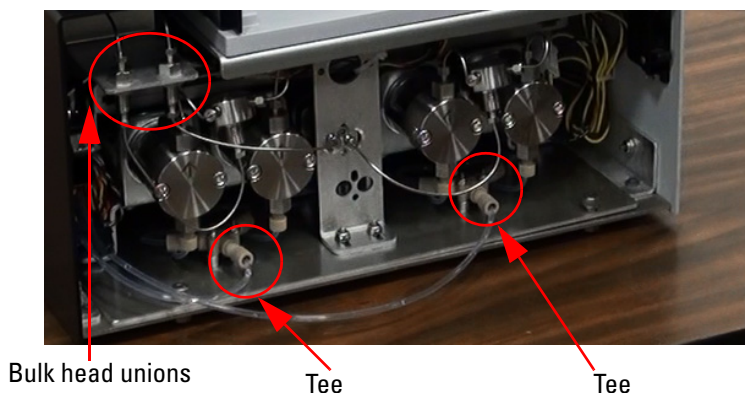


Figure 2-7 Line connections on the pumps

3. Connect the *shorter* FEP inlet solvent line to the left side pump's PEEK tee. Slide the open side of the wrench (included) over the tubing and FEP fitting and make small turns as to not bend the tubing (Figure 2-8).
4. Connect the *longer* FEP inlet solvent line to the right side pump's PEEK tee. Slide the open side of the wrench (included) over the tubing and FEP fitting and make small turns as to not bend the tubing (Figure 2-8).

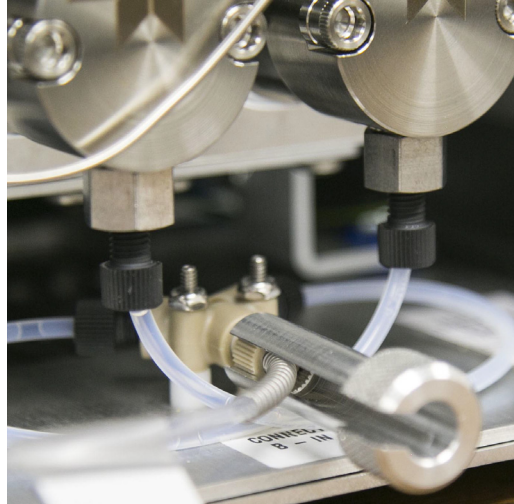


Figure 2-8 Wrench used to tighten the FEP inlet line

5. Once complete, install the faceplate onto the unit by snapping the side mounted connectors into place.

2.2.3 Fluid Line Installation

Note

The solvent lines are installed at the factory and include labels identifying the lines A1, A2, and A3 for the A solvents and line B1, B2, and B3 for the B solvents when using the Solvent Selector Valve Module.

1. Remove the two screws that secure the solvent lines for shipment.
2. Place the solvent lines in the desired containers.

2.2.4 ACCQPrep Equipped with Optional ELSD

Note

Teledyne ISCO recommends >99% pure nitrogen from a source that can deliver 2.5 SLPM at 60 to 70 psi.

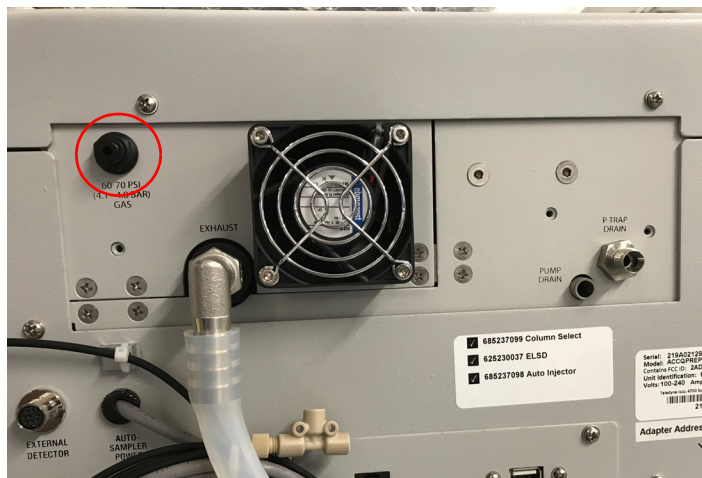


Figure 2-9 Location of ELSD gas port (Nitrogen input line circled)

The ACCQPrep ELSD system has several additional connections.

1. Install the ELSD P-trap vent assembly found in the accessory package (PN 3-250004) by placing the short tube into the port on the rear panel, orienting the vent as shown and tightening the fitting nut finger tight plus a small amount more with a wrench (Figure 2-10).



Figure 2-10 Back of ACCQprep with optional ELSD

2. ELSD operation requires a carrier gas. To connect the carrier gas to the ACCQPrep:
 - Locate the $\frac{1}{8}$ inch O.D. FEP tubing (PN:023-0503-02) from the accessory package.
 - Push one end of the tubing into the NITROGEN INLET (Figure 2-9) port on the back of the system. The tubing should be fully seated in the port.
 - Cut the tubing to length and connect the other end to the user-supplied carrier gas. An assortment of $\frac{1}{8}$ inch adapters are supplied in the accessory kit to complete the connection to your gas source.
3. The P-trap must be filled with fluid to prevent sample loss. To accomplish this, raise the drain tubing attached to the P-trap drain vent assembly above the instrument and place 10 mL of liquid, such as isopropyl alcohol, into the tube. 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. Lift it high enough so the fluid enters the P-trap drain (Figure 2-11).

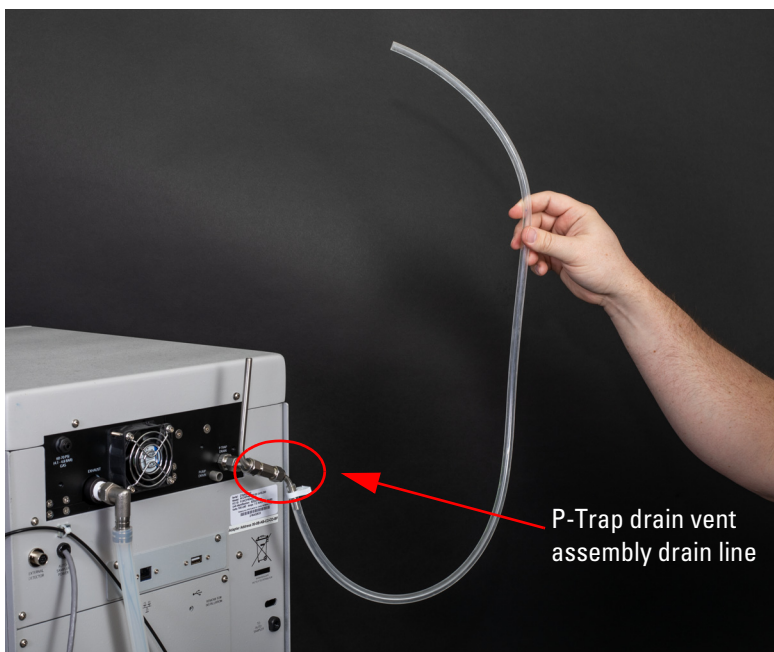


Figure 2-11 Keep liquid level lower than the top of the system

4. Route the end of the P-trap drain tube to a suitable waste fluid collection container. Please be aware that as the tubing is lowered, several mL of isopropyl alcohol will run out of the tubing. This is normal and means that the P-trap is properly filled. Depending on your application, the P-trap fluid may need periodic replenishment to ensure maximum signal strength of the ELSD.

✓ Note

If accessible, the outlet end of the P-trap drain tube may be used to refill the P-trap.

5. Route the attached tubing from the ELSD exhaust port away from the system. This will prevent unnecessary solvent vapor alarms.

✓ Note

The ELSD exhaust will contain fine particles of compound. If using outside of the hood, ensure that the exhaust port tubing is routed to an exhaust handling system that meets your safety and environmental requirements. Do not use tubing smaller than the exhaust tube. If the exhaust is restricted, some of the gas flow could be diverted out the P trap drain resulting in reduced sensitivity and the need to route the P trap drain to an exhaust. In normal operation, the exhaust gas is dry and does not require a waste collection container.

⚠ CAUTION

Discontinue use of the ACCQPrep if liquid is present at the Pump Drain. Contact Teledyne ISCO technical service for assistance with correcting the leak.

2.2.5 Priming Steps

The system will require priming with the desired solvents. To prime the system:

1. Press the red switch, next to the front USB port, to the ON position to boot up the system (Figure 2-12).

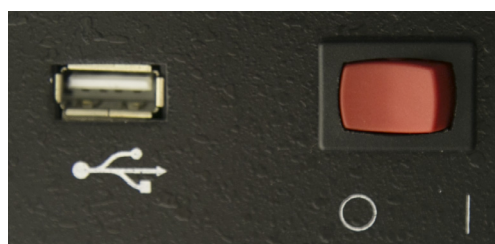


Figure 2-12 Front USB and On switch

2. Turn the prime valve to the PRIME position (Figure 2-13).



Figure 2-13 Turn the valve to the Prime position

- If the solvent select option is not installed, the system will immediately prime both solvent lines.
 - If the solvent select option is installed, a dialog box opens when the valve is switched to the PRIME position. The dialog box allows you to select which of the A solvents and B solvents will be primed.
 - If you have not already assigned names to the A and B solvents, they can be named at this time using the drop downs. If the name you want isn't listed, it can be added in the CONFIGURATION screen. This action can be completed at a later time.
3. Once priming is complete, a new screen will appear directing you to turn the valve from the PRIME position to the RUN position.

Note

The system will prime both pumps simultaneously. There is no method to prime A and B individually.

2.3 Installing the Manual Injection Port

Manual sample injection can be performed with the system even when equipped with an AutoInjector or AutoSampler.

If no AutoInjector or AutoSampler is installed, a luer injection port (209900002) is included in the accessory package. To install, place the fitting into the injection valve and tighten the nut snugly (Figure 2-14).

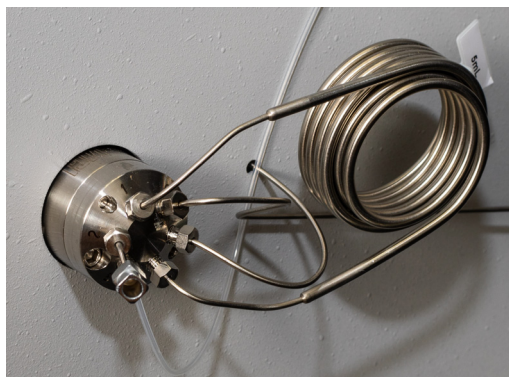


Figure 2-14 ACCQPrep with Manual Injection Luer Port installed

Connect the included check valve(209016629) to the inject valve waste line. Ensure that the arrow on the check valve points toward the waste container. This check valve ensures sample doesn't run from the port when the injection syringe is removed.

If an AutoInjector or AutoSampler is installed and manual injection is also desired, install the included check valve (209016629) in the waste line from either the inject valve (if no AutoInjector or AutoSampler is installed) or the waste line from the sample load pump. Ensure that the arrow on the check valve points toward the waste container. This check valve ensures sample doesn't run from the port when the injection syringe is removed.

2.4 Installing the AutoSampler

These instructions assume that the AutoSampler was purchased with a new ACCQPrep. In that case, the AutoInjector portion of the AutoSampler is already installed in the ACCQPrep. If not, please refer to installation instructions included with the AutoInjector.

To install the AutoSampler:

1. Carefully unpack the Z-Drive and remove the packing materials from the Y-axis arm (Figure 2-15).

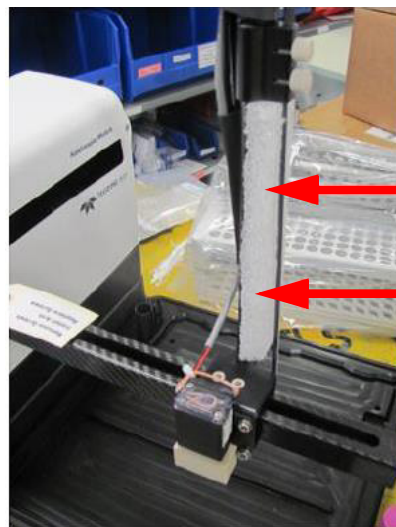


Figure 2-15 AutoSampler Module and Z-Drive

2. Find the Y-axis carriage on the arm of the AutoSampler Module. The Z-Drive will be attached to this carriage (Figure 2-16).



Figure 2-16 Z-Drive mounted on the Y-axis carriage

3. Remove the two screws from the Y axis carriage. Slide the Z-Drive onto the arm until the two holes align with the matching holes in the Y-axis carriage.
4. Using the two plastic thumbscrews, secure the Z-Drive to the carriage and finger tighten (Figure 2-17).



Figure 2-17 Z-Drive secured to the Y-axis carriage

5. Rotate the Z-Drive rotor back and forth to ensure that the Z-Drive moved up and down freely (Figure 2-18).

Note

If the rotor does not move freely, check that the other cables are not interfering with the movement of the green probe cable.

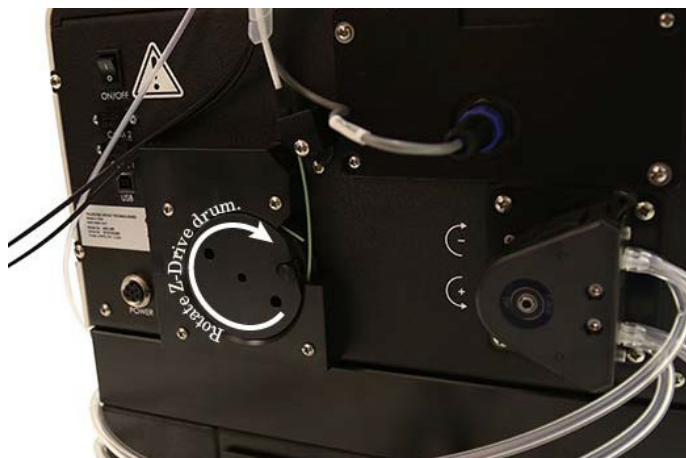


Figure 2-18 Z-Drive rotation

6. Mount the wash well by placing it into the receptacle located on the right-rear portion of the AutoSampler Module bed (Figure 2-19).

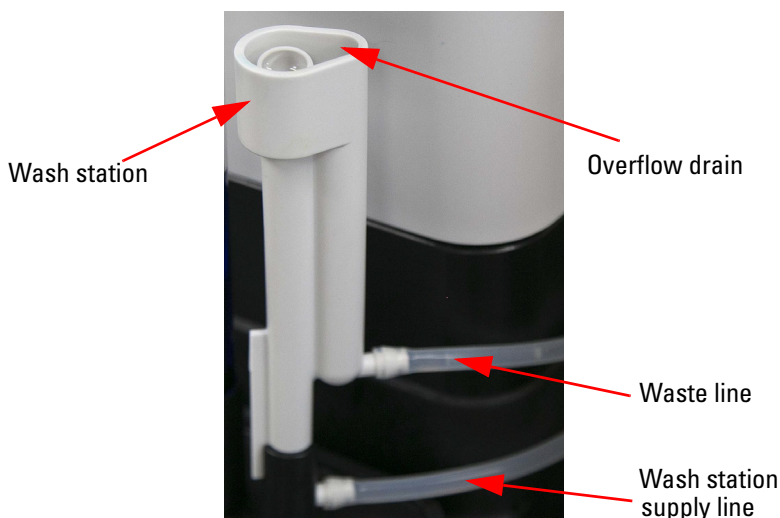


Figure 2-19 AutoSampler Module wash well with tubing attached (top tube is waste to port #2 while the bottom tube is supply port #3)

7. The wash well comes pre-plumbed to the peristaltic pump ports. If disconnected in the future, it is important to connect the wash well waste tube (upper tube on wash well labeled 2) to port “2” of the peristaltic pump and connect the wash well supply tube (lower tube on wash well labeled 3) to port 3 of the peristaltic pump.
8. Connect the tube labeled “Wash Supply” to the tube labeled “1” connected to port “1” of the peristaltic pump on the back of the Autosampler Module. The free end of the tubing can be placed in the wash solution container or tee'd into a solvent supply line (Figure 2-20).

✓ Note

The wash fluid peristaltic pump uses Pharm-A-Line™ tubing. Please ensure the wash fluid is compatible with this tubing, such as methanol.



Figure 2-20 AutoSampler Module peristaltic pump with tubing correctly attached

9. Connect the tube labeled “Wash Waste” to the tube labeled “4” connected to port “4” of the peristaltic pump on the back of the Autosampler Module. The free end of the tubing can be placed in the waste container or tee’d into an existing waste line (Figure 2-21).

