# ACCQPrep® SFC

## Operation Guide

TELEDYNE | LABS

Part #69-5263-074
Rev. B July 2025

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# Safety Overview

Refer to the document *ACCQPrep® SFC Important Information* (PN 69-5263-088) for the most current safety information. This document is included with your ACCQ*Prep* system, and the latest version is available at www.teledynelabs.com.

### 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. |
| (lightning flash symbol) | The lightning flash and arrowhead within the triangle is a warning sign alerting you of “dangerous voltage” inside the product. |
| (hot surface symbol) | The three wavy lines above the horizontal line within the triangle is a warning sign alerting you of a “hot surface” inside the product. |

| Hazard Symbols (Continued) | |
|---|---|
| **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. |
| (hot surface) | Ce symbole signale la présence d'une surface chaude à l'intérieur du produit. |
| **Warnungen und Vorsichtshinweise** | |
| ⚠ | Das Ausrufezeichen in Dreieck ist ein Warnzeichen, das Sie darauf aufmerksam macht, daß wichtige Anleitungen zu diesem Handbuch gehören. |
| ⚡ | Der gepfeilte Blitz im Dreieck ist ein Warnzeichen, das Sei vor "gefährlichen Spannungen" im Inneren des Produkts warnt. |
| (hot surface) | Der drei Wellenlinien über der horizontalen Linie Dreieck ist ein Warnzeichen, das Sei vor "heißen Oberfläche" 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. |
| (hot surface) | Esta señal alerta sobre la presencia de superficie caliente en el interior del producto. |



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# ACCQ*Prep* SFC Safety Considerations

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

## General Safety Guidance

**⚠ 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.**

Follow all applicable safety practices and regulations when handling and moving the system's shipping crate and associated containers, and when moving the system itself.

**⚠ CAUTION**

**Lifting or Moving the Equipment**
When carrying or moving the instrument, use the supplied system handles and remove when complete. Holding by the sides or bottom of the instrument may result in pinched fingers causing injury or damage the instrument.

**⚠ CAUTION**

**Installation**
Installation should be performed by Teledyne LABS qualified personnel. Do not carry multiple modules at the same time.

**⚠ WARNING**

***Safe Lifting Practice***
**Keep the unit upright when moving. Be sure to follow your company's procedures and practices regarding the safe lifting and relocation of heavy objects. Use a 2-person lift when moving or carrying the modules.**

**⚠ CAUTION**

When opening the oven drawer, be careful to not pinch your fingers.

- Locate the system away from potential spark sources.
- See the warning below regarding placing the system.
- Keep the system's power cord plug and outlet easily accessible in case the system needs to be disconnected quickly from AC power.

- Install external fire protection conforming to local regulations
- Have plans in place that conform to local regulations to address solvent spills or leakage at your site to prevent a fire or explosion hazard.

**⚠ WARNING**

***Use of Flammable and/or Hazardous Chemicals and Solvents***

**Chemicals used with this instrument may be classified as carcinogenic, bio-hazardous, flammable, or radioactive. Additionally, the use of flammable solvents or chemicals with this system may result in vapor concentration levels that exceed the maximum exposure levels as recommended by OSHA Guide 1910.1000.**

**Do not use solvents with an autoignition temperature below 200 °C.**

**In all cases, use good laboratory practices, appropriate personal protective equipment, and standard safety procedures.**

**Should these chemicals be used, Teledyne LABS highly recommends that these applications be performed in an isolated environment and/or a laboratory fume hood designed to reduce exposure to a safe level 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**

***Damage to System by Chemicals***

**Do not allow chemicals to come into contact with the system's power cord or cables. Solvents can degrade cord and cable insulation, causing a risk of electric shock, fire, and equipment damage.**

**⚠ CAUTION**

**Flowpath Under Pressure**

Before removing any component in the high-pressure flowline, such as tubing, the sample loop, in-line filter, column, etc. stop the pump and confirm the system pressure has fallen to zero. Depending on the position in the flow path the system may need to be vented via Manual Control to release the pressure completely. Removing any of these parts while high pressure remains could cause the mobile phase and the clogging particles to eject rapidly and injury could occur.



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**CAUTION**

**Unintended Use of this Equipment**
Use of this instrument in any way not specified in the manual or the user not following on-screen prompts and messages may impair the protection provided by the instrument.

Operators and maintainers of the system must be provided with all applicable health and safety regulations for use of the system, its accessories, and consumables. They must be educated, trained, and competent to use the machine as it is intended.

**CAUTION**

**Possible Carbon Dioxide Expansion in Sample Path**
Ensure that the cosolvent pump is properly primed before use, after changing or refilling solvents, and when directed.

If the cosolvent pump is not properly primed, $CO_2$ can accumulate in the cosolvent fluid path. $CO_2$ in this fluid path could enter the sample injection flow path, creating a possibility of sample loss via expansion of $CO_2$ to atmosphere through the sample probe.

Ensure all sample introduction is done in an appropriately ventilated hood or vapor enclosure and that appropriate personal protective equipment (PPE) is used in accordance with company's chemical hygiene protocols.

***Hot Surface***

**The column oven is able to reach temperatures up to 70 °C. Areas near the heating element at the bottom of the drawer may be hotter.**

**Open the top door of the oven drawer carefully to allow hot air to vent. Columns can be hot to the touch and should be changed only after they cool.**

*Hazards Unique to SFC: Using Carbon Dioxide*

The ACCQ*Prep* SFC system is designed to perform supercritical fluid chromatography using carbon dioxide ($CO_2$) as the supercritical fluid. The use of other supercritical fluids is prohibited.