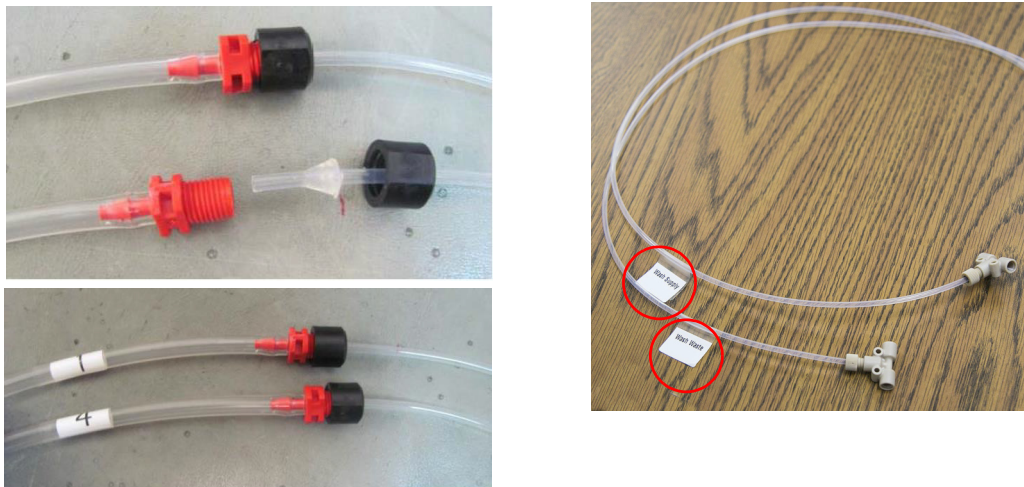


Figure 2-21 Wash and waste tubing connections and labels

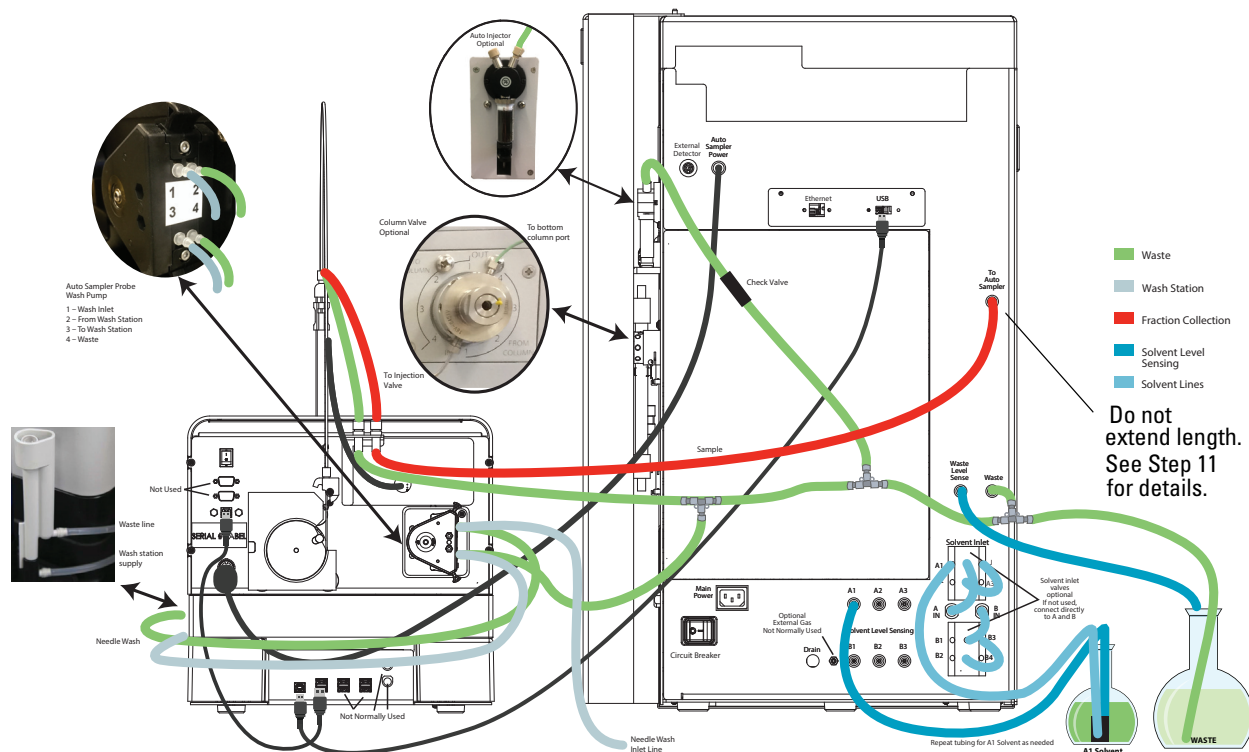


Figure 2-22 Tubing diagram of the AutoSampler connected to the ACCQPrep (See Appendix A, Figure A-1 for a larger diagram)

10. Place the AutoSampler Module on the right side of the ACCQPrep.
11. Connect the AutoSampler tubing labeled “To ACCQPrep” to the connection on the ACCQPrep’s rear panel labeled “To AutoSampler”. The length of this tubing is critical to control the delay volume for fraction collection on the AutoSampler. The overall length of the tubing to the diverter valve should be 67” (170 cm).
12. Route the “To Waste” tubing to a suitable waste container or tee to either the Wash Waste Line from the AutoSampler Module (from step 8 above or seen in Figure 2-22) and/or the ACCQPrep waste line and injection valve waste line with the supplied tee(s).

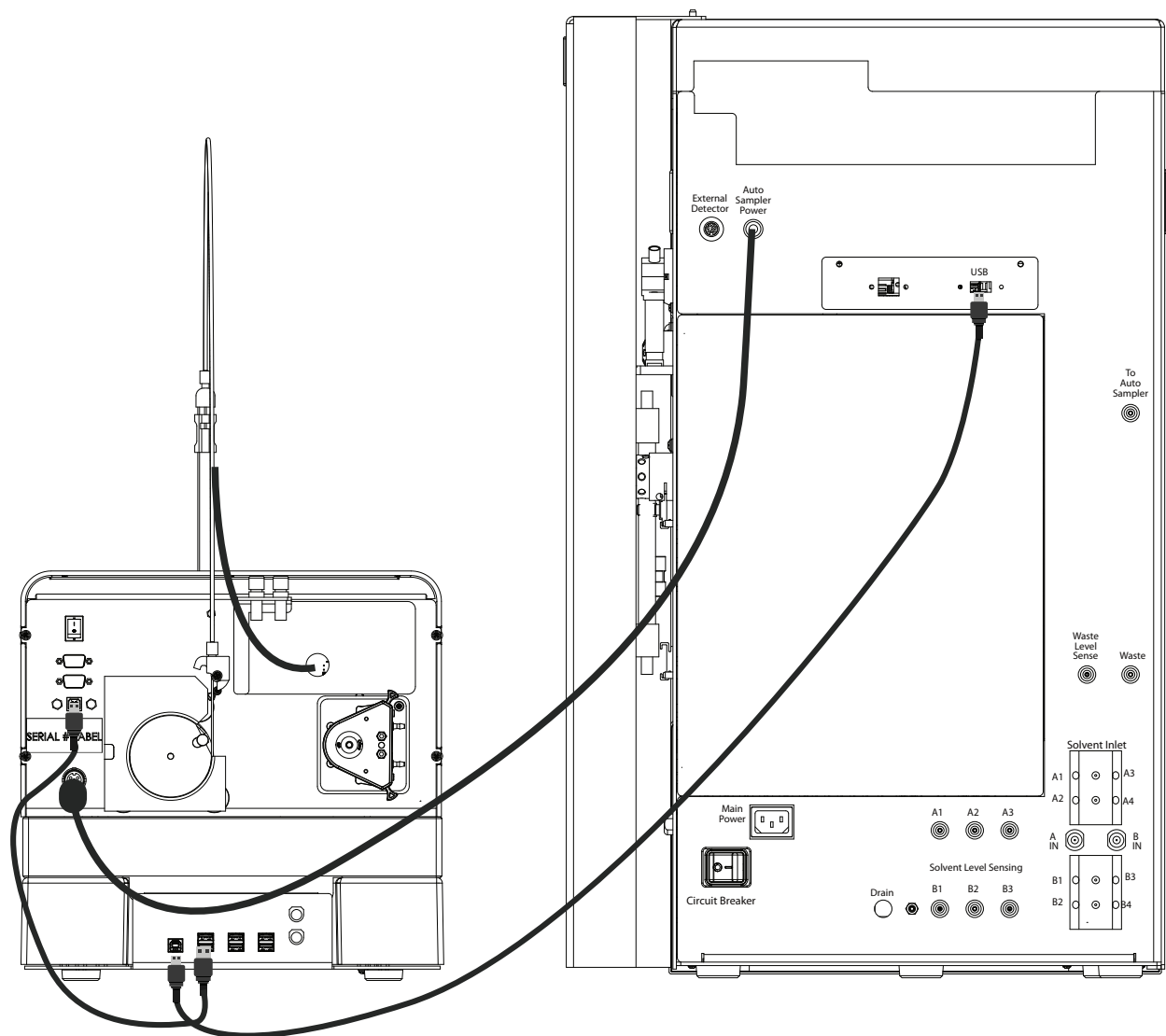


Figure 2-23 Diagram of the AutoSampler and ACCQPrep USB connections (See Appendix A, Figure A-2 for a larger diagram)

13. Connect the long USB cable (provided) from the USB-A port on the back of the ACCQPrep to the USB-B port on the lower portion of the back panel on the AutoSampler Module (Figure 2-23). See Appendix A, Figure A-2 for a larger diagram.
14. The short USB cable connecting the USB-B port on the top portion of the back panel of the Autosampler Module to the USB-A port on the lower portion of the back panel of the Autosampler Module should already be pre-installed. If not, make connection as seen in Figure 2-23.

✓ Note

The power supply accompanying the AutoSampler will not be used, the AutoSampler will receive its power supply through the ACCQPrep.

15. Power for the AutoSampler is provided by the ACCQPrep. On the rear panel of the ACCQPrep, the power cord is coiled and retained (Figure 2-24). Remove the retainer and route the power cable to the mating connector on the AutoSampler Module.

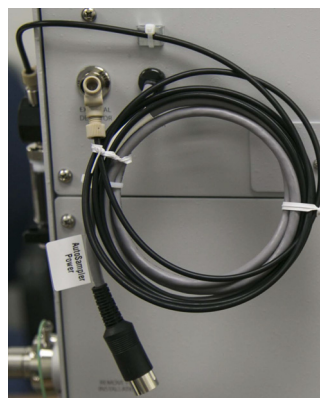


Figure 2-24 Power cable on the ACCQPrep

2.4.1 Establishing the Fluid Connections

To establish the necessary fluid connections:

1. Connect the sample probe fitting to the inlet port #2 of the injection valve on the ACCQPrep (Figure 2-25).

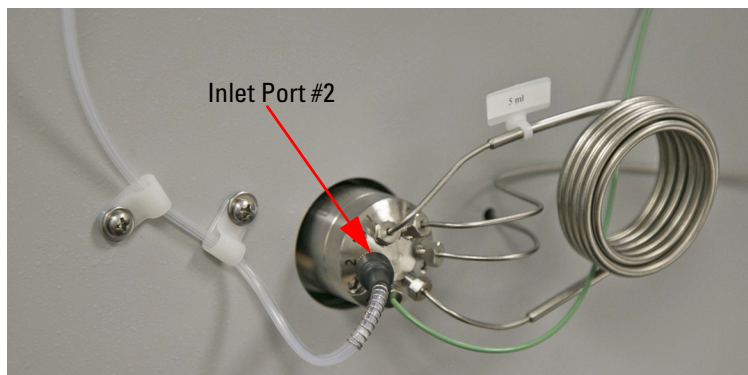


Figure 2-25 Probe fitting connected to inlet port #2

2. Secure the probe tubing to the side of the ACCQPrep by removing the housing screw, fitting the tubing bracket to the screw, and replacing the screw (Figure 2-25).
3. The tubing bracket should be affixed to the ACCQPrep so that the probe tubing threads vertically through the bracket. This ensures that the probe tubing will not be obstructed by the other end of the probe attachment (Figure 2-26).

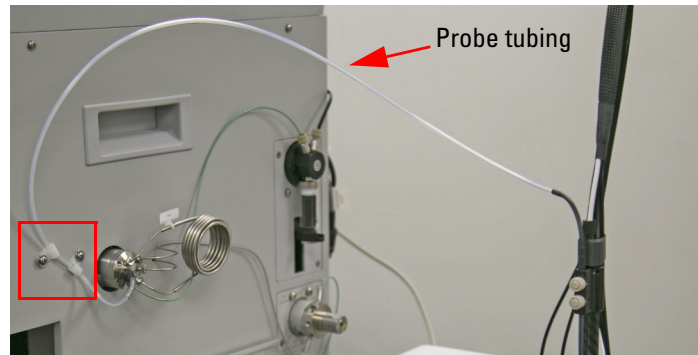


Figure 2-26 Probe tubing affixed to the side of the ACCQPrep

4. Install the included 18 mm test tube in the receptacle at the right rear corner of the AutoSampler platform just to the left of the wash well (Figure 2-27). Fluid in this tube will be used for bracketed solvent injections. In this technique, the injected sample is separated from the chromatographic solvents by a small amount of air and a “bracket” solvent during the sample loading and injection process.

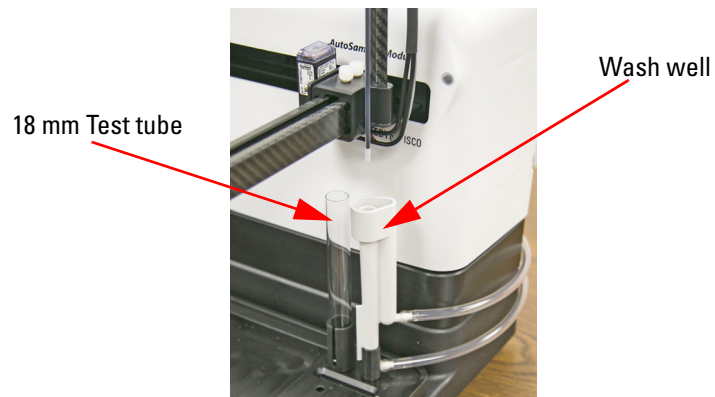


Figure 2-27 Location of the test tube and the wash well on the AutoSampler

To Power on the System

The AutoSampler gets its power from the ACCQPrep.

1. Turn ON the power switch on the AutoSampler (Figure 2-28).



Figure 2-28 On switch for the AutoSampler

2. Turn ON the power to the ACCQPrep. A red light on the AutoSampler will light up, the arm will move to the HOME position, and then the light will turn blue (Figure 2-29).

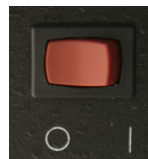


Figure 2-29 Power switch for the ACCQPrep

Prime AutoSampler Wash Station

1. Ensure the wash station supply line is placed into a suitable solvent (typically the strong chromatographic solvent) and the waste line is routed to a suitable container.
2. Go to TOOLS | AUTOMATION MANUAL CONTROL, and select the START WASH button (Figure 2-30).

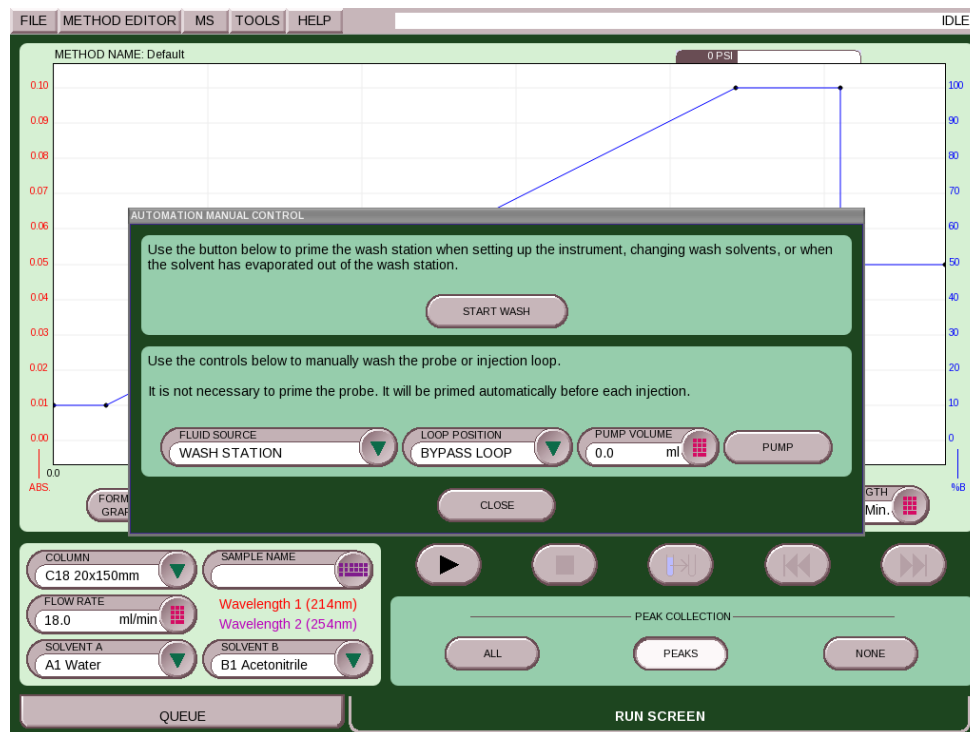


Figure 2-30 Screen of Start Wash button

3. Ensure the wash solvent is pumped into the center portion of the wash station and when full, the fluid drains into the overflow drain (Figure 2-31).

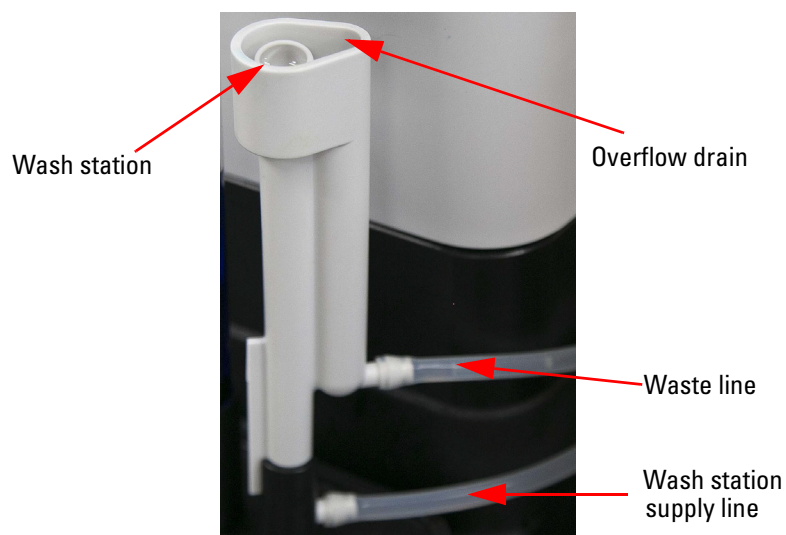


Figure 2-31 Wash station and overflow drain

2.5 Column Installation

The ACCQPrep is compatible with HPLC columns ranging from 4.6 mm up to 50 mm diameter using typical 10-32 HPLC style fittings. Columns with different fittings may be used with adapters available from HPLC fitting manufacturers. Column lengths of up to 250 mm can be mounted to the instrument using the included hardware. The high pressure capability of the ACCQPrep may enable the use of longer columns than typical preparative HPLC systems, but these may be difficult to mount with the included hardware.

Larger diameter columns can also be used on the system at below optimal flow rates and will require the column to be supported outside of the instrument. Column packing materials of 5 or 10 μm work well on the ACCQPrep due to its high pressure capability, but any packing can be used if compatible with the flows and pressures supported by the ACCQPrep.

2.5.1 Column mounting

The ACCQPrep includes hardware to support 4.6 mm, 10 mm, 20 mm (or 21mm), 30 mm, or 50 mm column diameters. 40 mm columns can be mounted on the system using a “universal” column mount (P/N 60-5234-636 not included) (Figure 2-32).

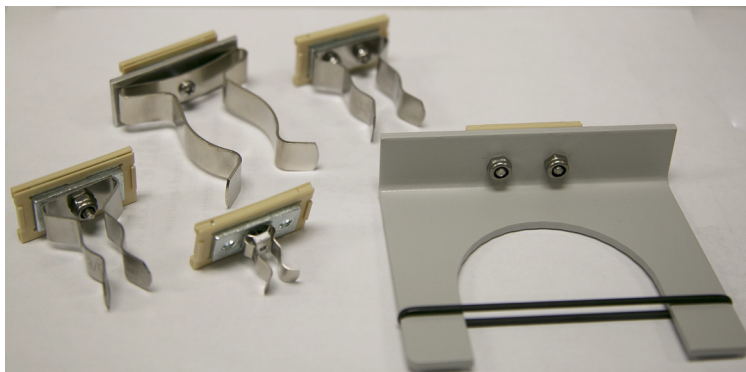


Figure 2-32 Column Mounting Parts

To install a column onto the ACCQPrep:

1. Select the proper sized column mount and simply slip the mount into one of the two column mounting tracks on the side of the ACCQPrep (Figure 2-33).



Figure 2-33 Column track on the ACCQPrep

2. Remove the union connecting the column fluid supply line to the column effluent line.
3. Connect the line from the injection valve to the inlet of the column and connect the remaining tubing to the column outlet (Figure 2-34).



Figure 2-34 Column with inlet and outlet lines connected

Note

Metal tubing and fittings for the column connection are recommended. The high pressure capability of the ACCQPrep could cause a rupture if polymer tubing or fittings are used and a blockage occurs. If you would like to use polymer tubing and fittings, we recommend the column be configured with a lower pressure limit in the PeakTrak[®] software.

If a guard column is desired, this can be connected between the injection valve and the column inlet. This may require an additional column mount and tubing which is not included. A kit containing a complete set of column mounts can be obtained (P/N 60-5234-790). In addition an assortment of tubing lengths and nuts can be obtained (P/N 60-5239-025).

2.5.2 Column Configuration

The ACCQPrep software supports default methods for individual columns. This allows easy selection of default methods with a single key touch. This process is described in section 3.4.1.

2.5.3 Column Select Valve (Optional)

The ACCQPrep's efficiency can be enhanced by the addition of the Column Select Valve Module (CSVm). This optional accessory supports up to four columns on the system that can be selected for a separation from a simple drop down menu. When coupled with the AutoSampler, every programmed sample can utilize the column best suited for each sample. This assumes the fluids used for each column are miscible and small amounts are compatible in sequentially used columns.

 **Note**

Mixed reverse phase and normal phase solvents may require manual flushing of the pumping system between separations.

- If the CSVM was ordered with the instrument, it will arrive installed in the ACCQPrep.
- If you decide to add this capability at a later time, this can be easily accomplished in a manner of minutes following the instructions included with the valve upgrade (PN 68-5237-102).

Columns are mounted onto the CSVM in the same manner as a system without the CSVM (Section 2.4.1). The CSVM comes with additional column mounts and an assortment of precut tubing and fittings to support the additional columns. When the columns are connected, the inlet and outlet of the columns are connected to the column select valve instead of directly to the injection valve. The columns are identified on the CSVM as 1 through 4 and can be assigned a meaningful name in the PeakTrak software (such as 20x150 Hi pH).

If you would like to flush the internal tubing of the ACCQPrep between solvents (such as when changing from a low pH to a high pH), one of the column positions (such as position 4) can be connected with a length of tubing. For ease of use, the column defined for the position could be called “Flush”. When changing from a low pH to a high pH solvent, a line can be placed into the AutoSampler queue to use a method with neutral pH solvent to flush the fluid lines. This assumes that the system has the CSVM option installed.

2.6 Installation Troubleshooting

ACCQPrep won't turn on using the front panel power switch -

- Ensure power cord is connected.
- Verify that rear panel circuit breaker is in the “1” position. (The breaker looks like a white rocker switch on the rear panel near the cord inlet.) Switch breaker to off and then back on to ensure the circuit breaker is reset.
- Ensure the lab power connection has power.

Error: “Unable to communicate with pump controller module” -

- Check the pump communication connector at the top, left corner and the pump power connector at the top right corner of the pump subassembly. Ensure the connectors are oriented so that the locking tab is at the top.

AutoSampler control not visible on the ACCQPrep software – The ACCQPrep only loads the AutoSampler control features if it can detect the presence of the AutoSampler. Check the AutoSampler pilot light (upper right corner of AutoSampler) to determine the next step.

- If the light is off:
 - Verify that the AutoSampler power switch is on. This is located on the rear panel of the AutoSampler.
 - Check that the AutoSampler power cord connects from the ACCQPrep to the power connection on the rear panel of the AutoSampler.
- If the light is blue the AutoSampler is functioning but not communicating with the ACCQPrep:
 - Check the USB cable connection from ACCQPrep to the square USB connector along the rear, bottom edge of the AutoSampler (Figure 2-22).
 - Check the USB cable connection from the lower, rear panel of the AutoSampler to the USB connector on the upper left corner of the rear panel (while viewing the AutoSampler from the rear panel).
 - Recycle the power on the ACCQPrep. The PeakTrak software only checks for the presence of the AutoSampler on power up. If the AutoSampler is powered up after the ACCQPrep, the software will not detect the presence of the AutoSampler.
- If the light is red the AutoSampler is not able to reach the home position:
 - The sampler arm is not able to move to the left rear corner to home. Verify that there is nothing blocking its motion.
 - Inspect the flag on the rear of the probe mechanism to ensure the flag is perpendicular to the rear panel. It must enter into a receptacle to reach the home position. Its mounting screw may be tightened, but too much torque could break the plastic screw.
 - The sample probe is unable to reach the top of its travel. Verify that there is nothing blocking its motion.

Table 2-1 Installation Qualification Checklist

| Step | Section | Description | Installer Initials | Operator Initials |
|--|---------|--|--------------------|-------------------|
| 1 | | Unpacking the unit | | |
| 2 | | Instrument location | | |
| 3 | | Connect power | | |
| 4 | | Install AutoSampler (if applicable) | | |
| 5 | | Set up solvent lines | | |
| 6 | | Set up waste lines | | |
| 7 | | ELSD/Purlon connections and gas supply (if applicable) | | |
| 8 | | Connect and route drain lines | | |
| 9 | | Position the system | | |
| 10 | | Installation of the collection tube racks | | |
| 11 | | External detector (optional) | | |
| 12 | | Turn on the power | | |
| 13 | | Configure the system | | |
| 14 | | Prime the solvent lines | | |
| 15 | | Prime AutoSampler wash station (if installed) | | |
| 16 | | System verification | | |
| Certification of Section 2 Completion | | | | |
| Installer Name (print): | | | | |
| Installer Signature: | | | | |
| Date: | | | | |
| Operator Name (print): | | | | |
| Operator Signature: | | | | |
| Date: | | | | |
| Comments: | | | | |
| | | | | |
| | | | | |
| | | | | |

ACCQPrep HP125

Section 3 Configuration

3.1 Configuration of the ACCQPrep

Instrument Configuration

Instrument Configuration can be found under:

TOOLS > CONFIGURATION > INSTRUMENT CONFIGURATION

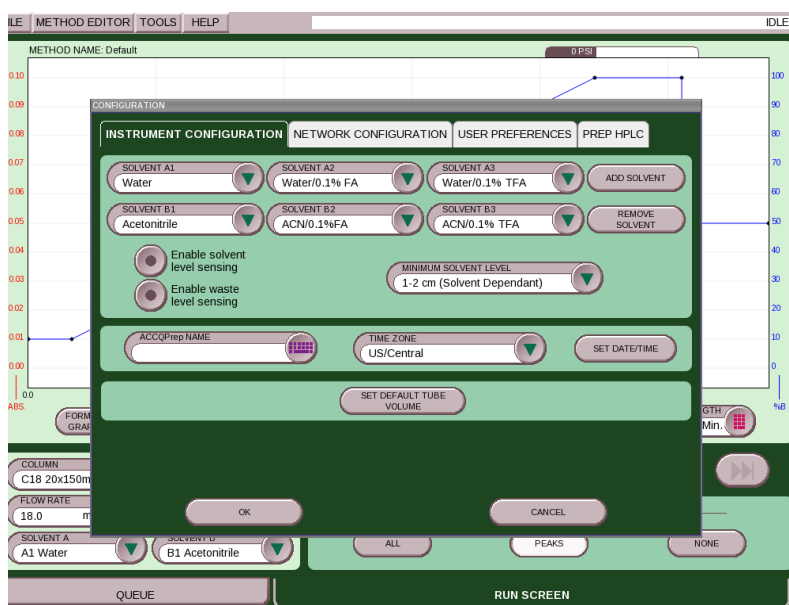


Figure 3-1 Instrument configuration screen

From this menu you can select the solvents for both solvent A and B (Figure 3-1). This screen will appear when the Solvent Selector Valve Module is present. If it is absent the solvents are selected on the MAIN RUN screen and the drop downs are not shown in the CONFIGURATION screen. The CONFIGURATION screen 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, click on ADD SOLVENT and type in the solvent name. There is also an option to remove solvent names you no longer use.

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.

Naming the System – Under the ACCQPREP NAME option, you can name the system so when reports print you know what system was used.

Setting Time Zones and Date/Time – The time zone will automatically change the time during daylight savings time. Set the Date/Time to your local day and current time since all separations are tagged with the date and time of operation. If the sample is not named previously, the date and time will be used as its identifier.

Set Default Tube Volume – This 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 will then become the default used for new methods, but can be changed within the method editor.

3.2 Network Configuration

Network Configuration can be found under:

TOOLS > CONFIGURATION > NETWORK CONFIGURATION and is used if you want to connect the system to a network. By doing so, you can remotely view the user interface for monitoring or controlling the system, print to a network printer, or automatically save data files right to the network.

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

3.3 User Preferences

The following options are found on the USER PREFERENCES tab and can be found under: TOOLS > CONFIGURATION > USER PREFERENCES

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

Automatically Print Report – You can print 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, it is recommended that this is not selected. This information is always stored with the run data for later review, so if a problem is suspected, this parameter can be enabled 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 many times, the profile of the trace is adequate for troubleshooting.

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

Pressure Units – You can choose between PSI and Bar.

Enable Automatic Peak Hold – Allows the system to go into an isocratic condition whenever a peak is detected to get the current compound off while not rushing the other compounds off the column. The length of the separation is increased by the amount of time the system is in the isocratic hold condition. At the end of the detected peak, the system will automatically exit the hold condition.

 **Note**

If a Purlon mass spectrometer detector is attached, you can set the DEFAULT MS GRAPH settings. The X-AXIS START control denotes the starting mass displayed in the mass spectrum graph. The X-AXIS END control denotes the ending mass displayed in the mass spectrum graph. In either case, the entire mass spectrum (10 Da through 1200 (2000 Da PURION L only)) is always acquired and stored and can be viewed later opening the format graph button on the results screen.

3.4 Prep HPLC

Prep HPLC can be found under: TOOLS > CONFIGURATION > PREP HPLC

Loop Volume – Ensures that the samples entered do not exceed the loop volume amount per injection. A warning will display if half of the loop volume is exceeded, but it will not limit the injection amount that can be programmed. 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 which columns 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 – This defaults to the standard, “Teflon” probe supplied with the instrument. It allows selection of an optional “Steel” needle available for piercing vials with an aluminum foil cover to prevent evaporation. This optional probe is NOT able to pierce a silicone septa. The steel probe has a slightly different internal volume that requires a different compensation volume.

Bracketed Sample – This allows the sample injection to be “bracketed” with air and a user supplied solvent to address potential solubility issues when injected. If this button is activated, 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 4.3.5.

3.4.1 Define a Column

To define a column:

1. Press the NEW button. This will open a keyboard where you can enter the name of the column.
2. Once the name is complete, press ENTER.
3. To complete the column definition, enter the media size and column dimensions.
4. Select the inside column diameter. A flow rate for a default method will be 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 will provide the suggested default method with ~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 can be changed for each column to a suitable pressure not to exceed the system pressure limit of 6000 psi.
7. Press SAVE, to save the default method associated with the column.

To customize the just created method, press DEFINE METHODS followed by EDIT to view and modify all the method parameters. Further information can be found in Section 5.2.2.

3.4.2 Delete a Column

To delete a column:

1. Ensure the column to be deleted is not selected in the column position field(s).
2. Press the column drop down and select the column you would like to delete.
3. Press the DELETE button.
4. In some cases you may get an error message stating the method is currently in use. To address this, leave the CONFIGURATION screen and go to the RUN screen and select a different column on the RUN screen.

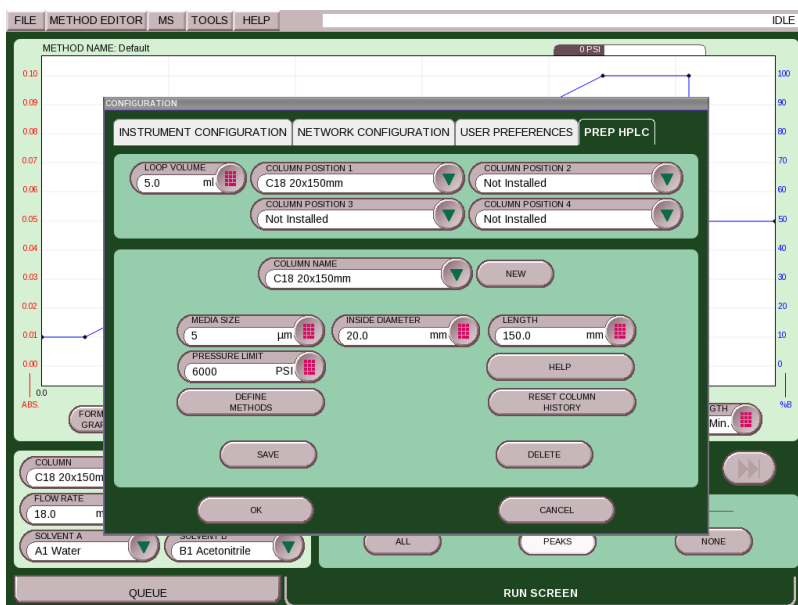


Figure 3-2 Prep HPLC screen

3.4.3 Creating Other Methods

Methods created in this screen 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 will be shown in the column selection drop down menus in the order the methods were created.

To create the new method:

1. Select the column the new method will be associated with.
2. Press the DEFINE METHODS button (Figure 3-3).

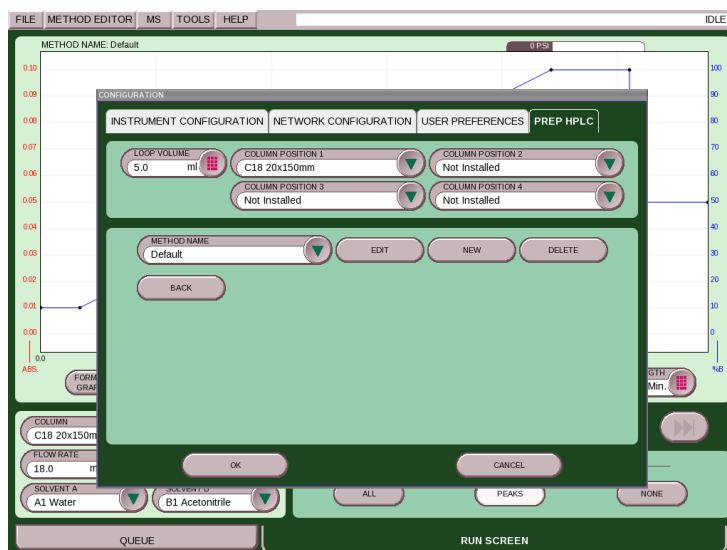


Figure 3-3 Define Methods screen

3. Press NEW.
4. Enter in the name you have chosen.
5. Once named, press ENTER,
6. The newly created method uses the default method parameters. To modify the new method:
 - a. Select EDIT.
 - b. Make changes as desired in the method editing screen.
 - c. Exit when completed.

3.4.4 Creating Focused Gradients

Focused gradients are a convenient way of minimizing method development. Typically, a universal gradient separation can be performed on an analytical HPLC system. Based on the retention time of the analytical separation, a method with an ideal narrow range gradient (such as 20-30%B) can be selected for optimal separation of a target peak.

When creating a set of focused methods, keep in mind that the methods will be displayed in the drop down in the order created. For example, if you want methods for “10-30%B” and “20-40%B”, etc. you may want to create them in sequential order. To create other methods see Section **3.4.3**.

ACCQPrep HP125

Section 4 Operation

4.1 Introduction

The ACCQPrep HP125 is unique in the Preparative HPLC market. There are several methods of operation possible that can be selected 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 ACCQPrep is that a defined method file is not required for operation. A default or existing method can be loaded and user modified for the current separation without the need for saving 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.

4.2 Method Selection

Several options can be used to select a method:

- Use a pre-programmed column that has several methods associated with it. Once the column is selected, you can simply 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 by going to FILE > OPEN and sort for the method file that fits your needs.
 - Use the file dialogue to open the run file from a previous separation and select the EXTRACT button on the lower portion of the screen.

If the method selected is adequate, simply press the PLAY button 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. Once edited, the method can be used directly for the immediate separation(s) or saved with a name for future reuse.

4.3 Starting a Separation

To separate a single sample, press PLAY to start a separation. The MINIMUM RUN REQUIREMENTS screen is displayed. This allows you to select an injection technique and select where the fractions will be collected (the starting rack and tube position). In addition, the system projects the amount of solvent required and the separation duration. To proceed from this screen, the START EQUILIBRATION button is selected.

For an ELSD

If an ELSD is installed on the system, this screen has the option of using the ELSD or disabling the ELSD. ELSD method parameters are typically based on the solvents used and therefore are somewhat universal when doing preparative separations of non-volatile 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 based on the names assigned to the solvents.

| |
|---|
| <input checked="" type="checkbox"/> Note |
|---|

The METHOD EDITOR should be used to change the ELSD parameters.

For a PurIon

If a PurIon mass spectrometer is connected, the RUN REQUIREMENTS screen 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. Due to the high concentrations of sample in a preparative separation this is adequate for most samples with good ionization.) Further information can be found in Section 5.2.11.

If No AutoInjector or AutoSampler are Connected

If you do not have an AutoInjector or AutoSampler, the ACCQPrep will give 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. Press START EQUILIBRATION, and walk away.
- **LOAD AFTER EQUILIBRATION**- Select this if you want to give your column time to equilibrate while you finish preparing your sample. Once the equilibration is complete, the system will pause and prompt you to inject your sample.

The next section will cover how to start a separation when the additional modules are set up.

4.3.1 Separation with an AutoInjector

To use the AutoInjector Module:

1. Place the sample probe into your sample container.
2. Press PLAY to start a separation as you would for an individual separation.

3. If you want to automatically perform multiple injections (or perform a single injection using the AutoInjector Module), select the AUTO INJECTOR drop down as shown in Figure 4-1. This is the default selection if an AutoInjector Module is present. If an Auto Sampler Module is present, you will select the AUTO SAMPLER dropdown.