Your $CO_2$ gas supplier should be consulted for proper design, installation, and safety guidance regarding $CO_2$ supply infrastructure.

The use of supercritical fluids brings risks that are beyond that typical of traditional HPLC or flash chromatography techniques. Carbon dioxide as a gas is colorless, odorless, and tasteless. While $CO_2$ offers several advantages in safety being non-toxic and non-flammable, it does present the risk of asphyxiation.

Typical concentrations of $CO_2$ in a room can vary between 350 and 1,000 ppm. As levels rise above 2,000–5,000 ppm, they can create health problems like headache, sweating, rapid breathing, increased heart rate, shortness of breath, dizziness, visual disturbances, or shaking. NIOSH recommended exposure limit (REL) is 5,000 ppm time-weighted average (TWA) over a 10-hour period; while NIOSH REL for short-term exposure is 30,000 ppm TWA over any 15-minute period. Higher concentrations (above 100,000 ppm) can result in unconsciousness or death.[1]

The pressure of a $CO_2$ cylinder is about 860 psi at room temperature. A typical $CO_2$ cylinder stores approximately 50 lbs. of liquid $CO_2$. One kg of liquid $CO_2$ expands to around 535 L of $CO_2$ gas at atmospheric pressure. In the case of a cylinder or tank rupturing or releasing into an enclosed space, the room can quickly fill with $CO_2$ and either poison an individual or displace all the oxygen available for breathing.

***Risk of Frostbite***

**If carbon dioxide leaks from the system tubing or fitting, dry ice may form, which may cause frostbite if touched. Should leakage occur, stop the pumps and take appropriate action after the dry ice dissipates.**

1. https://www.cdc.gov/niosh/npg/npgd0103.html
https://www.osha.gov/chemicaldata/183



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**⚠ WARNING**

***Pressurized Gas***

**It is the customer's responsibility to ensure that the ACCQ*Prep* SFC and any $CO_2$ supply and/or connections are placed in an appropriately sized room and well-ventilated space.**

**$CO_2$ is denser than air and can accumulate in higher concentrations in low spaces.**

**$CO_2$ monitors should be installed in rooms where the ACCQ*Prep* SFC system is installed and any other areas that house $CO_2$ supply cylinders or tanks with an alert function in case the measured $CO_2$ level exceeds 5,000 ppm.**

**The system and any $CO_2$ supply lines to the instrument may be is pressurized, even after power shut-off. Venting of pressurized gases and solvent is possible.**

**Keep $CO_2$ systems gas-tight. Seal any leaks immediately.**

**Since some $CO_2$ remains in the eluate stream that either goes to the fraction collector, AutoSampler, or to waste after the gas-liquid separator, fractions should be collected into:**

**1) open test tubes or bottles using the AutoSampler (enclosed in either a vapor enclosure vented to an appropriate exhaust source or fume hood);**
**2) bottles with a GL-45 thread using the modified GL-45 bottle caps provided with the system using the fraction collection valve with the bottles vented to an appropriate exhaust source or enclosed in either a vapor enclosure vented to an appropriate exhaust source or fume hood.**

**Additionally, waste streams from the fraction collection valve and/or the AutoSampler should be directed into an appropriate waste container that is non-glass, non-sealed, and vented to an appropriate exhaust source, or enclosed in either a vapor enclosure or fume hood vented to an appropriate exhaust source.**

**⚠ WARNING**

***Aerosolization***

**Depressurization of $CO_2$ from the eluate during fraction collection may result in aerosolization of compounds/sample or solvent found in the eluate. Do not inhale aerosolized particles and observe safety data sheets for all substances being used.**

**The ACCQ*Prep* SFC system should be properly exhausted to a suitable exhaust source and the AutoSampler or fraction collection bottles should be used in an appropriately exhausted fume cabinet, hood, or vapor enclosure.**

☑ ***Note***

The ACCQ*Prep* SFC has an automatic solenoid shut-off valve for the $CO_2$ inlet supply with a redundant closed state when not powered. Shutting off the system or removing power to it will result in the valve shutting off in an emergency. This valve may malfunction if blocked with particle from a contaminated $CO_2$ source. Follow installation guide instructions and ensure the $CO_2$ supply is filtered before entering the instrument. Have a back-up shut-off valve near the instrument.

## Electrical Hazards: Electrostatic Discharge

*Maintaining the System to Prevent Static Electrostatic Discharge*

Clean the collection tube racks and tray monthly. They are made of conductive plastic that must be kept clean to dissipate static electricity. Use distilled water with a mild detergent. For tougher stains, use isopropyl alcohol.

*Preventing Person-to-System Electrostatic Discharge*

Observe the following precautions to prevent person-to-system electrical static discharges:

1. Wear anti-static clothing and shoes when operating the system. Stand on an anti-static floor mat.
2. Touch a grounded object before touching the system or before handling any of its parts (such as columns). For example, metal water pipes are typically grounded. This will discharge any static electricity you may have accumulated.
3. Maintain humidity above 65% at the instrument location so that static buildup will be generated less readily.



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*Preventing Static Electrostatic Discharge During System Operation*

Static electricity can also be generated during system operation, such as when non-polar liquids flow through it. To prevent static buildup as the system operates, observe the following precautions:

1. Do not exceed the flow rate specified in the documentation for the preparative chromatography column.
2. Prevent air bubbles from accumulating in the flow lines. These can significantly increase electrostatic charge.