Note

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

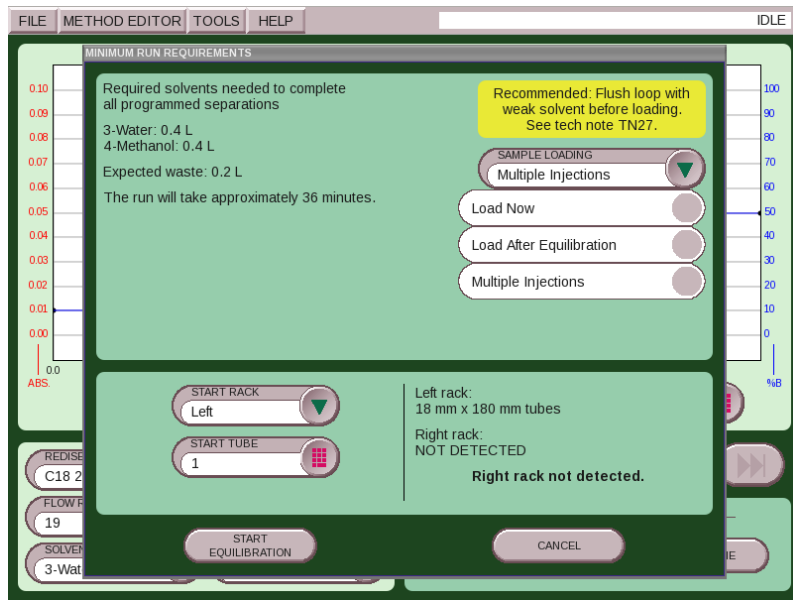


Figure 4-1 Selecting for multiple injections

4. In the dialogue box, enter the total sample volume and the number of injections (Figure 4-2).

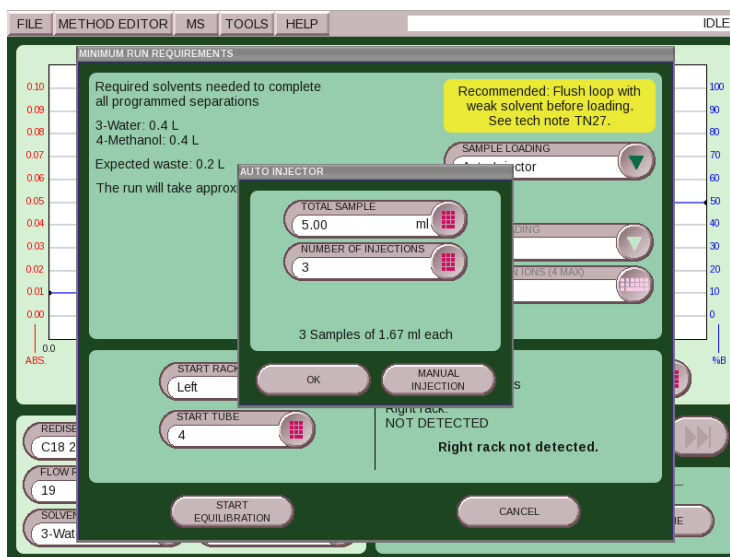


Figure 4-2 Selecting the sample volume and number of injections

PeakTrak will divide the total volume by the number of injections to determine the injection volume for each separation. If this volume exceeds 50% of the loop volume, a warning message will appear recommending a change in the parameters. This message can be ignored at your discretion. PeakTrak will perform exactly as programmed. It will also compensate for the volume of tubing used for the supplied sample probe. If the sample probe is ever lost or damaged, it should be replaced by the AutoInjector Needle Assembly (PN 60-5234-657).

During the programmed separation sequence, any changes made to the method will be 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. Please be aware that changes of flow rate will 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. At the completion of the sequence, the final separation results will be displayed. To easily view previous separations, press the REWIND key to return to the HOME screen.
7. Select FILE | OPEN to view previous separations.

If a sample had multiple injections, the run sequence will be 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 wish 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.

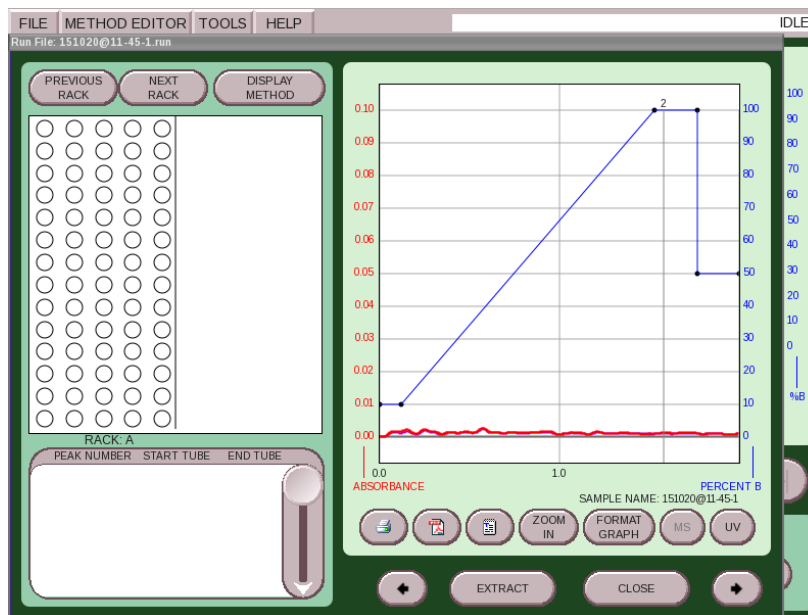


Figure 4-3 Viewing injections

4.3.2 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 above (from Step 2). During this process, the AutoInjector Module continues to aspirate the programmed injection volume.
7. 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, it is recommended that users program the injection sequence with about 0.5 mL more sample volume than the actual sample amount provided in the sample container. This will ensure all of your sample is processed.
 - If some sample remains in the sample probe (probe volume is approximately .43 mL), it can be recovered 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 is detailed as follows:

1. The system will prompt 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 will prompt 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 it cannot interfere with future separations.

4.3.3 Separation using an AutoSampler Module

Verify that the wash station contains fluid. If not, go to TOOLS | AUTOMATION MANUAL CONTROL and select the START WASH button. Once the wash station is primed, select the STOP WASH button. Ensure 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 ~100µl 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 simply press the PLAY button to start with a single sample. Accessing the RUN tab will result in a screen and operation much like the standard ACCQPrep (without an installed AutoSampler Module). Pressing PLAY will display the RUN REQUIREMENTS screen. On this screen, 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 will be created automatically. Selecting the QUEUE tab will allow 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.

4.3.4 Using the Queue Tab

This section outlines the operating procedures for the AutoSampler Module.

1. Install a sample vial rack before opening the QUEUE tab. This will allow the software to limit sample programming to the positions available for sample vials.
2. Open the QUEUE tab.

The following is an explanation of the available columns on this screen:

= - Allows you to start the separation from the Queue tab, to remove a single row of an existing queue, clear all rows of the queue, or enable or disable a scouting pause. The scouting pause halts the system after the first separation is completed for the sample. This allows you to adjust the separation parameters based on the results of the initial separation. This is especially useful to allow adjustment of the quantity of sample injected for each separation to optimize the loading on the column. After the initial pause, you can modify the method as needed and disable the pause for the remaining injections, or leave it enabled to allow a second condition scouting separation.

Sample Name – Allows you to name each sample. Naming is optional. If left blank, the sample is named based on the date and time the separation was started.

Column (drop down menu) – If a Column Selector Valve Module (CSV-4) is installed, this menu allows you to select a separation column and associated method for use. If a CSV-4 is not installed, this menu allows you to select different methods that may be associated with the configured and installed separation column.

Method – Once 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, touch the method name and a list of methods associated with the column will appear. These methods will be listed in the order they were created. Alternatively, select CUSTOMIZE CURRENT METHOD or BROWSE to populate this field.

Select CUSTOMIZE CURRENT METHOD to create a modified version of the method for use in the current queue. To use this modified method, exit the method editor and select SAVE when prompted. A new method named “Temporary 1” will be created. This method will be used for all injections of the current sample and will be discarded after the sample is complete. All method parameters are saved with each separation. If you would like to save the modified method for later use, select SAVE AS before you exit the method editor and create a unique name for the new method.

Sample Position – This field indicates where the sample for this separation is located. 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 – Enter 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 – Allows you to optimize rack usage by sharing racks for multiple samples or optimizing work flow by placing each sample’s fraction into individual racks to support multiple users.

3. Once the queue is complete, you can select the RUN tab and press the PLAY button or select the “=” menu on the first unfinished run and select the START RUN option. In either case, the RUN REQUIREMENTS screen will be shown. This allows you to accept or modify the starting rack or tube for fraction collection.
4. While a separation is in process, the QUEUE screen can be accessed to add samples to the queue. Samples that have been completed cannot be edited. They can be viewed, how-

ever, by touching the sample name corresponding to that run.

5. During the separation, completed fraction racks can be removed when full and replaced with empty racks to allow continuous separations. If a single rack containing fractions is removed and then replaced into the rack position, PeakTrak will display a prompt to determine if the rack contains empty tubes. If you respond that the tubes are empty, PeakTrak will consider this rack available for future separations. Otherwise, PeakTrak will continue 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.

 **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 are displayed at the top and bottom of the screen which allow access to hidden rows.

6. Once the entire queue has completed, the RESULT screen is displayed with the last separation. To easily view previous separations, select the separation on the QUEUE screen or select FILE | OPEN to view previous separations.

If a sample had multiple injections, the run sequence will be displayed within the FILE | OPEN dialogue 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.

4.3.5 AutoSampler Injection Techniques

This section describes operating protocols for the AutoSampler Module.

 **Note**

The below sequence of operation is valid for software versions starting with 4.1.13.

The AutoSampler supports 2 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. Therefore, 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. Once 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 will begin.
14. The next injection of the sample is accomplished by repeating the above process.

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

1. The inject valve will be 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 will flush 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 will use 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 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 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. Therefore, 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. Once 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 ~.05 mL of air to ensure all off 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 will begin.
17. The next injection of the same sample is accomplished by repeating the above process.

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

1. The inject valve will be 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 will flush 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 will use 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.

4.3.6 Fraction Collection with the AutoSampler

The fraction collection racks in the AutoSampler double the fraction collection ability of the ACCQPrep. They are treated as an extension of the racks present in the ACCQPrep. In general, if racks are placed in both the ACCQPrep and AutoSampler, available racks will used in the following order: ACCQPrep left rack (A), ACCQPrep right rack (B), AutoSampler left rack (C), AutoSampler right rack (D). 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 mentioned above.

When the system moves from the ACCQPrep 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 ACCQPrep). This may leave a few tubes unused in the previous rack. If the system is mid peak or the separation is using 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 and then the system advances to the next rack.

4.4 Operation Troubleshooting

Injection Valve Leak- refer to on rotor replacement if needed – (See Section 6.5.1 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 inject pump 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.

Un expected 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, 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 dummy injection and watch injector pump to determine if fluid is being aspirated. Disconnect tubing at different points and place into fluid to determine location of problem.
 - If loose fitting, tighten or replace if needed.
 - If plugged, replace part or connect to a high pressure pump to dislodge 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, check valves, confirm solvent lines in correct bottles, look for leaks.
- Solvent gradient method too weak to elute 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 could be trapped in the detection gap due to immiscible solvents. When changing from normal to reverse phase, flush system with an intermediate solvent miscible with both phases such as isopropanol, or flush 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 will have a corresponding error message.

Peaks elute too early –

- Solvent A not being delivered properly. Check solvent level, inspect or clean pump A check valves, confirm solvent lines in correct solvents, look for leaks of air into the lines.
- Sample injected in a strong solvent that carries the sample down the column.
- Column 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.

Peaks elute too late –

- Solvent B not being delivered properly. Check solvent level, inspect or clean pump B check valves, confirm solvent lines in 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 still unsuccessful, open the front cover and tap on the heads of the problem pump.
- Column not suitable for the compounds separated.
- Incorrect method.

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/bacterial growth, or ignore the peak.

Drifting baselines –

- Mobile phase absorbance which may be due to 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 5.2.7 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 5.2.7 before adjusting the ELSD operating temperatures.
 - Verify 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 (see section 2.2.4)
 - Increase ELSD sensitivity and gain in the method editor to see if the peaks are simply too small to be easily seen.
 - Flow splitter tube blocked. This .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 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 5.2.7 before adjusting the ELSD operating temperatures.
- Check P-trap (see discussion on “No Peaks Detected”).
- UV and ELSD signals aren’t aligned in time
 - The peak widths or shapes of the 2 signals may vary due to varying sensitivity of the 2 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 .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 will cause arcing resulting 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. Check 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, 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 particle 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 1 solvent shows ½ 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 in 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 due to particulates in the flow stream or 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 finger nail and replaced (PN: 209009917). Inspect the face of the stator for scratches. In rare cases the stator must also be replaced (PN: 209009919).

ACCQPrep HP125

Section 5 PeakTrak

5.1 Overview

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

5.1.1 PeakTrak Window Elements

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

Menu – The topmost item in the window is the PEAKTRAK MENU from which you can access all of PeakTrak's features.

System Status – PeakTrak displays the system status 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 – This area is where you can view or modify method file settings. Frequently used method settings are displayed on the MAIN window ADVANCED METHOD settings are displayed on the METHOD EDITOR window.

Many of the PEAKTRAK WINDOWS contain these main elements, or a subset of them.

Note

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

5.1.2 Method File

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 powered up, it has a default method in the system associated with the column selected as a default in the CONFIGURATION screen. In a single column system this should reflect the column mounted on the system. This default method exists on the system hard drive and a copy of the method is automatically loaded into a temporary use area. If the default method meets your needs, simply press play to start a separation. On the other hand, if you want to customize the method for use on this separation only, simply edit the method (editing 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 don't save the method, but later want to reuse the method, simply open the data file that used the method and extract the method for reuse. The system uses the method file in the temporary area to direct the system operation after you click the PLAY button.

PeakTrak categorizes the method settings as Basic or Advanced. Basic settings are the frequently used controls accessed through the MAIN window. 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, use the FILE > OPEN menu command. Method files can be transferred to the system from an external storage device using the FILE > IMPORT METHOD menu command.

Method files use an *.pmtd filename extension.

Default Methods

When PeakTrak is started, the system loads a DEFAULT METHOD. A DEFAULT METHOD contains Teledyne Isco's recommended basic and advanced settings for the use of a default column (as selected in the config screen). There can be a DEFAULT METHOD for each size and type of column defined in the config 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 DEFAULT METHODS can be changed to meet your specific requirements.

5.1.3 Run File

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, use the FILE > OPEN menu command. Run files use an *.run filename extension.

5.2 PeakTrak Menu Options

PeakTrak menu options include:

- File
- Method File
- MS
- Tools
- Help

5.2.1 File

The File menu lists the following commands:

New – Select the NEW command to open a new method file. PeakTrak will open the MAIN window using the default program settings for the default configured column size.

Open – Choose this command to open a METHOD FILE (.pmt) or a RUN FILE (.run) stored on the system's internal hard drive.

Manage Files – Choose this command to open 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 the Copy or Move files to flash drive option.

- To remove a file from the internal hard drive, highlight the file or enter the filename and click the DELETE button.
- To remove multiple files, you can drag to highlight multiple files, or click the DELETE BY AGE button. Then specify the age limit in months and click 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 – This opens the SET DATA PATH window. Use this window to specify the default storage folders for users. These folders contain multiple METHOD FILE and RUN FILE resources created by system users.

Save Method – Choose this menu command to save the settings of the current method file to the system's internal storage if the file is already named. If not already named, you will be prompted to give the method a name and it will be saved.

 **Note**

DEFAULT METHODS are an exception and cannot be overwritten by the SAVE METHOD command. 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 – From this window you can rename the current method and save it on the system's internal hard drive.

Save Run As PDF – Choose this command to save the displayed run on a remote computer's hard drive or a USB Flash storage device connected to the system. When selecting this menu command from the touch screen, the system saves the file on the USB storage device. The default file name will be the same as the RUN file, except with a "PDF" file name extension. PeakTrak displays a file

download window so you can select a location and enter a file name. (You can open and print PDF files with Adobe Reader.)

Save Run As TXT – Choose this command to save the displayed run on a remote computer’s hard drive or a USB Flash storage device connected to the system. When selecting this menu command from the touch screen, the system saves the file on the USB drive. The file name will be the same as the RUN FILE, except with a “TXT” file name extension. PeakTrak displays a file download window so you can select a location and enter a file name. The “TXT” file is actually XML. These files can be opened, with text editing or word processing software. These can also be imported into a spreadsheet for data manipulation.

Import Method – Choose this command to load a METHOD FILE from an external source onto the system’s internal storage. From the touch screen, this command opens the FILES window so you can locate and select the file on a USB Flash storage device. When selecting this command from a remotely connected computer, PeakTrak displays a window so you can select the method file. After selecting the file, click the OPEN button to save the METHOD FILE on the system’s internal hard drive.

Export Method – Choose this command to save a METHOD FILE in a location other than the system’s internal hard drive. This can be done to archive METHOD FILES or to transfer the method to another system using the menu IMPORT METHOD command. From the touch screen, this command opens the FILES window so you can save the file on a USB Flash storage device. When selecting this command from a remote computer, PeakTrak displays a window from which you can save the METHOD FILE.

Print – From the touch screen, this command prints the completed run on the network printer. When selecting this command 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, click the PRINT button to print the completed run.

Logout – Choose this command to logout of the system. After logging out, the system displays a login screen and waits for the next user to log in.

5.2.2 Method Editor

Selecting the METHOD EDITOR menu command will open the METHOD EDITOR window. Use this window to view and modify the basic and advanced METHOD FILE settings.

5.2.3 MS

The MS menu item is only available when a PurIon Mass Spectrometer is added to the ACCQPrep system. The following options are available:

Method Development – Choose this command to open the MS METHOD DEVELOPMENT window to verify ionization conditions for the compounds to be purified. This command is not available from a remotely connected computer via a web browser.

Ion Finder – Enables you to find fragments and adducts based on the molecular weight of their desired compound.

- DESIRED MASS- enter the molecular weight of your compound, rounded to the nearest integer.
- DETECTED IONS- list of ions to be detected.
- DETECTION IONS - list of possible adducts or fragments based on the value entered in the desired mass control and detected by the system. Checking one of these values adds it to the DETECTED IONS list.

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

Ionization settings – This command opens the IONIZATION SETTINGS window to allow creation of 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 – This command sets the status of the PurIon mass spectrometer from standby to an operational mode by applying power to the heaters and dynode detector. The nitrogen gas is also supplied to the nebulizer. If the mass spectrometer is already in operate mode, the STANDBY button will display.

Standby – This command is available when the PurIon is in the OPERATE mode. It turns off all high voltages within the PurIon including the dynode detector which prolongs its life. In addition, it sets the inlet capillary heater temperature to 50 °C and the remaining heaters off and lowers the gas flow rate to ~.2 liters/minute. When in standby mode, the operate command is used to return the PurIon to the operational state (high voltages on, heaters on, and gas flow at the proper rate of ~4 liters/min).

Shutdown – This command removes power to the heaters, and removes power from the turbomolecular pump. A window will appear confirming that you want to initiate the shutdown procedure. This command is used to shut down the PurIon mass spectrometer for maintenance or to move the system.

 **WARNING**

Do not shut down or disconnect the vacuum line to the roughing pump until the Purlon is fully vented as indicated by PeakTrak.

5.2.4 Tools

The Tools menu has the following options:

Manual Control – Choose this command to open the MANUAL CONTROL window. From this window you can operate the system manually. Manual control can assist with priming or purging the internal plumbing, and aid system troubleshooting.

Auto Zero – Choose this command to zero the detector trace(s) during a run.

Configuration – Choose this command to open the CONFIGURATION window. From this window you specify the solvents used with the system, set default volumes for collection tubes, set the system date and time, configure network settings, etc.

Set Administrator Password – Choose this command to open the SET ADMINISTRATOR PASSWORD 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 protect unauthorized modifications to:

- User management
- System date and time
- System password

Before any of the above can be accessed, you must enter a password. Password protection can be disabled so all users can modify the entry.

User Management – Choose this command to open the USER MANAGEMENT window. You can use this window to add or remove users from the system.

- To add a user, click the ADD NEW button and enter the user name in the window that appears. Another window will appear to enter a password for the new user. Enter the password, or leave the text empty if no password is desired. When you add a user a working folder for that user is automatically created. When you log in, their data files will automatically be stored in their user folder. It will be reset when logging out. Use the FILE > SET DATA PATH command to select your folder. To prevent unauthorized changes to user management, this function is password protected.
- To delete a user, click the DELETE button next to the user’s name and confirm the action.
- To change a password, click the CHANGE PASSWORD button to change the password for a user.
- To delete a password, leave the text box empty.

Once users are created, they can be assigned access levels for the system (Figure 5-1).

| 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 5-1 Table of user level access

5.2.5 Help

The HELP menu has the following options:

Service – Qualified service personnel use this command to access the system’s service functions. These service functions are password protected to restrict their use to only trained personnel. This uses the administrator password.

Software Update – Choose this command to open a window used to install patch files to update your ACCQPrep system software.

Export Log Files – During operation, the system writes operating data to a log file. Teledyne Isco service personnel can interpret this data to optimize system performance or to troubleshoot difficulties. Teledyne Isco recommends that you use the menu command only when advised by a qualified service technician.

About PeakTrak – Choose this command to view information about PeakTrak.

About Isco – Choose this command to view contact information for Teledyne Isco.

5.2.6 PeakTrak Windows

This section contains descriptions of the windows used for most PeakTrak tasks. In this section you will find information on the following:

- MAIN WINDOW
- METHOD EDITOR
- GRADIENT OPTIMIZER
- COLUMN DATA
- BATCH FILES
- SET DATA PATH
- MS METHOD DEVELOPMENT
- MS MANUAL IONIZATION SETTINGS
- AUTO PRIME/VALVE WASH
- MANUAL CONTROL
- CONFIGURATION
- SET ADMINISTRATOR PASSWORD
- USER MANAGEMENT

Main Window – The MAIN window gives you control of the frequently used functions before and during the run. After a run, or when viewing previous runs, the MAIN window displays peak collection data as well as the settings used for that run. The MAIN window dynamically changes to display the controls required for the current state of the system. The top region of the MAIN window always displays a GRADIENT PLOT area. The bottom region of the window displays the RUN SETTINGS, RUN CONTROL buttons, and PEAK COLLECTION buttons. 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.

Gradient Plot – The gradient plot area depicts the current gradient that will be used for the run. The X-axis of the plot displays the run time in minutes. 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 clicking the FORMAT GRAPH button and setting the desired values.

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 function.)

The gradient plot area also provides a convenient method to edit the gradient curve. You can click 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 5.3.3 *Defining a Gradient*.

MS – The MS button displays the current mass spectrum on PurIon systems. This spectrum hides the flow rate and solvent selection controls. Pressing 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.

- The LIVE DATA button shows the current spectrum.
- The ADD SPECTRUM button 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 depicted with a triangle pointing down allows a user to cycle 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 – The UV (UV-Vis on ACCQPrep systems with a UV-Vis detector) button displays the UV spectrum on systems. This spectrum hides the flow rate and solvent selection controls. Pressing the UV 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. The LIVE DATA button shows the current spectrum. The ADD SPECTRUM button saves the spectrum at a point for inclusion in a run report screen. Up to 4 spectra can be saved in a run report. The button depicted with a triangle pointing down allows a user to cycle through saved spectra.

Run Settings – •

- **Column** — Select the name of the column used on the system. A second level menu will allow you to select from methods already associated with the selected column. If the system has a Column Selector Valve Module, this control allows you to select the column that will be used for the 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.).

Spaces are not allowed in the name. If you do not type a Sample Name, PeakTrak will generate a date/time stamp for the name when you click the PLAY button to begin the run.

- Flow Rate — Type or select the desired flow rate for the run.
- 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 drop down list. The solvents in the list are those defined by the CONFIGURATION window. If the Solvent Selector Valve Module is installed, this control allows you to select a solvent for use in the current separation.

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

- PLAY — The PLAY button starts or resumes the run. After a run has started, this button is replaced by the Pause button.
- PAUSE — The PAUSE button holds the %B at the current value while the system continues to operate (sometimes called an isocratic hold).

 **Note**

Pausing the run extends the run length. While in the paused state, you can resume the gradient by clicking the PLAY button, or stop the run by clicking the STOP button. If you resume the run, the system continues the gradient curve from the %B when the system was paused.

- STOP — This button suspends the entire run. Unlike the Paused state, the pump, peak detection, and fraction collection will not operate. While stopped, you can abort the run by clicking the REWIND or FAST FORWARD buttons, or resume the run by clicking the clicking the PLAY button. In either case, the data is always saved. Using the FAST FORWARD button 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.
- NEXT TUBE — This button advances the fraction collector to the next tube position. This allows you to conveniently collect eluant of interest in new tube.
- REWIND — This button is active when the system has completed the run, or if the run was Stopped by clicking the STOP button. If you want to terminate the separation prematurely the FAST FORWARD button is recommended. The REWIND button returns you to the MAIN window.

Unlike the REWIND button, the FAST FORWARD button will not modify the current method's run length setting.

- **FAST FORWARD** — Click this button to jump to the next step of a run. Once 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 will enter the sample probe cleaning step. Clicking the FAST FORWARD button will cause 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** — Click this button to collect all eluent in the fraction collection tubes. Detected peaks will advance to the next tube automatically to maximum peak concentration and purity
- **PEAKS** — Click this button to collect only eluted peaks in the fraction collection tubes.
- **NONE** — Click this button to divert all eluent to the waste port. This is sometimes used to divert all peaks except the peak of interest to waste. This action can also be accomplished with the Initial Waste or Time Windows functions.

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. Clicking on a test tube with the mass spectrum window displayed displays the mass spectrum for compounds collected into that tube (PurIon systems only). Clicking on adjacent tubes allows a user 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 during the run more than one set of tube racks was filled, use the NEXT and PREVIOUS RACK buttons to view the additional racks. The table below the rack diagram displays the peak data in tabular form.
- **DISPLAY METHOD** — Click the DISPLAY METHOD button to view a summary of the method settings for the run.

You can return to the rack and tube display by clicking the DISPLAY RACK button.

5.2.7 Method Editor

The METHOD EDITOR window has several sections. The BUTTON BAR at the top of the window gives you quick access to file operations, and column data. Below the BUTTON BAR are the RUN SETTINGS and the RUN NOTES. The lower part of the window contains the GRADIENT PLOT AREA and settings for PEAK COLLECTION and PEAK DETECTION.

Button bar – The METHOD EDITOR button bar contains the following:

- **NEW** — Click this button to open a new method file using the default method settings for the selected column.
- **OPEN** — Click this button to open a method file stored on the system's internal hard drive.
- **SAVE** — Click this button to save any modifications to the current method file. If you attempt to save modifications to a default method file, the FILES window will open instead so you can rename the file. This preserves the default method.
- **SAVE AS** — Click this button to open the FILES window. From this window you can rename the current method and save it on the system's internal storage.
- **EXIT** — Click this button to close the METHOD EDITOR and return to the MAIN WINDOW.
- **COLUMN DATA** — Click this button to open the COLUMN DATA window. This window reports information about the column selected on the main screen.

Run Settings – The Run Settings section contains the following:

- **Column** — The name of the currently selected column is displayed. 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 will generate a date/time stamp for the name when you click the PLAY button to begin the run.
- **Flow Rate** — Type or select the desired flow rate for the run. When using the Isco 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. Data is not collected while this volume is being pumped.

- **INITIAL WASTE & TIME WINDOWS** — Click this button to open 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. After this volume has been diverted, the system is ready to collect peak or all fluids in the collection tubes. Use this feature to conserve collection tubes by diverting all fluids until the first peak is expected.

Time Windows can limit the fraction collection to specific time durations of the run. To use time windows, first enter a start time and an end time. Note that all times are relative to start of the run, just following the sample injection. Then choose a peak collection mode. If you select Peaks, the module will collect fluid only when the time window is active and a peak is present. If you select All, the module will collect all fluid during the time window regardless of the peak state. Detected peaks will still trigger tube advances. You can define up to three time windows.

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

Gradient Plot Area – The 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. The X-axis scale can be adjusted by typing or selecting the Run Length. The left Y-axis displays absorbance units and the right Y-axis indicates the percentage of solvent B.

The gradient plot area also provides a convenient method to edit the gradient curve. You can click 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 function.)

The following controls appear in the Gradient Plot Area:

- **INSERT** — Click this button to enable the gradient point insert mode. When this mode is active, click the gradient curve to add a single point. You can then drag the new point to any desired position. Click the INSERT button

once for each gradient point that must be added to the plot area.

- DELETE —Click this button to enable the gradient point delete mode. When this mode is active, the system will delete the point nearest the next click on the gradient curve. Click the DELETE button once for each point that must be deleted from the plot area.
- Run Length — Type or select the length of the run.

 **Note**

This will change the scale of the X-axis on the gradient. Points that define the gradient will be automatically scaled to fit the new run length. The run length can be expressed in.

- Solvents A and B— Select the desired solvent from the list. The available solvents are those defined by the CONFIGURATION settings. You can program mid-run solvent B changes by clicking the EDIT GRADIENT button to open the GRADIENT TABLE.
- EDIT GRADIENT — Although the gradient may be edited directly within the plot area, you can also edit the gradient in tabular form. Click this button to open the GRADIENT TABLE. The GRADIENT TABLE also lets you program solvent changes in the gradient.
- For complete instructions on defining gradient curves, see Section 5.3.3 *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 will be 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 click the appropriate GRADIENT button for the action you desire.
- Insert Point — This command or button will insert a row below the selected point.
- Delete Point — This command will delete the selected row. 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, click the EDIT GRADIENT button. See Section 5.3.3 *Defining a Gradient*.

Peak Collection – Use the PEAK COLLECTION buttons to set the collection mode.

- ALL — Click this button to collect all fluids in the fraction collection tubes during a run.

- **PEAKS** — Click this button to collect only eluted peaks in the fraction collection tubes during a run.
- **NONE** — Click this button to divert all fluids during a run to the waste port.
- **Tube Volume** — You can also specify 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 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 click the NEXT TUBE button.

- **Peak** — Type or select the desired volume to be collected in each tube when the system detects a peak.
- **Non-peak** — Type or select the desired 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.

Peak Detection – This section of the window contains option buttons to enable and disable various peak detection options. When an option is enabled, the window also allows you to modify the setting details for that peak detection option. All enabled options will be displayed on the chromatogram. The options and settings for the ACCQPrep systems, are described below.

Note

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

- λ_1 and λ_2 (wavelength 1 and 2) — Enable these options 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 enabled, type or select the peak detection wavelength in nanometers, then click the DETAILS button to configure additional settings described below.
 - **Slope Based** — Select this option to enable
 - **Slope-based peak detection**. When enabled, peaks will be recorded if the slope algorithm detects a peak similar to the Peak Width setting.
- **Threshold Detection** — Select this option to enable Threshold peak detection. When enabled, peaks will start when the detector signal exceeds the programmed

value. The peak will end when the detector signal drops to .01 AU below the programmed value. This reduces the potential of multiple peaks if a noisy signal oscillates around the threshold value.

 **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** — Enable this option to only use the detection source as a monitor. When enabled, the detection source will be displayed as a trace on the gradient plot area, but will not be used to cut peaks.
- **Peak Width** — Select the average peak width setting. Peak widths are measured at the baseline. The slope detector will typically detect 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, you should enter a peak width of 1 minute. 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.
- **All Wavelength Detection** — Enable this option to detect peaks within a user-selected range of wavelengths. When enabled, click the **DETAILS** button to configure additional settings. These settings include the slope-based and peak width options described in λ_1 and λ_2 above. 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 will be subtracted as an assumed baseline spectrum. Additionally, you can type or select the minimum and maximum wavelengths limits in nanometers. This eliminates areas of little spectral information from the all wavelength detection signal resulting in a stronger signal.
- **Mass Spectrometer** – Enable this option to monitor or detect compounds with a PurIon mass spectrometer system (PurIon systems only).
 - **Threshold** – signal level used to detect a peak. This is based on the baseline noise. The baseline noise is measured during the first column volume; this is multiplied by the value entered in the threshold control to generate a trigger value. A peak is collected if the signal is greater than this trigger value.

- Monitor – Clicking on this option prevents fractionation based on the mass spectrometer signal.
- Terminate On Target – stops the run after all mass spectrometer detection ions have been detected.
- Detection Ions – used to set ions for detection or 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.
- Ion Settings – set ionization parameters to enhance detection of molecular ion peaks.
- External Detector — Enable this option to use a 0 to 1 volt analog signal from an external detector. When enabled, click the DETAILS button to configure additional settings. These settings include the slope-based and peak width options described in λ_1 and λ_2 above. The system will use its internal algorithms to cut detect and cut peaks based on the analog input signal. Refer to the External Detector instruction sheet for cable and plumbing requirements.

The external detector option is not available on ACCQPrep systems with ELSD or PurIon detectors.

- ELS Detector — Enable this option to use the evaporative light scattering (ELS) detector on an ACCQPrep system. When enabled, click the DETAILS button to configure additional settings:
 - Sensitivity- Can be selected as NORMAL to keep large peaks on scale or HIGH to detect small peaks. The default setting is HIGH to ensure most compounds are detected.
 - Signal Gain – You may modify the gain to scale the detector signal.
 - Slope Based and Threshold – Refer to these features described in λ_1 and λ_2 above.
 - 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.
 - 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 using the ELSD ON button 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.

- Purity Measure — If using two absorbance wavelengths, check “Show Ratio” to display a ratio of the selected wavelengths. The ratio trace is often a useful indicator of purity. Use the SHOW SPECTRAL PURITY control to measure purity by using a comparison of the entire
 - UV-spectra measured at differing times as a purity measurement. The SPECTRAL PURITY DETECTION button allows fractionation based on spectral purity.

5.2.8 Column Data

The Column Data window reports information about the RediSep ACCQPrep 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” will help 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 click the COLUMN DATA button.

5.2.9 Files

The Files window is modal. That is, its function and features change according to the command used to open the window. Menu commands such as FILE > OPEN and SAVE METHOD AS, or clicking OPEN and SAVE AS buttons will open this window. Use this window to browse the system’s files and folders. The following controls appear on the window:

- Current Path — The top-left corner of the window displays the path (current folder). As you browse through the files, the path will update as you go. You can click the folder names to return to upper folder levels.
- File and Folder Operation buttons:
 - COPY — Click this button to copy a highlighted file to the system’s clipboard memory.
 - PASTE — Click this button to paste a file from the clipboard memory. If the file already exists in the current folder, the system will ignore the Paste command to prevent the original file from being overwritten.
 - DELETE — Click this button to delete a highlighted file or a folder and its contents.

- **NEW FOLDER** — Click this button to create and name a new folder.
- **UP** — Click this button to browse the contents of the next-higher folder level.
- **SEARCH** — Enter a keyword and click the **SEARCH** button to find matching file names. Click the **CLEAR SEARCH** button to clear the results.
- **File Management options** — Click the **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. Click the **Delete by Age** to specify an age limit, beyond which will be deleted from the internal hard drive.
- **File/folder info scroll box** — This box lists the contents of the current folder. The contents can be sorted by clicking the column headings.
- **File Type** — This option is shown when you can limit the display to certain types of files. Select the desired file type from the list.
- **File Name** — This text entry box is used to identify the currently selected (highlighted) file or folder when browsing and opening files. When using a **Save As** command, use this box to name the file.
- **OPEN/SAVE/DELETE** — This button performs the listed action.
- **Load Previous Run from Detected Rack** — This option appears when using the **FILE > OPEN** command. If you click this option, the system reads 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 ACCQPrep.

- **CANCEL** — Click the **CANCEL** button to close the window without saving or opening the file.

5.2.10 Set Data Path

The **FILE > SET DATA PATH** menu command opens the **SET DATA PATH** window. Use this 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.

The Set Data Path primarily is used with the **USER MANAGEMENT** feature. **USER MANAGEMENT** will automatically create 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 — The top-left corner of the window displays the path (current folder). As you browse through the files, the path will update as you go.
- NEW — Click this button to add and name a subfolder within the currently selected folder.
- DELETE — Click this button to delete the selected folder.
- Folder selection box — This box lists the available folders and selection buttons.
- OK — Click this button to save your selection as the data path and close the window.
- CANCEL — Click the CANCEL button to close the window without changing the data path

5.2.11 MS Method Development

The MS->METHOD DEVELOPMENT menu command opens the PurIon mass spectrometer method development window (PurIon equipped systems only). This enables a user to test and verify ionization conditions for their compound. This command is not available from a remote connection. This window contains the following controls:

- Polarity—On the PurIon system, this control changes the probe polarity between positive and negative ionization. On PurIon S and PurIon L systems, this button toggles the displayed mass spectrum between positive and negative ionization.
- Ion Settings— Allows selection of different ion settings to maximize the intensity of the desired 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. User defined ion settings may also be loaded.
- Ion Finder- Identifies potential adducts and fragments for the compound of interest when the compound of interest does not show up in the spectrum graph. This algorithm identifies the mass of the 10 most intense ions and compares that to the desired compound’s molecular weight. The difference in mass is compared to a table of potential adducts or fragments. This list is displayed to the user to determine if any of these potential adducts or fragments corresponds to the mass of interest and could be used as the detection ion.
- Detection Ions—After a spectrum is collected, clicking on a peak adds that ion to this control. Alternately, masses can be entered using a keypad control.
- Clear Ions—clears the last value entered into the Detection Ions control.
- Mass spectrum graph- this 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 position”

- Inject the sample (>20 uL) 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 μ g/mL.

- **Format Graph**—Set 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 based on 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**- the file name for the mass spectrum is only needed 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** - save the displayed spectrum on either a USB drive or a remote computer’s hard drive. The file name will be the same as the spectrum name except it has a PDF extension. PeakTrak displays a file download window so you can select a location and change the file name.
- **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.

5.2.12 MS Manual Control

The MS->MANUAL CONTROL menu command allows the fluid interface carrier solvent pump to be run for priming and to purge the carrier solvent. This window can also be used for trouble shooting.

- **Start Carrier Pump**- Turns the carrier pump on for priming or to replace one carrier solvent with another one. A purge is complete in ~5 minutes. This changes to read 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 changes to read 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.

5.2.13 Ionization Settings

The MS->IONIZATION SETTINGS menu command allows a user to adjust various settings to improve the ionization of a particular compound by reducing fragmentation or adduct formation.

- Mass Spectrum Graph - this 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 uL) 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- Set 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 based on 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 the desired 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. User-defined settings may also be loaded.
- Make Changes- Pressing this button allows a user to change the settings to improve the intensity of an ion.
- Save- save changes to an existing ion settings file.
- Save As- save 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- restore the “typical”, “Robust”, and “Fragile” ion settings to the factory default values.
- Probe - The probe nebulizes and ionizes the sample. There is a choice of ESI (electrospray interface) or APCI (atmospheric pressure chemical ionization) probes. The software will change 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 will display a single temperature for both positive and negative ionization).
- Voltage (Current) - This 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 - The source voltage settings have the greatest effect on fragmentation. Higher values induce more fragmentation but also reduce adduct formation.

- Offset - Offset is 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.