**⚠ CAUTION**

**Static Electricity**
Do not modify any of the tubing/fittings used on or within the system. System tubing/fittings is designed to meet specific pressure requirements.

When using the ACCQ*Prep* SFC preparative chromatography system, take precautions to avoid static electricity buildup. Discharges of static electricity could ignite vapors, especially when using the system with flammable, non-conducting solvents operating under high flow rate conditions.

Read, understand, and follow all local and national codes and regulations to avoid static electricity hazards.

**⚠ WARNING**

**Substitution of Tubing**
**Never substitute the black tubing on ACCQ*Prep* SFC systems. The black tubing (P/N 023-0503-06) is anti-static. This tubing is required to dissipate static electricity. Discharges of static electricity could ignite vapors.**

**⚠ WARNING**

***Solvent Leaks and Spills***
**Do not wipe solvents from system or column surfaces while the system is operating or while it is plugged in.**

**In the event of such solvent leaks, immediately turn off the system and disconnect the power cord. The combination power switch and circuit breaker is located on the rear panel.**

**In event of column leakage, allow all solvent vapor to dissipate before removing the column. Failure to do so could cause a discharge of static electricity that could ignite vapors.**

**Have plans in place that conform to local regulations to address solvent spills or leakage at your site.**

### Electrical Hazards: General

**⚠ WARNING**

***Placing the System***

**Do not locate this instrument near potential spark sources such as equipment with mechanical thermostats or line level power switches. Vapors that occur during normal operation due to open fraction collection vials may be ignited by external spark sources.**

**⚠ WARNING**

***Resetting of GFI Devices and Circuit Breakers***

**If the lab power outlet circuit breaker or GFI (Ground Fault Interrupter) is tripped, follow your company's procedures to ensure no hazardous conditions occur, such as an electrical spark igniting solvent vapors in the area.**

**If the rear panel circuit breaker is tripped, the area should be cleared of solvents and vapors before resetting the circuit breaker. If the breaker trips again, follow your company's guidance on lock out/tag out to prevent operation until the instrument can be repaired by a qualified service technician.**

*For Additional Information*

Technical assistance for the Teledyne LABS ACCQ*Prep* SFC 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



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# ACCQ*Prep*® SFC

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# Section 1 Introduction

## 1.1 Overview

This Operation Guide provides the following information:

- safety information
- instrument configuration options, including network configuration options
- operating instructions, including information on methods, separation, and fraction collection
- an overview of the PeakTrak® interface
- maintenance instructions

## 1.2 Product Overview

The Teledyne LABS ACCQ*Prep* SFC 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.

The ACCQ*Prep* SFC is configured to meet your needs. The Solvent Selector Valve supports up to four different choices for the co-solvent. Detection options include UV and UV/Vis., ELSD and a PurIon mass spectrometer capable of detecting your compounds with up to six individual mass signals, including a range of masses. Automation options include the AutoInjector, the AutoSampler, and the Column Selector modules.

*AutoInjector Module*

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

*AutoSampler Module*

The AutoSampler is an optional module for the ACCQ*Prep* system. When installed, it automates purification when you have several different samples that require multiple injections and different chromatographic conditions. It can be used unattended: it moves its probe robotically and automatically washes it

between samples to eliminate carry-over. The AutoSampler uses RFID rack swapping technology to allow you to replace completed racks.

*Column Selector Module*

The Column Selector module supports up to four Prep SFC columns ranging from 20 mm up to 50 mm inner diameter. (Larger columns may be used at less than optimum flow rates.) PeakTrak software operates the module's column selector valve.


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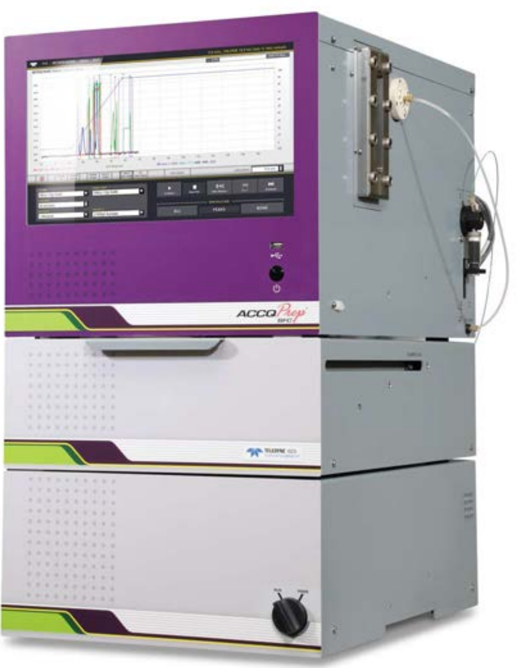

*Figure 1-1 ACCQPrep SFC*

**ACCQ*Prep* SFC –** This Prep SFC system has flow rates from 50–200 mL/min, with up to 6000 psi (413 Bar) capability. An integrated mass-flow meter allows for reproducible chromatography every day. A two component gradient can be formed between $CO_2$ and 4 different cosolvents. The system includes active solvent level sensing and detection of a full waste container. The base system includes an 8-port fractionation valve. Two different AutoSampler options allow open-bed fraction collection into either two or four collection racks. The RFID tagged racks can be replaced while in operation with new racks for practically limitless fraction collection.