5.2.14 Manual Control

Manual Control can assist with method development, maintenance of the system plumbing, and system troubleshooting. The Manual Control window can be opened by selecting **TOOLS > MANUAL CONTROL** from the PeakTrak menu. The Manual Control Window has several controls:

- Solvent Selections – If the Solvent Selector Valve Module is installed, you can select which solvent is pumped 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 # – As a default setting, solvent is pumped to the waste port during manual control. If you would like to collect the solvent in a tube, select the **OPTION** button and enter the tube number. This feature can be useful when trying to recover a compound that has precipitated or “crashed” somewhere in the fluid path.
- Prime A – Click the “PRIME A” button to pump 100% of solvent A at the selected flow rate.
- Prime B – Click the “PRIME B” button to pump 100% of solvent B at the selected flow rate.
- PRIME XX% B – Click this button to pump a mix of Solvent A and B.
- STOP – Click this button to stop the pump. This button is only active 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. Click the **CLOSE MANUAL CONTROL** button to close the window.

5.2.15 Configuration

Network Configuration – •Network configurations settings require assistance from your network administrator. Contact your Information Technology department before changing the settings. For more information on Network Configuration, see Technical Note 28 available at www.isco.com.

Instrument IP Address Configuration – •Use these settings to specify the instrument’s network address and its parameters:

Network Type – •Select the “Static IP” option.

- The “Static IP” option will require an IP address, Netmask, and Gateway provided by you network administrator. The IP address, Netmask, and Gateway will be automatically retrieved from the DHCP server when selecting the DHCP option.

IP Address – •Enter the static IP Address for the system. Static IP option only.

Netmask – •Enter the Netmask for the system. Static IP option only.

Gateway – •Enter the Gateway for the system. Static IP option only.

Network Printing – •The system can print to a network printer and supports JetDirect and line printer (LPR) queues.

Consult with your network administrator to determine:

- The IP address of selected printer.
- If the printer uses an LPR print queue, you must also find out the queue name.
- If the printer uses a JetDirect print queue, also ask for the port number.
- 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 using the FILE > PRINT menu command 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 will be able to 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.

5.2.16 Set Administrator Password

This window, opened by selecting the TOOLS > SET ADMINISTRATOR PASSWORD menu command, is used to enter and change the system 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. Click the OK button to save your settings and close the window.

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

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

5.3 Examples of PeakTrak actions

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

- EDITING A METHOD
- ALTERNATIVE WAYS TO CREATE METHOD FILES
- EDITING A DEFAULT METHOD
- DEFINING A GRADIENT
- REAL-TIME GRADIENT EDITING
- MONITORING THE PURITY MEASURE
- VIEWING RUNS
- MANUAL CONTROL OF THE ACCQPrep

5.3.1 Editing a method

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

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

Basic Method Settings – The basic settings allow you to control the %B gradient mix, flow rate, solvents, and run length. These settings are part of the MAIN window. These settings 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 5.3.3 *Defining a Gradient*. for more details. The other settings are:

- 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 the desired solvents from the lists. The 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 peak collection mode.

Equilibration Volume – 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 eluant 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. To use time windows enter a start time and an end time. All times are relative to start of the run, just following the sample injection. Then choose a peak collection mode. If you select Peaks, the module will collect fluid only when the time window is active and a peak is present. If you select All, the module will collect all fluid during the time window regardless of the peak state. Define up to three time windows.

Peak Collection – There are three peak collection options:

All, Peaks, or None. The All option will collect peak and non-peak fluid in tubes. The Peaks option will collect only peak fluid in tubes and divert all other fluids to the waste port. The None option will divert all fluids, peak and non-peak, to the waste port. The None option 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 will advance to the next tube whenever a peak is detected. Also note that the system will advance 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.

Peak Detection – This section of the window contains option buttons to enable and disable various peak detection options. When an option is enabled, the window also allows you to modify the setting details for that peak detection option. All enabled options will be displayed on the chromatogram. T

Note

You can select up to four peak detection options (excluding the External option) on the ACCQPrep systems. If using more than one option, such as λ_1 with λ_2 , the system considers a peak to be present when either option is true (a logical OR).

λ_1 and λ_2 (wavelength 1 and 2) – Enable these options to use and configure primary and secondary wavelength detection. When enabled, type or select the peak detection wavelength in nanometers, then click the DETAILS button to configure additional settings described below.

Slope Based – this option to enable Slope-based peak detection. When enabled, peaks will be recorded if the slope algorithm indicates a peak within the Peak Width setting.

Peak Width – Select the average peak width setting. Peak widths are measured at the baseline. The slope detector will detect 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, you should enter a peak width of 1 minute. For most flash chromatography, 1 minute is a good starting point for a peak width.

Threshold – Select this option to enable Threshold peak detection. When enabled, peaks will be 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 – Enable this option to only use the detection source as a monitor. When enabled, the detection source will be displayed as a trace on the gradient plot area, but will not be used to cut peaks.

All Wavelength Detection – Enable this option to detect peaks within a user-selected range of wavelengths. When

enabled, click the DETAILS button to configure additional settings. These settings include the slope-based and peak width options described in $\lambda 1$ and $\lambda 2$ 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 – Enable this option to use a 0 to 1 volt analog input signal from an external detector. When enabled, click the DETAILS button to configure additional settings. These settings include the slope-based and peak width options described in $\lambda 1$ and $\lambda 2$ above. The system will use its internal algorithms to cut detect and cut peaks based on the analog input signal. Refer to the External Detector instruction sheet for cable and plumbing requirements.

ELS Detector – Enable this option to use the evaporative light scattering (ELS) detector on an ACCQPrep Lumen system. When enabled, click the DETAILS button to configure additional settings:

- **Signal Gain:** You may modify the gain to scale the detector signal.
- **Slope Based and Threshold:** Refer to these features described in $\lambda 1$ and $\lambda 2$ above.
- **Spray Chamber Temperature:** The default settings are 30 °C (normal phase default methods) and 15 °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. 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.

Mass Spectrometer – (PurIon systems only) Enable this option to monitor or detect compounds with a PurIon mass spectrometer system.

- **Threshold:** A signal level used to detect a peak. This is based on the baseline noise. The baseline noise is measured during the first column volume; this is multiplied by the value entered in the THRESHOLD control to generate a trigger value. A peak is collected if the signal is greater than this trigger value.
- **Monitor:** Clicking this option prevents fractionation based on the mass spectrometer signal. Terminate on Target – Stops the run after all mass spectrometer detection ions have been detected.

- **Detection Ions:** Sets ions for detection or to be monitored. Up to 4 single ions may be chosen, or a range of ions and up to 3 single ions may be chosen. On PurIon S and PurIon L systems, detected ions may be a mixture of positive and negative ions.
- **Ion Settings:** Sets ionization parameters to enhance detection of molecular ion peaks.

Purity Measure – If using two absorbance wavelengths, check this option to display a ratio of the selected wavelengths. The ratio trace is often a useful indicator of purity. Use the SHOW SPECTRAL PURITY control to measure purity by using a comparison of UV-spectra measured at differing times as a purity measurement. The SPECTRAL PURITY DETECTION button allows fractionation based on spectral purity.

Saving Changes to the Method File – After you have edited the method file, you can save the changes for future use. Click the SAVE AS button, give the method file a descriptive name, then click the SAVE button. The method file will be stored by the ACCQPrep system and will be available for future runs.

5.3.2 Alternative Ways to Create Method Files

Other than editing a method file on the ACCQPrep system, there are more ways to create method files.

Importing Method Files from another System – If a method file is located on another ACCQPrep system, you can use the FILE>EXPORT METHOD menu command to save the method file on an external storage device. From the touch screen panel, this storage device is a USB Flash Drive connected to the USB port below the display. If connected to the 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 the FILE>IMPORT METHOD menu command 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 and click the EXTRACT button. The system will load a new method with identical run parameters. You can then save the method using the FILE>SAVE METHOD AS menu commands.

5.3.3 Defining a Gradient

The simplest way to change the gradient is to click and drag the inflection points that define the shape of the gradient. Add a point by clicking the INSERT button, then click the gradient curve

to add a point. You can then drag the new point to the desired location. Delete a point by clicking the DELETE button and then clicking the undesired inflection point.

The above methods 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. Click the EDIT GRADIENT button to open this view, then use the controls to modify the settings. Click the EDIT GRADIENT button again to close the table view.

5.3.4 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, click and drag the points on the gradient plot area of the MAIN window. You can also add or remove points by clicking the INSERT or DELETE point buttons. The INSERT button allows you to add a single point when you click on the gradient profile. Repeat this action to add more points. The DELETE button allows you to remove a single point when you click on it.

 **Note**

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

5.3.5 Bypassing the Injection Loop

The LOOP BYPASS button is used to override 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 will require you to perform sufficient method development to know when all of the compounds elute from the column.

As a simple example, assume that a separation is developed with 2 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 simple example, the 2 injections can be separated in 16 minutes instead of 20 minutes. The process can be repeated as needed to 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.

5.3.6 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. Refer to the following discussion.

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 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 will be 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 a case where 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, one can therefore assume that if the ratio is not constant for the entire duration of the peak, the compound eluting may not be pure.

The SHOW SPECTRAL PURITY control measures purity by using a comparison of UV-spectra measured at differing times. The algorithm used is the "similarity index". The SPECTRAL PURITY DETECTION button allows fractionation based on spectral purity. The spectral purity algorithm doesn't will fail on saturated peaks (flat on top due to detector saturation).

To display the ratio, open the METHOD EDITOR window and select the Show Ratio option.

5.3.7 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 click the OPEN button. The FILES window is displayed.
2. Choose a Run file and click the OPEN button. 3. PeakTrak displays the run in the RUN VIEWER window. The PeakTrak RUN VIEWER window will include:
 - 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, click the DISPLAY RACK button to

view this information. On PurIon equipped systems, the mass spectrum of the fraction contents can be displayed by pushing the MS button and clicking on a tube.”

- Method parameters — Click the DISPLAY METHOD button to view a summary of the peak detection and collection settings for the run.
- Chromatogram — The right side of the window displays the chromatogram, identified by the Sample Name in the title bar of the window.
- OPTION buttons — The OPTION buttons give you quick access to frequently used commands while viewing a run. The buttons are:
 - PRINT — Click this button to print 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 — Click this button to save the run summary in PDF file format. 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 — Click this button to save the run summary in an ASCII text file format. 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. Pan requires the use of 2 fingers to differentiate a pan action from a select action
- Format Graph— Click this button to open 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. Clicking 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.
- MS - Click this button to display a mass spectrum (PurIon systems only). Clicking in the Chromatogram window displays a mass spectrum at that point during the run. Clicking on a tube in the rack map shows the mass spectrum of the contents of that fraction.
- UV (will be displayed as vis on UV-Vis equipped systems) - Click this button to display a UV or

- UV-visible spectrum (UV-Vis systems only). Clicking in the Chromatogram window displays a mass spectrum at that point during the run.
- EXTRACT METHOD button — Click the EXTRACT button to load a new method file based on the parameters for the run you are viewing.
- CLOSE button — Click the CLOSE button when you are done viewing the run.

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. Below each collected peak there is a color bar that 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 wish to identify the tube by number, refer to the table below the rack diagram.

If the run used multiple racks, the PREVIOUS and NEXT RACK buttons are active. Click these buttons 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 may be visible if you are monitoring a second wavelength.
- Green traces are from ELSD (Lumen systems) or external detectors.
- The PurIon traces are 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 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.

5.3.8 Manual Control of the ACCQPrep

Manual Control can assist with method development, maintenance of the system plumbing, and system troubleshooting. To manually control the ACCQPrep system, first open the MANUAL CONTROL window by clicking the TOOL > MANUAL CONTROL menu command.

Pumping Solvents

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

You can control the flow rate by adjusting the Flow Rate mL/min setting.

By default, the system pumps the solvent directly to the waste port., you can also pump the solvent into a collection tube. To do so, select the Pump Into Tube # option and then select which tube (“next” or 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.

ACCQPrep HP125

Section 6 Maintenance

6.1 Introduction

This section will cover some common maintenance routines for the ACCQPrep HP125.

6.1.1 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. Each 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.


6.1.2 Collection Rack and Tray Cleaning

 **WARNING**

Risk of fire or equipment damage. Unclean collection racks and tray might inhibit their 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 possibly result from an electrostatic discharge, clean the racks and tray monthly. Use distilled water with a mild detergent. For tougher stains, use isopropyl alcohol.

6.2 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 touch screen 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.

6.2.1 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 should 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 6-1).

Correct any deficiencies before returning the instrument to operation.

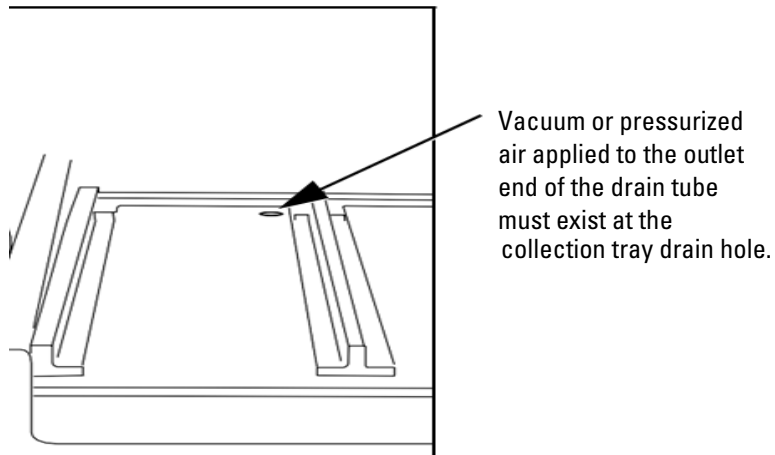


Figure 6-1 Fraction collection tray drain hole

6.3 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 6.1.1).
- Quick flow cell cleaning when recommended by a system alert message (Section 6.4).
- Wipe cone on PurIon system with wipe soaked with methanol or water to remove visible residue near 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 6.4.
- Allow 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 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 6.2.1).
- Collection rack and tray cleaning (Section 6.1.2).
- Monthly flow cell cleaning (Section 6.4.3).

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

- Change roughing pump oil (PurIon systems only).

6.4 Flow Cell Cleaning

6.4.1 Post Separation

As a preventive measure, all default column methods finish the separation run with a high percentage of solvent B (Figure 6-2). This brief time (one to six column volumes) of strong solvent flushes residual compounds from the column, flow cell, and internal tubing.

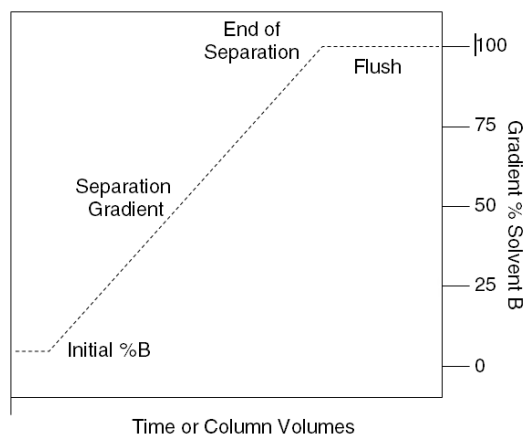


Figure 6-2 Default post-separation column and flow cell flush

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.
- Frequent messages recommending flow cell cleaning (Figure 6-3).

Typically, chemists STOP and then TERMINATE the run after the last compound elutes. This action skips the post-separation flush. If any of the above conditions 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.

6.4.2 Quick Cleaning when Recommended

When the lamp energy is lower than normal, the system will recommend flow cell cleaning (Figure 6-3) before starting a separation run.

When the system displays this message you can:

- **Cancel Run** (recommended) – Click the CANCEL RUN button so you can perform a quick cleaning described in the following steps.
- **Continue Collect All** – Click this button to 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** – Click this button to display the flow cell cleaning on-line help topic.

To perform a quick cleaning:

1. After clicking the CANCEL RUN button, select the TOOLS>MANUAL CONTROL menu option.
2. From the MANUAL CONTROL window, note the Raw Lamp Energy level at 254 nm. The Raw Lamp Energy gauge has two ranges: red and green. (Figure 6-3),

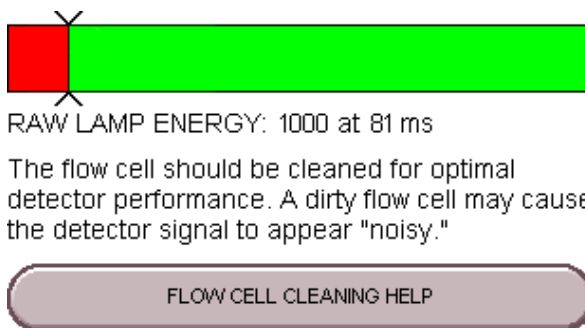


Figure 6-3 Raw lamp energy gauge

- **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 6-4).

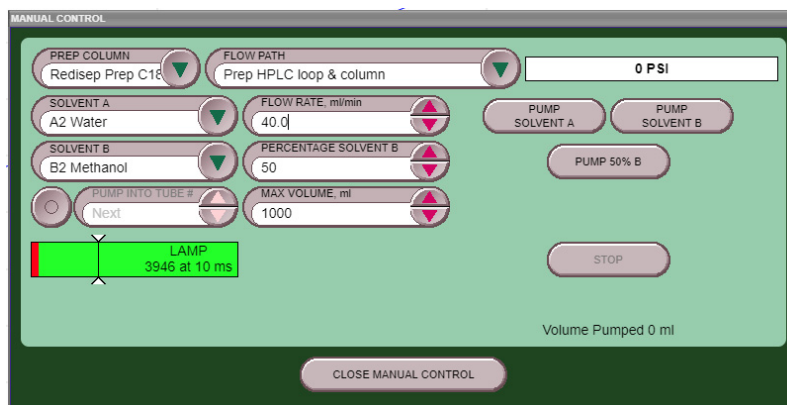


Figure 6-4 Manual Control Settings - Quick Cleaning

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. Click the PUMP SOLVENT B button 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 will usually solubilize 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 6.4.3).

Note

If the numerical values of the Raw Lamp Energy (Figure 6-3) do not change, or if the first number remains at zero, contact Teledyne ISCO's Technical Service department.

6.4.3 Monthly Flow Cell Cleaning

Perform this procedure as part of your scheduled preventive maintenance, or when QUICK CLEANING AS REQUIRED (section 6.4.2) 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. Click the PUMP SOLVENT B button to pump 100% Solvent B through the bypass tube and into the collection tubes.
8. After three minutes, click the STOP button. 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 6.4.2 and monitor the Raw Lamp Energy. (Figure 6-3)

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.

6.5 ACCQPrep HP125 Maintenance

6.5.1 Injection Valve Rotor Replacement

The following instructions are for the removal and replacement of the injection valve rotor. (PN: 209009917).

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 6-8). 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 $\frac{9}{64}$ " hex key to remove the 2 screws that retain the stator (the portion of the inject valve with the tubing connections). These should be loosened in quarter turn increments until the tension is removed and a gap starts to appear (Figure 6-5).

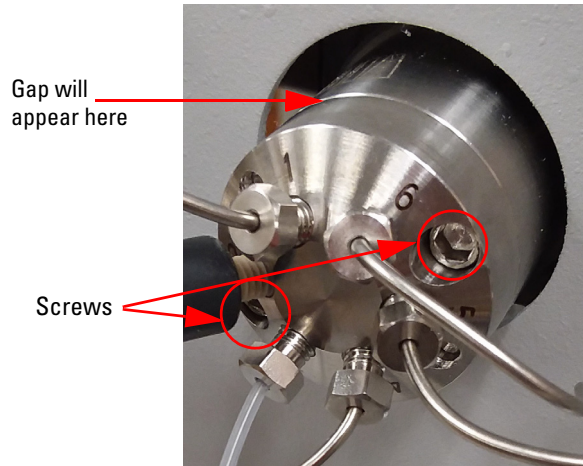


Figure 6-5 Location of screws on the stator retainer

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 6-6).