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

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

## 1.3 Operating Overview

The ACCQ*Prep* SFC system is equipped with a capacitive touch-screen display for local control.

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

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

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

### 1.3.1 Multiple Control Possibilities

The ACCQ*Prep* SFC system can be accessed from the built-in touch panel or, alternatively, by using a mouse connected to one of its USB ports. Additionally, it can be accessed by up to ten network devices. The system performs the most recent command from any control input.

### 1.3.2 File Storage

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

## 1.4 Safety Components

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

Also, refer to the document *ACCQPrep SFC Important Information*, (PN 69-5263-088) for the most current safety information. This document is included with your ACCQ*Prep* system, and the latest version is available at www.teledynelabs.com.

### 1.4.1 Power disconnects

The back panel power cords are safety disconnects for the ACCQ*Prep* SFC system. There are two: one on the control box (Figure 1-2) and one on the oven module.

To remove power from the system, remove *both* power cords by pulling them straight out from their power inlet connectors. Additionally, circuit breaker rocker switches are located adjacent to the power inlet connectors. Pressing the end of a switch labeled “O” will remove power from that module’s internal operating components. If an internal circuit fault occurs, a breaker will trip. It can be reset pressing the end of the switch to the “|” position.


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*Figure 1-2 Location of the control box power cord and breaker*

### 1.4.2 System Pressure Management

The ACCQ*Prep* SFC monitors the pressure at several different points within the system. In addition to predefined pressure limits within the system, the user may input a lower column pressure limit in order to use and prevent damage to columns with lower than system-defined limits.

The ACCQ*Prep* SFC system has redundant safety devices to limit system pressure, the maximum system pressure is the lowest of these two values:

- 6,000 psi (412 bar) maximum system pressure; or
- the maximum pressure of the column as defined by the user in the Prep SFC Configuration.

*Overpressure Conditions*

Exceeding the system pressure limit results in a hard stop of the system.

The system pressure limit is a dynamic value based upon the column PRESSURE LIMIT + measured BPR PRESSURE.

The Pressure Gauge above the Gradient Plot area of the MAIN screen (Section 4.3.1) reports the system pressure, the measured BPR pressure, and the actual system pressure limit.

## 1.5 Specifications

| Table 1-1 ACCQ*Prep* SFC | |
|---|---|
| Dimensions of ACCQ*Prep* SFC<br>(Footprint without column installed) | 41 x 56 x 73 cm (16.25 x 22.25 x 28.75 in) (W x D x H) |
| Weight<br>(Including AutoInjector, Column Selector Valve Module, Pump Module, Column Oven, and Control Module) | 83.3 kg (183.5 lbs)<br>89.0 kg (196.3 lbs) with optional Evaporative Light Scattering Detector (ELSD) |
| Dimensions of ACCQPrep SFC AutoSampler AS 2x2 | 34 x 56 x 66 cm (13.5 X 22.25 X 26 in) (W x D x H) |
| Weight of ACCQ*Prep* AutoSampler AS 2x2 | 14.1 kg (31.1 lbs) |
| Dimensions of ACCQPrep SFC AutoSampler AS 4x2 | 58 x 56 x 66 cm (23 x 22.25 X 26 in) (W x D x H) |
| Weight of ACCQ*Prep* AutoSampler AS 4x2 | 19.1 kg (42.2 lbs) |
| User Interface | 15" high-resolution touchscreen |
| Power | Input voltage range: 120(+-10%) or 240 (+-10%) VAC;<br>300 VA maximum.<br>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)<br>(Environmental requirements for any optional modules are the same as those for the ACCQ*Prep* SFC system, except that the $CO_2$ chiller has a ambient temperature range of 5° to 35 °C (41° to 95 °F); and relative humidity maximum of 80%, noncondensing) |
| Humidity (when connected to power) | 90% relative humidity maximum at 20 to 40 °C (non-condensing) |
| Altitude | 2000 m (6561 feet) maximum |
| Flow Rate Range (system) | 50–200 mL/min |
| Flow Rate Accuracy | ± 2% |
| System Pressure Limit | 414 bar (6000 psi). The system pressure limit is the combination of the column pressure limit and the back pressure regulator (BPR) setting. |
| Pressure Accuracy | 5% of full scale |
| Gradient Formation | Binary gradient 4 solvent inlets. |
| Gradient Accuracy: | ±1% of full scale (typical) |
| Peak Detection Modes | Slope or threshold |
| Flow Cell Pathlength | 0.5 mm, ±25% (Standard) |
| UV Detection Wavelength | Variable UV PDA (200-400 nm) or<br>Variable UV-VIs PDA (200-800 nm) |
| UV Lamp Source | Deuterium |
| Wavelength Accuracy | ±5 nm |


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| Table 1-1 ACCQ*Prep* SFC | |
|---|---|
| **Optional ELSD** | |
| Gas Inlet Pressure | 60 to 70 psig |
| Gas Consumption | <2.5 SLPM |
| Drift Tube Temperature | Setting range: 30 to 90 °C<br>Minimum temperature is 5 °C above ambient<br>Maximum temperature is 60 °C above ambient |
| **Electrical Safety per EN 61010-1** | |
| Pollution Degree | 2 |
| Installation Category | II |
| Maximum Altitude | 2000 meters |
| Note 1. All specifications are subject to change. | |

| Table 1-2 ACCQ*Prep* SFC with PurIon System | |
|---|---|
| Dimensions (H x W x D) | ACCQ*Prep*: 27.5 x 14.0 x 20.0 in (69.9 x 35.6 x 50.8 cm)<br>Mass Spectrometer 26 x 11 x 22 in (66 x 28 x 56 cm)<br>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,<br>1 Dalton Resolution |
| | Electrospray Ionization (ESI) or optional Atmospheric Pressure Chemical Ionization (APCI) |
| | Positive or 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) |
| Flow Cell | PEEK, quartz, FEP |
| 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 |

## 1.6 FCC Statement

ACCQ*Prep* SFC systems with AutoSamplers are equipped with RFID rack recognition and contain modules with FCC ID number 2ADZBID-2 and are subject to the following statements:

1. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:
   a. This device may not cause harmful interference.
   b. This device must accept any interference received, including interference that may cause undesired operation.
2. Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.


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# ACCQ*Prep*® SFC

## *Section 2 Configuration*

### 2.1 Configuration of the ACCQ*Prep SFC*

The system can be configured using PeakTrak. For more about the user interface, see Section 4, *PeakTrak*.

### 2.2 Turning the System On and Off

For power control, ACCQ*Prep* SFC systems use a combination power switch and circuit breaker located on the rear panel of the control module and rear panel of the oven module.

For normal operation, the switch can be left on while the right side panel RUN/STANDBY push button is used to control most power circuits within the instrument. Power consumption while in Standby is about 6 W.

Use of the rear panel power switch basis is also acceptable. If used for power control, the system should be placed in Standby until the screen goes dark before switching the back panel power switch off to allow the internal operating system to complete an orderly shutdown.

**⚠ CAUTION**

Do NOT turn ON the main power of the AutoSampler while the ACCQ*Prep* SFC is turned ON.
Ensure that the AutoSampler is turned on before the ACCQ*Prep* SFC unit in order to avoid damage to the system.

***☑ Note***

The rear panel mounted circuit breaker is the power disconnect device.

## 2.3 Instrument Configuration

Instrument Configuration (Figure 2-1) can be found at TOOLS > CONFIGURATION > the INSTRUMENT CONFIGURATION tab.

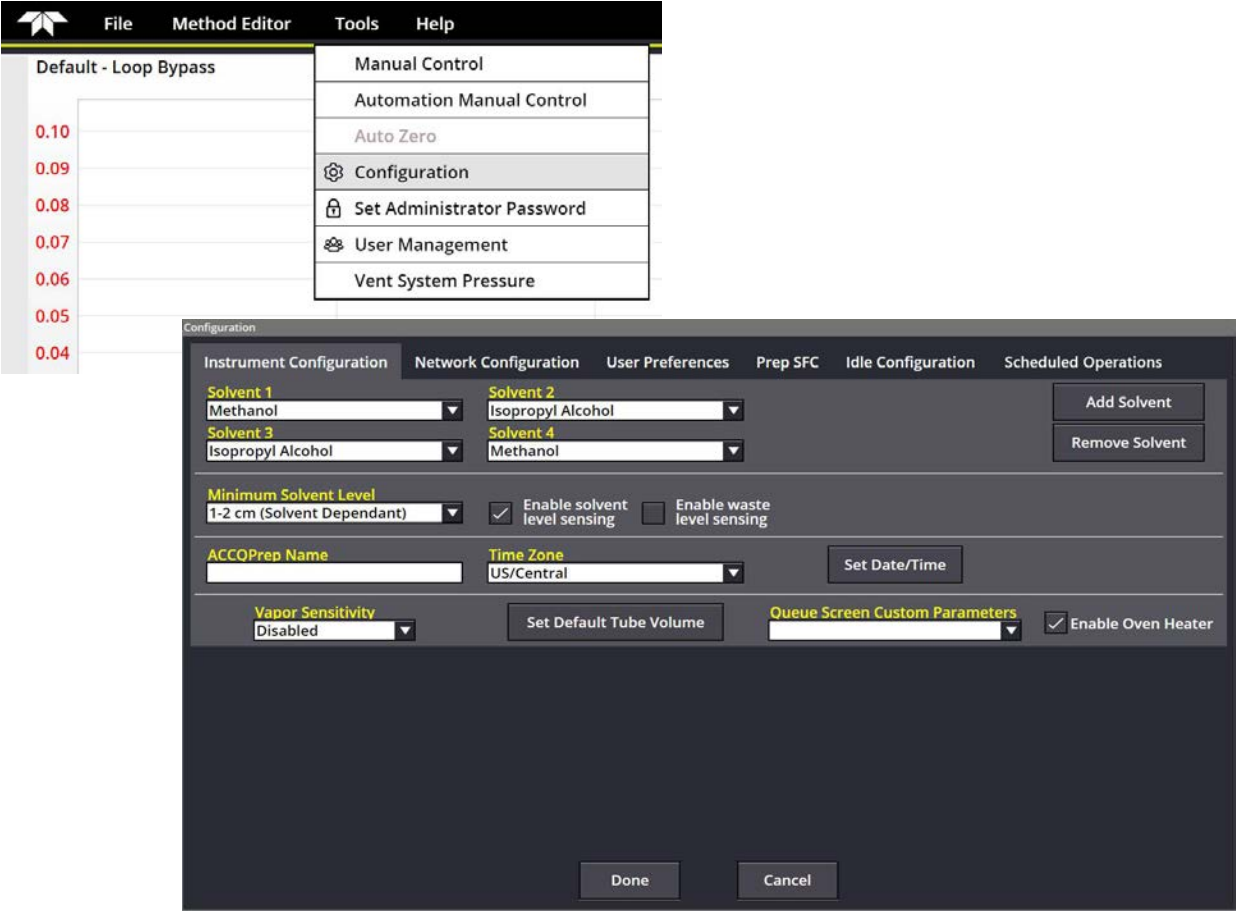


*Figure 2-1 The Instrument Configuration tab*

**☑ Note**

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

From the INSTRUMENT CONFIGURATION tab, you can select the cosolvents.