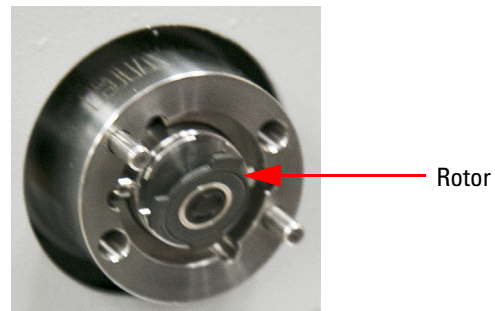


Figure 6-6 Removing the rotor

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

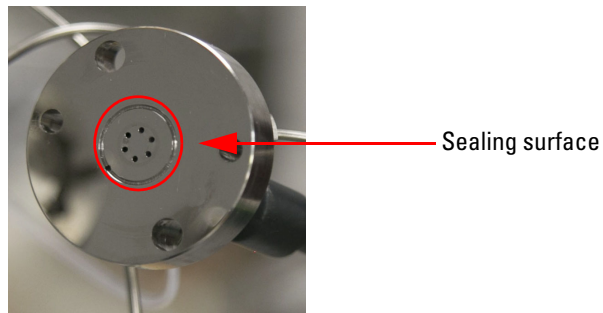


Figure 6-7 Sealing surface of the stator

*Installing the New
Injection Valve Rotor*

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

1. Place the new rotor into the recess with the passages facing outward. The tabs on the rotor are not symmetric so the rotor will only fit 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.

**6.5.2 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 6-8).



Figure 6-8 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 6-9).

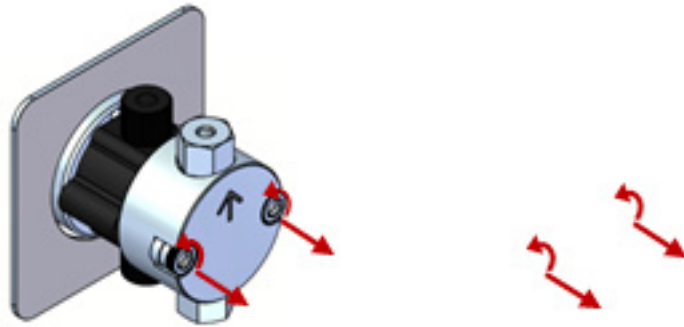


Figure 6-9 Removing the fasteners

9. Carefully pull the pump head forward and off the guide pins. Pull straight and slowly to prevent damage to the piston (Figure 6-10).

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.

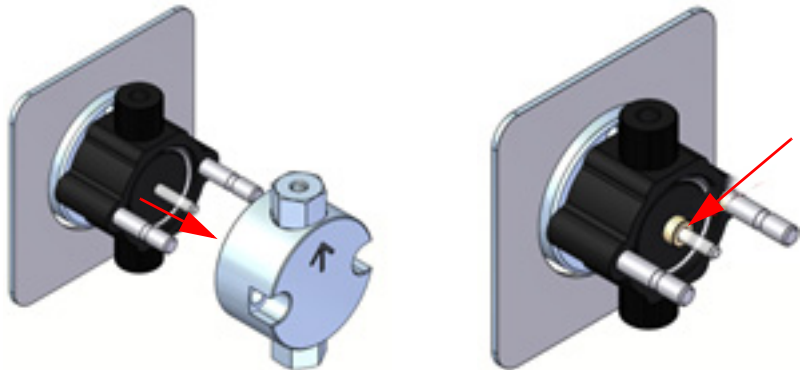


Figure 6-10 Removing the pump head

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 6-11).

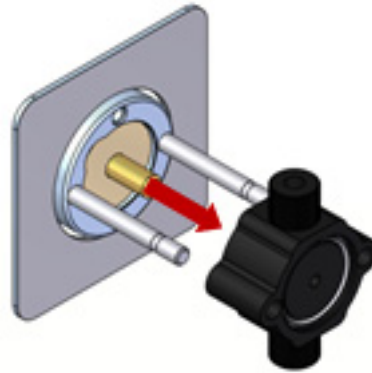


Figure 6-11 Removing the pump spacer block

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, and pull out the seal (Figure 6-12).

 **CAUTION**

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

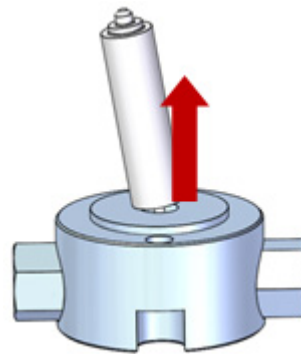


Figure 6-12 Removing the seals

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 6-13).

 **Note**

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

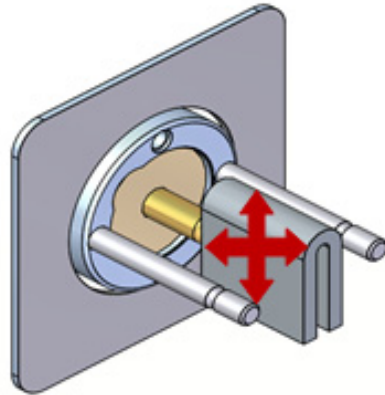


Figure 6-13 Cleaning the piston

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 6-14).

Note

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

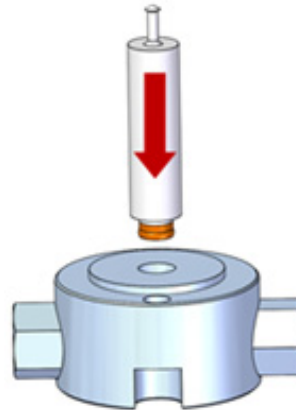


Figure 6-14 Replacing the seal

14. Carefully replace the pump spacer block, making sure the O-ring is properly installed (Figure 6-15).

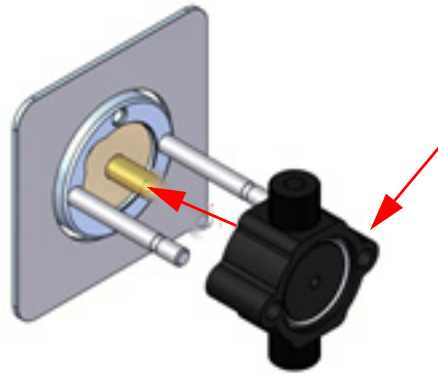


Figure 6-15 Reinstalling the pump spacer block

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 6-16).

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.

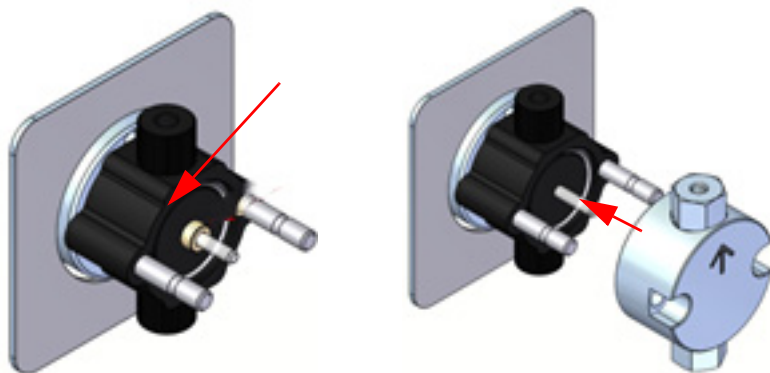


Figure 6-16 Reinstalling the pump head

16. Reinstall fasteners. As you tighten, alternate side-to-side until snug. Turn 1 flat past snug using a $\frac{3}{16}$ " hex key (Figure 6-17).

Note

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

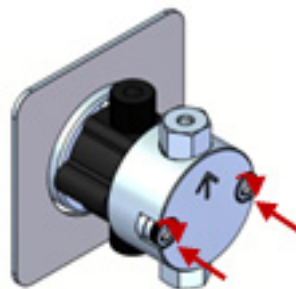


Figure 6-17 Reinstalling the fasteners

6.5.3 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 ~1000 psi. Then run the pump for another 15 minutes at a flow rate that results in 3000 psi.

6.5.4 Check Valve Replacement

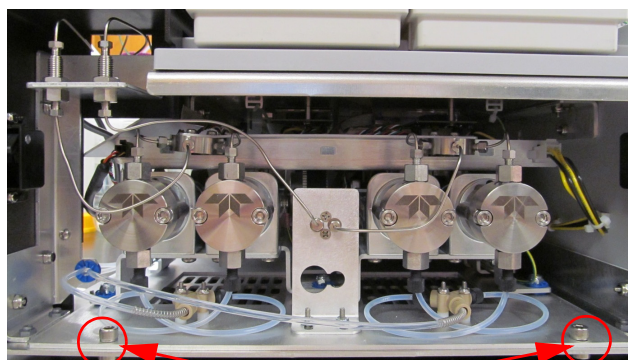
The following instructions are for the removal and replacement of the pump check valves for the ACCQPrep HP125 system.

1. Turn the power OFF.
2. Access the pump compartment by grabbing the edges of front panel and pulling forward on both sides (Figure 6-18).



Figure 6-18 Front panel of the ACCQPrep

3. The solvent supply lines to the pumps have fluid in them that will be drained during this procedure. If it is acceptable to drain this fluid back into the supply bottles, follow step 'a' below, if not, go to step 'b'.
 - 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 will allow 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 6-19 using the included $\frac{3}{16}$ " hex key.



Pump assembly mounting screws

Figure 6-19 Location of the pump assembly mounting screws

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

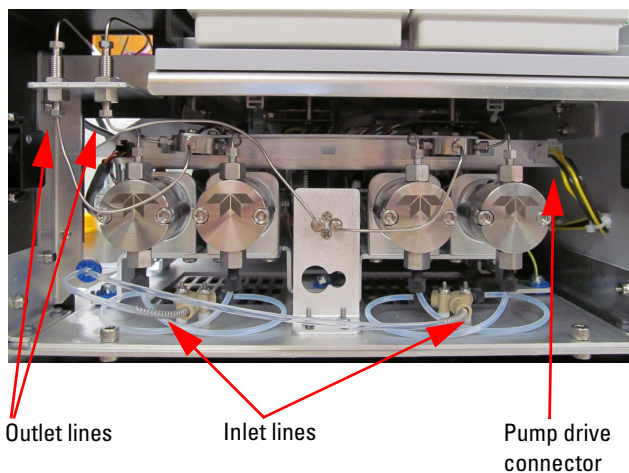


Figure 6-20 Pump assembly

7. Pull the pump assembly forward to the position shown in Figure 6-21.



Figure 6-21 Pump assembly

8. With the pump assembly pulled out, remove the inlet fittings connected to the bottom of the pump head. Using a $\frac{1}{4}$ " wrench, remove the stainless steel outlet lines on the top of the pump head (Figure 6-22).

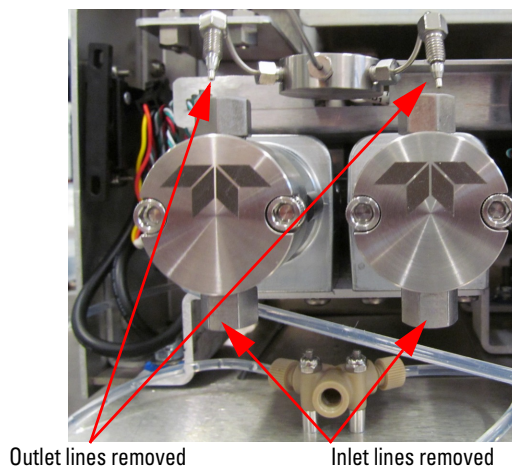


Figure 6-22 Location of fittings

- Using a phillips screwdriver, remove the 'Y' fitting located above pump heads (Figure 6-23).

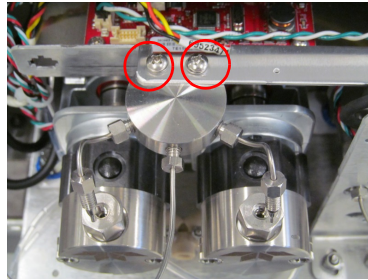


Figure 6-23 Location of tee mounting screws

- Using a $\frac{1}{2}$ " wrench, remove the check valve housings from the top and bottom of the pump heads (Figure 6-24). 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 6-24).

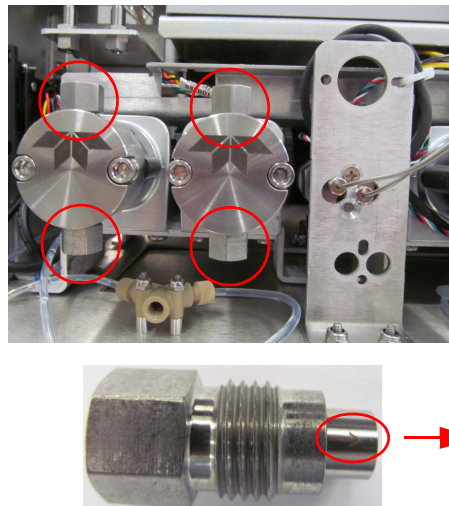


Figure 6-24 Check valve housings (top) and inlet check valve and housing (bottom)

Note

On an outlet check valve, the arrow is reversed.

- 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.
- Place the solvent supply lines into the proper connectors and reprime the system.

6.6 AutoSampler Maintenance

Peristaltic Tubing Replacement

The Peristaltic pump on the back of the AutoSampler may need to be replaced.

The peristaltic tubing P/N SP7477.



Figure 6-25 Back of the AutoSampler

1. Removed the outer band of the peristaltic pump (Figure 6-26).

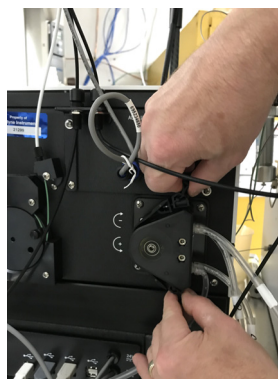


Figure 6-26 Removing the outer band

2. Lift up to remove the peristaltic tubing from the pump, repeat on the bottom (Figure 6-27).

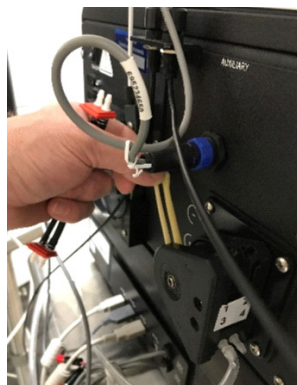


Figure 6-27 Removing the tubing

3. Slide the new peristaltic tubing with the thicker tubing towards the AutoSampler (Figure 6-28).



Figure 6-28 Attaching the new tubing

4. Replace the outer band of the peristaltic pump (Figure 6-29).



Figure 6-29 Replace the outer band

6.7 ELSD Maintenance

Periodic cleaning of the spray chamber will keep the ACCQPrep operating at maximum performance. Use the following steps if spray chamber cleaning is recommended.

6.7.1 Cleaning the ELSD Detector

1. From the run screen select the METHOD EDITOR.
2. In the Peak detection window, select the ELS DETECTOR radio button then select DETAILS.
3. Set the spray chamber temperature to 40°C.
4. Exit the Edit Method screen and accept the changes, then allow approximately 5 minutes for the chamber to reach the set temperature.

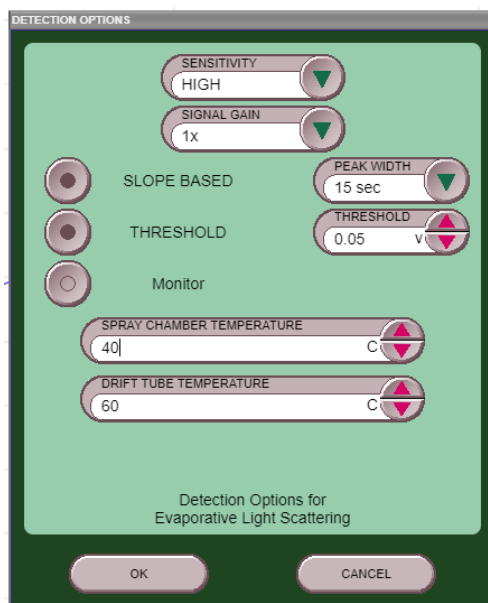


Figure 6-30 ELSD Detection Options screens

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 acetone. 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.

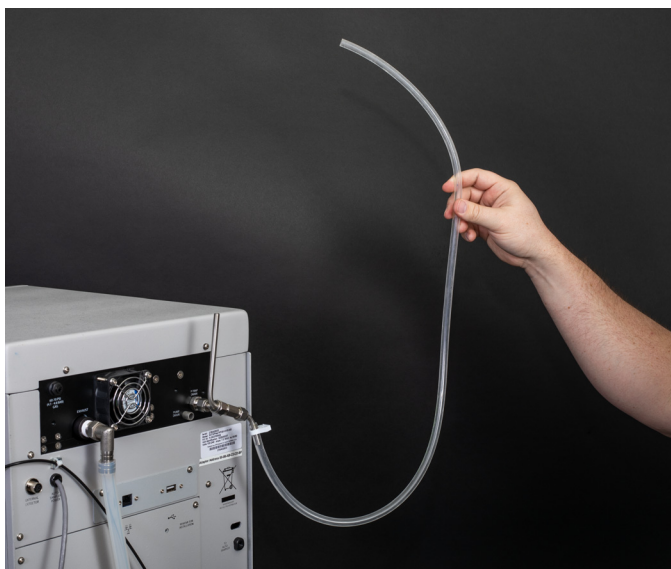


Figure 6-31 Line filled with 40 ml of acetone

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



Figure 6-32 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. Use the DETAILS menu to 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. Go to the MANUAL CONTROL window to turn the gas on and let run 10 minutes. Turn off the gas.
15. Press 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.

6.8 PurIon Maintenance

6.8.1 ESI and APCI Removal from PurIon S and PurIon L

Refer to Figure 6-33.

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.

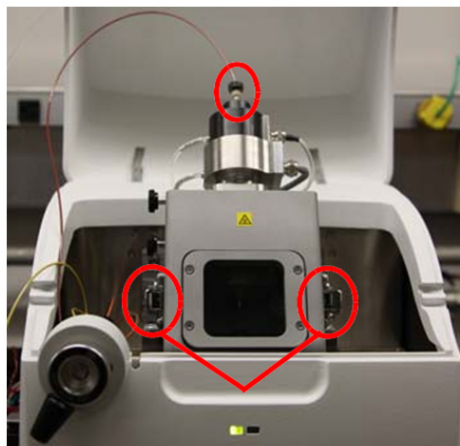


Figure 6-33 ESI and APCI removal from PurIon S and PurIon L

6.8.2 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.

6.8.3 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. Connect a 1 mL syringe, using adaptors, as needed to the ESI or APCI capillary and 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.

6.8.4 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.