- ADD SOLVENT and REMOVE SOLVENT edit the list. If you do not see your solvent name on the list, select ADD SOLVENT and type in the solvent name. Select REMOVE SOLVENT to remove solvent names that you no longer use.

**Note**

The physical properties of each cosolvent require unique system settings for each cosolvent to ensure proper system performance, including peak cutting and fraction collection.

PeakTrak software automatically sets and compensates several system settings, depending on the chosen cosolvent. For proper system performance it is imperative that the proper cosolvent is chosen.

If a solvent you intend to use is not on the current solvent list, contact Teledyne LABS for guidance.

**Solvent Levels –**

- MINIMUM SOLVENT LEVEL allows you to specify a minimum level in centimeters.
- ENABLE SOLVENT LEVEL SENSING monitors the solvent level in each of the supply containers. If Solvent Level Sensing is enabled, the $CO_2$ inlet supply pressure is also monitored to notify the user that a low $CO_2$ supply condition is possible.
- ENABLE WASTE LEVEL SENSING stops the system when the waste level reaches approximately 1" (2.54 cm) above the sensing line.

**⚠ CAUTION**

If ENABLE WASTE LEVEL SENSING is unchecked, the waste collection container could overflow.

**System –**

- ACCQPREP NAME— Names the ACCQ*Prep* SFC system. At sites where multiple systems are in use, entering a name tells users which system a printed report came from.

**Setting Time Zones and Date/Time –**

- TIME ZONE — Sets the system to your time zone. The choice made here automatically changes the time during daylight savings time.
- SET DATE/TIME — Sets the system to your local day and current time. If an administrator password is set, setting the date and time will be required. All separations are tagged with the date and time of operation. If

the sample was not named previously, the date and time will be used as its identifier. Therefore, the system time provides a way of identifying samples.

**Vapor Sensitivity –** The ACCQ*Prep* SFC system has an internal vapor sensor in each of the system modules to detect organic vapors inside the system. The sensor is intended to monitor the system for leaks of internal plumbing connections. Fluid vapors external to the system may be detected when ambient air is drawn into the systems by the cooling fans. When the vapor level limit is exceeded, the system will stop the pumps to minimize a hazardous condition.

Currently, Teledyne LABS ships all systems with this vapor limit feature enabled to the default setting of HIGH sensitivity.

Sensitivity settings and the approximate percentage relative to the lower explosive limit (LEL) of hexane are provided in the table below.

**Table 2-1 Vapor Limits**

| Sensitivity Setting | Percentage (relative to the LEL of hexane) |
|---|---|
| Low | 45% |
| High | 5% |

Vapor sensor alerts from the oven module may occur when removing or installing columns or may be due to loose fittings. Alerts from other system modules or repetitive alarms from the oven module may indicate an internal leak to the system requiring evaluation from a qualified service engineer.

High sensitivity settings may result in false alarms (i.e., without an internal leak in the system) when the ambient vapor level in the area is high. If PeakTrak displays a vapor limit alarm (shown below), perform the following checks on your laboratory and on the instrument:

- Ensure that no open containers or spills of organic solvent are in close proximity to the system.
- Ensure that the system is located in a well-ventilated area.
- Ensure that there is no visible solvent leakage from the system.


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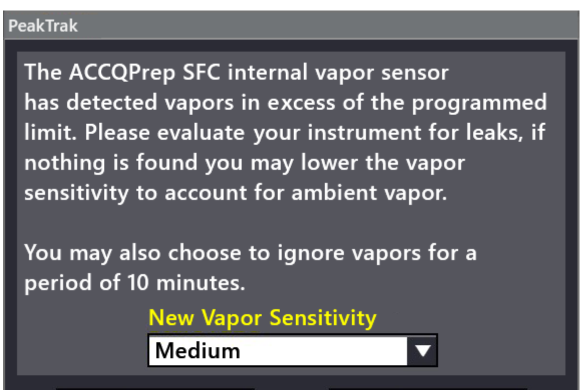


*Figure 2-2 PeakTrak vapor limit alarm*

After you have remedied the cause for the vapor sensor alarm, you may choose to change the default vapor sensitivity level, or ignore the vapor alarm and continue operation without the vapor sensor disabled for 10 minutes.

If PeakTrak continues to display the Vapor Limit alarm after you have made these checks and corrected any problems found, it is likely that excessive organic vapors are present in the ambient environment of your laboratory, or the laboratory has higher ambient humidity and/or temperature conditions. In such cases, choosing a lower sensitivity Vapor Limit setting is possible. Low sensitivity settings are appropriate for laboratory environments with a somewhat elevated background solvent vapor concentration. By changing the Vapor Limit sensitivity level, you assume any additional risk and reponsibility for taking any additional steps to mitigate the increased risk of disabling or lowering the vapor limit sensitivity level.

If you start a run when VAPOR SENSITIVITY is set to Disabled, the MINIMUM RUN REQUIREMENTS window will warn you of that fact.

**Queue Screen Custom Parameters** – A custom column option. The parameter chosen here displays as a column on the QUEUE tab of the MAIN screen.

**Set Default Tube Volume** – Determines how much is collected in each test tube. A suggested amount is preset, but if you prefer another level of fluid in each test tube, you can enter the tube volume you would like to use. This becomes the default volume used for new methods, but that can be changed within the METHOD EDITOR. PeakTrak automatically tracks the total liquid volume (cosolvent and make up flow) collected in each fraction vessel or test tube. Be sure to leave sufficient height in the vessel or tube for the z-axis fraction collector probe to direct fluid flow to the wall of the vessel without splattering.

PeakTrak automatically accounts for the amount of $CO_2$ that is part of the eluate and compensates so that it is only tracking the liquid volume.

**Enable Oven Heater –** Enables the column heater. Useful when tight temperature control is desired. The heater should be enabled for consistent retention times.

**Queue Screen Custom Parameters –** This sets extra parameters for the QUEUE tab (Section 3.3.4). These parameters change fraction collection options:

- None (no additional parameters)
- UV Threshold – level, as absorbance units (AU), to start peak collection
- Detection Ions – The ions used for peak collection (PurIon equipped systems only)

## 2.4 Network Configuration

**Network Configuration –**
Settings to connect the system to a network can be found under TOOLS > CONFIGURATION > the NETWORK CONFIGURATION tab.

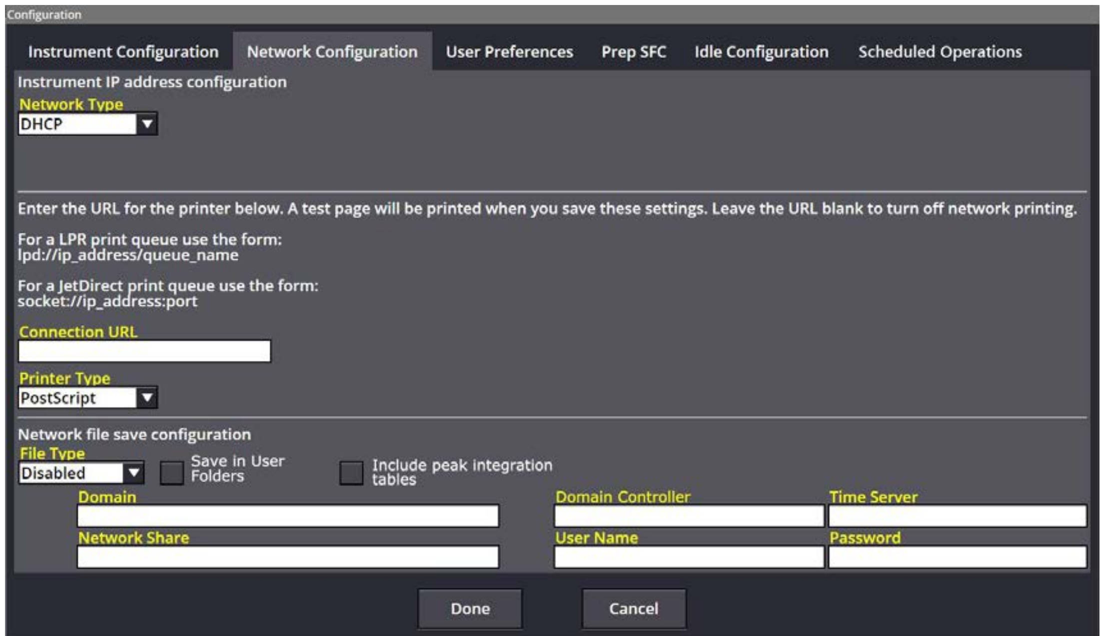


*Figure 2-3 The Network Configuration tab*

Once the system is connected to a network, you can remotely view the PeakTrak user interface to monitor or control the system, print to a network printer, or automatically save data files right to the network.

To connect to a network, you need access to IP addresses and network information from your Information Technology (IT) department. Contact your IT department before changing these settings. For more information on Network Configuration, see Technical Note 28, available at www.teledynelabs.com.

**Instrument IP Address Configuration –**
Specifies the instrument's network address and its parameters:


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- NETWORK TYPE –
  - Static IP: Requires an IP address, Netmask, and Gateway provided by your network administrator.
  - DHCP: Automatically retrieves the IP address, Netmask, and Gateway from the DHCP server.
- IP ADDRESS – The static IP Address for the system. This applies only when the NETWORK TYPE is STATIC IP.
- NETMASK – The Netmask for the system. This applies only when the NETWORK TYPE is STATIC IP.
- GATEWAY – The Gateway for the system. This applies only when the NETWORK TYPE is STATIC IP.

**Network Printing –** Configures the system for use with a network printer. JetDirect and line printer (LPR) queues are supported. Consult with your network administrator to determine printer information:

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

When this information is known, you can proceed with configuring system for network printing. Follow the on-screen instructions for entering the CONNECTION URL and PRINTER TYPE information. After successfully printing a test page, the network printer will be available for printing using FILE > PRINT and the AUTOMATICALLY PRINT AT END OF RUN feature.

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

## 2.5 User Preferences

Options for each user are found under TOOLS > CONFIGURATION > the USER PREFERENCES tab. The user for whom the settings will be made is listed at the top of the tab.