6.8.5 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 troubleshoot an overpressure error, complete the following:

- If the error occurs during a run, select CONTINUE WITHOUT PurIon, and the purification can continue without the PurIon signal or peak detection, but will rely on any other detector selected such as UV or ELSD. This allows the run to be completed before troubleshooting the plug.
- Use the menu item MS-> Manual control.
- Press the START CARRIER PUMP button.
- 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 to the splitter valve until the error is corrected.

6.8.6 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 6-34), and then remove the check valve holders. Pliers may be needed to remove the holders.



Figure 6-34 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 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 $\frac{1}{4}$ 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 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. Once operating pressure is reached, the pump has a rapid refill stroke approximately that occurs ~ 8 seconds.

6.8.7 Replacing Check Valves

1. Install new check valve cartridges (P/N: 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 6-35).



Figure 6-35 Correct orientation of cartridge

2. Tighten check valve holders finger tight, then with pliers tighten an additional $\frac{1}{4}$ turn.

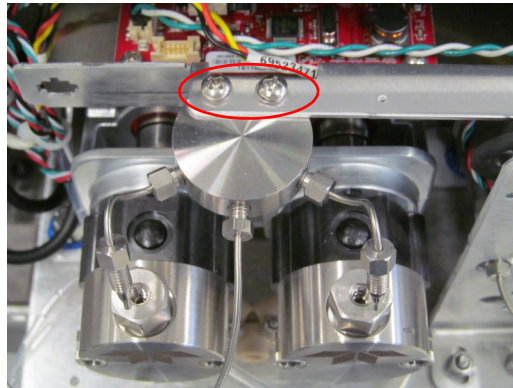
6.8.8 PurIon Cone Cleaning

This error occurs if the capillary inlet to the PurIon vacuum region is restricted causing a higher vacuum reading than normal. The 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 Isco recommends keeping a spare capillary inlet cone assembly (Teledyne ISCO part number 25-0000-085) to allow rapid replacement to minimize downtime while cleaning the plugged capillary.

6.8.9 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, section 6.8.3.
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. Many times 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. The flats are not large and may be difficult to see and keep a wrench seated on the flats.



5. Remove the O rings from the capillary inlet cone.

6.8.10 Capillary Inlet Cone Cleaning

1. Remove the o-ring under the capillary base, then sonicate the capillary in a methanol:water (50:50) mixture for 30 minutes (Figure 6-25).



Figure 6-36 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.

6.8.11 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.

6.8.12 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 6-1 Common Purlon Error Codes and Resolutions | |
|---|--|
| <p>Purlon temperatures are stabilizing. [r] seconds remain. (where [r] is a number)</p> | <p>The Purlon 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.</p> <p>During the standby condition most heaters are set to OFF except the inlet capillary with is set to 50 °C during standby.</p> |
| <p>The Purlon vacuum level is too low to operate. Please verify that the roughing pump is on and operating correctly. Pirani Pressure: [s1] mbar. (where s1 is the vacuum reading).</p> | <p>The Pirani pressure must be below 5.5E-3 mbar before the Purlon turbo pump will operate. If trying to place the Purlon in operate without turning on the roughing pump, this message will appear.</p> <p>This error generally occurs if the user forgets to turn the roughing pump back on after cleaning the capillary or changing the pump oil.</p> |
| <p>The Purlon has been shut down. It will be unavailable for use until the NextGen has been rebooted. Please ensure the Purlon and fluid interface are both turned on before rebooting the NextGen.</p> | <p>This message is displayed after the Purlon has been successfully shut down through the SHUTDOWN command. It serves as a reminder that an NextGen reboot will be necessary for the Purlon to be enabled again by the NextGen.</p> |
| <p>The splitter valve seals have exceeded their recommended life. The Purlon will continue to operate, but there is an increased possibility of leakage at the splitter valve and loss of Purlon detection during a separation.</p> | <p>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 (P/N:60-5234-629) or continue to use and monitor for leakage. Leakage should not drip out the bottom of the fluid interface front cover.</p> |
| <p>No ion source is detected on the Purlon. Please ensure that the ion source is properly installed and connected. If the source is still not detected, contact a qualified service technician. Error 309</p> | <p>The Purlon 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.</p> <p>This generally occurs upon changing or cleaning probes.</p> |

**Table 6-1 Common Purlon
Error Codes and Resolutions**

| | |
|---|--|
| A plug has occurred in the Purlon fluid lines. The separation can be continued without Purlon detection or continued if the plug is corrected. Error 310 | This error message is displayed if a plug is detected during a separation. |
| The Purlon inlet cone may be plugged which could prevent detection. Continued operation will not cause damage. Please 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 (6.8.3). |
| A plug has occurred in the Purlon 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 (6.8.3). |
| The ionization probe (ESI or APCI) isn't fully seated into the ion source housing. Please ensure the probe is fully seated, then press OK to continue. Error 317. | On Purlon systems (not Purlon S or Purlon 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. |

ACCQPrep HP125

Appendix A

A.1 Diagrams for the AutoSampler and ACCQPrep

The following diagrams are for connecting the AutoSampler to the ACCQPrep system. These diagrams include:

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

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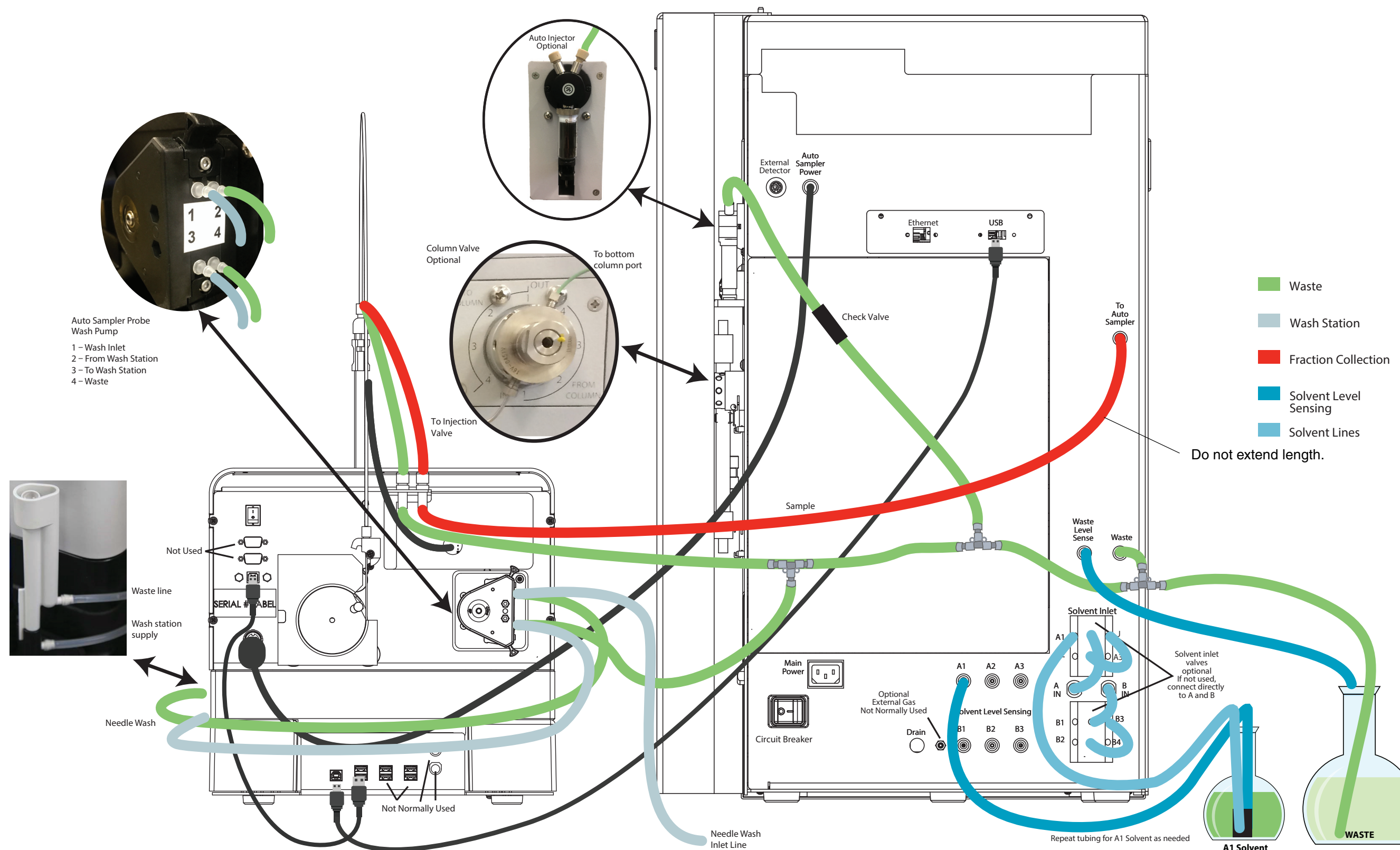


Figure A-1 Tubing diagram of the AutoSampler connected to the ACCQPrep

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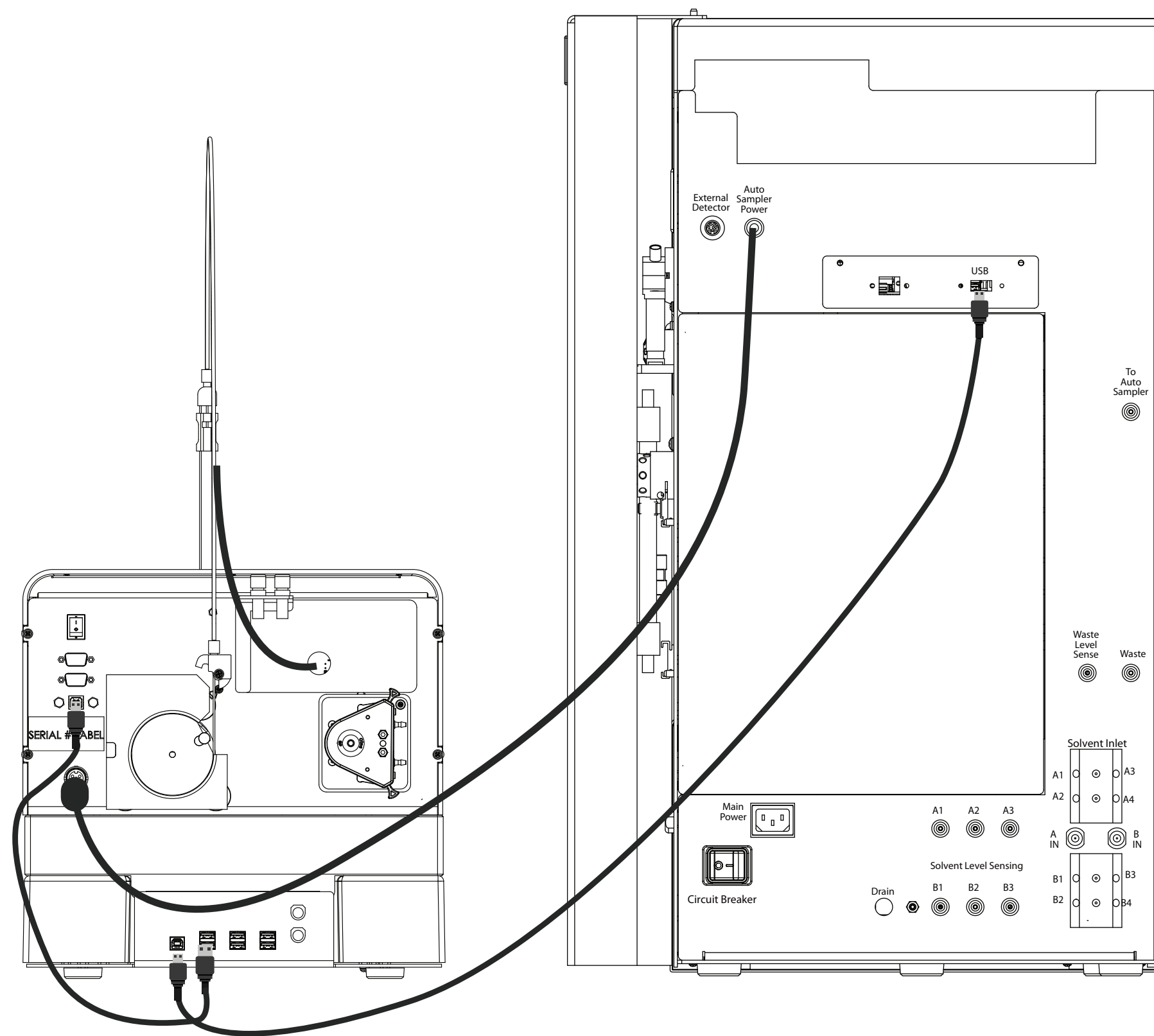


Figure A-2 USB wiring diagram of the Autosampler connected to the ACCQPrep

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DECLARATION OF CONFORMITY



Application of Council Directive: 2014/30/EU – The EMC Directive
2014/35/EU – The Low Voltage Directive
2011/65/EU – The RoHS Directive

Manufacturer's Name: Teledyne Isco
Manufacturer's Address: 4700 Superior, Lincoln, Nebraska 68504 USA
Mailing Address: P.O. Box 82531, Lincoln, NE 68501

Equipment Type/Environment: Laboratory Equipment for Light Industrial/Commercial Environments

Trade Name/Model No: ACCQPrep HP 125

Year of Issue: 2017

| | | |
|--|-------------------|--|
| Standards to which Conformity is Declared: | EN 55011:2016 | EMC Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use |
| | EN 61326-1:2013 | EMC Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use |
| | EN 61010-1:2010 | Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use |
| | EN 61010-2:2015 | Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes |
| | EN 61000-3-2:2014 | Limits for harmonic current emissions (equipment input current ≤ 16 A per phase) |
| | EN 61000-3-3:2013 | Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤ 16 A |

I, the undersigned, hereby declare that the design of the equipment specified above conforms to the above Directives and Standards as of July 17, 2017.

Edward J. Carter
USA Representative



Edward J. Carter
Director of Engineering
Teledyne Isco, Inc.
4700 Superior Street
Lincoln, Nebraska 68504

Phone: (402) 464-0231
Fax: (402) 464-3799

60-5232-256
Rev A

产品中有毒有害物质或元素的名称及含量
Name and amount of Hazardous Substances or Elements in the product

| 部件名称 Component Name | 有毒有害物质或元素 Hazardous Substances or Elements | | | | | |
|-------------------------|---|-----------|-----------|-----------------|---------------|-----------------|
| | 铅 (Pb) | 汞 (Hg) | 镉 (Cd) | 六价铬 (Cr(VI)) | 多溴联苯 (PBB) | 多溴二联苯 (PBDE) |
| 液晶显示 LCD Display | O | O | O | O | O | O |
| 线路板 Circuit boards | O | O | O | O | O | O |
| 接线 Wiring | O | O | O | O | O | O |
| 内部电缆 Internal Cables | O | O | O | O | O | O |
| 主电源线 Line Cord | O | O | O | O | O | O |
| 步进电机 Stepper Motor | O | O | O | O | O | O |
| 氘气灯 Deuterium lamp | O | O | O | O | O | O |
| 阀体 Valve Body | O | O | O | O | O | O |

产品中有毒有害物质或元素的名称及含量：Name and amount of Hazardous Substances or Elements in the product

O: 表示该有毒有害物质在该部件所有均质材料中的含量均在ST/ 标准规定的限量要求以下。

O: Represent the concentration of the hazardous substance in this component's any homogeneous pieces is lower than the ST/ standard limitation.

X: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出ST/ 标准规定的限量要求。

(企业可在此处，根据实际情况对上表中打“X”的技术原因进行进一步说明。)

X: Represent the concentration of the hazardous substance in this component's at least one homogeneous piece is higher than the ST/ standard limitation.

(Manufacturer may give technical reasons to the “X”marks)

环保使用期由经验确定。

The Environmentally Friendly Use Period (EFUP) was determined through experience.

生产日期被编码在系列号码中。前三位数字为生产年(207 代表2007年)。随后的一个字母代表月份：A 为一月，B为二月，等等。

The date of Manufacture is in code within the serial number. The first three numbers are the year of manufacture with the second digit removed (218 is year 2018) followed by a letter for the month. "A" is January, "B" is February and so on (I is not used).

Teledyne ISCO One Year Limited Factory Service Warranty*

This warranty exclusively covers Teledyne ISCO instruments, providing a one-year limited warranty covering parts and labor.

Any instrument that fails during the warranty period due to faulty parts or workmanship will be repaired at the factory at no charge to the customer. Teledyne ISCO's exclusive liability is limited to repair or replacement of defective instruments. Teledyne ISCO is not liable for consequential damages.

Teledyne ISCO will pay surface transportation charges both ways within the 48 contiguous United States if the instrument proves to be defective within 30 days of shipment. Throughout the remainder of the warranty period, the customer will pay to return the instrument to Teledyne ISCO and Teledyne ISCO will pay surface transportation to return the repaired instrument to the customer.

Teledyne ISCO will not pay air freight or customer's packing and crating charges. This warranty does not cover loss, damage, or defects resulting from transportation between the customer's facility and the repair facility.

The warranty for any instrument is the one in effect on date of shipment. The warranty period begins on the shipping date, unless Teledyne ISCO agrees in writing to a different date.

Excluded from this warranty are normal wear; expendable items such as desiccant, pH sensors, charts, ribbon, lamps, tubing, and glassware; fittings and wetted parts of valves; check valves, pistons, piston seals, wash seals, cylinders, pulse damper diaphragms, inlet lines and filter elements; and damage due to corrosion, misuse, accident, or lack of proper installation or maintenance. This warranty does not cover products not sold under the Teledyne ISCO trademark or for which any other warranty is specifically stated.

No item may be returned for warranty service without a return authorization number (RMA) issued by Teledyne ISCO.

This warranty is expressly in lieu of all other warranties and obligations and Teledyne Isco specifically disclaims any warranty of merchantability or fitness for a particular purpose.

The warrantor is Teledyne ISCO, 4700 Superior, Lincoln, NE 68504, U.S.A.

***This warranty applies to the USA and countries where Teledyne ISCO does not have an authorized dealer. Customers in countries outside the USA, where Teledyne Isco has an authorized dealer, should contact their Teledyne ISCO dealer for warranty service.**

Problems can often be diagnosed and corrected without returning the instrument to the factory. Before returning any instrument for repair, please contact the Teledyne Isco Service Department for instructions and to obtain a return material authorization number (RMA).

Instruments needing factory repair should be packed carefully and shipped to the attention of the service department. Small, non-fragile items can be sent by insured parcel post. **PLEASE WRITE THE RMA NUMBER ON THE OUTSIDE OF THE SHIPPING CONTAINER** and enclose a note explaining the problem.

Shipping Address: Teledyne ISCO - Attention Repair Service
4700 Superior Street
Lincoln, NE 68504 USA

Mailing Address: Teledyne ISCO PO Box
82531 Lincoln, NE 68501
USA

Phone: Repair service: (800) 775-2965 (lab instruments)
(866) 298-6174 (samplers & flow meters)
Sales & General Information: (800) 228-4373 (USA & Canada)
(402) 465-3001

Fax: (402) 465-3001

Email: IscoService@teledyne.com



March 2, 2016 P/N 60-1002-040 Rev J