**☑ *Note***

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

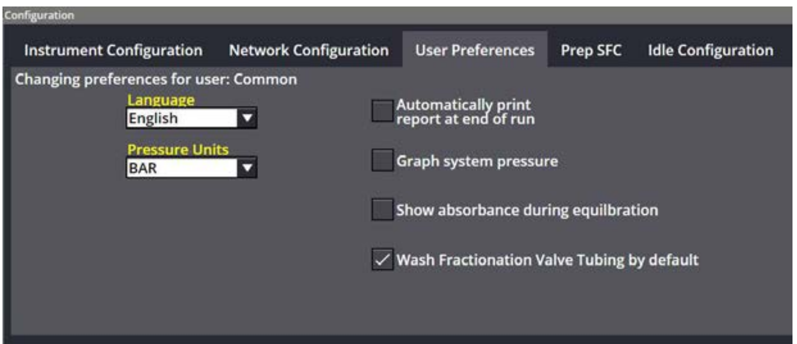


*Figure 2-4 The User Preferences tab (detail)*

**Language** – Allows selection from several languages for the user interface. If the system is set up for multiple users, each user can select a preferred language.

**Pressure Units** – Allows you to choose between PSI and Bar.

**Other User Preferences** –

- AUTOMATICALLY PRINT REPORT — Prints a report at the end of a run if the system is connected to a network.
- GRAPH SYSTEM PRESSURE — Shows the system pressure during a separation. This information is useful for troubleshooting a system that does not operate as expected.

  For normal operation, selecting GRAPH is not recommended. This information is always stored with the run data for later review, so if a problem is suspected, this parameter can be 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 the profile of the trace is often adequate for troubleshooting.
- SHOW ABSORBANCE DURING EQUILIBRATION — Shows absorbance of the system during equilibration to verify baseline stability.
- WASH FRACTIONATION VALVE TUBING BY DEFAULT — Makes a fractionation valve line wash the default for all runs that use the fractionation valve. It works by checking FLUSH FRACTIONATION VALVE TUBING AFTER SEPARATION by default on the COLLECTION DEFINITION window for each run, which directs the system to wash tubing after that separation. However, the wash step can still be turned off when starting an individual run by unchecking FLUSH FRACTIONATION VALVE.


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See Section 3.4.1 *SFC Fractionation Valve Line Wash* for more information about the wash process.

**☑ Note**

If a PurIon 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.

## 2.6 Prep SFC

Options to define and configure SFC columns and methods can be found under TOOLS > CONFIGURATION > the PREP SFC tab.

**☑ Note**

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

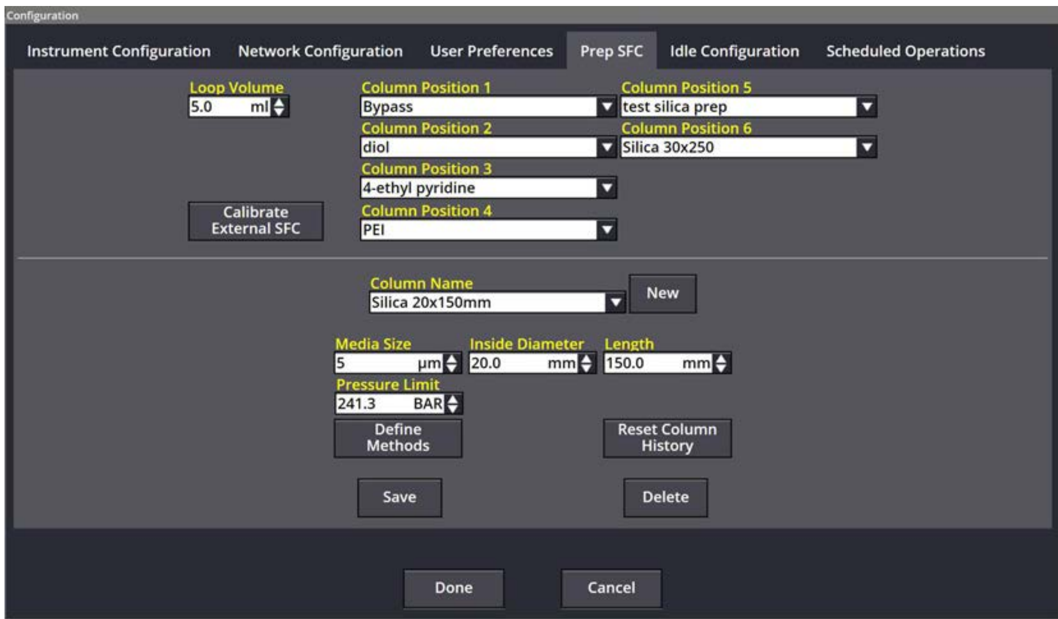


*Figure 2-5 The Prep SFC tab*

**Loop Volume** – The ACCQ*Prep* SFC system ships with a preinstalled 5 mL sample loop and a 1 mL loop (found in the accessory fittings package case). The installed loop volume is used for several calculations within the software, including for the Focus Gradient Generator tool. The 1 mL loop provides better injection accuracy and reproducibility when doing injections below 0.5 mL. Injections larger than this can effectively be done using the 5 mL loop.

If loops are changed on the system, it is important to update the LOOP VOLUME value to ensure that the system can identify any potential issues between the proposed injection volume and the size of the sample loop on the system.

- A warning is displayed if the injection volume inputted may exceed the sample loop capacity and sample is at risk of being lost through the injector.
- The system, however, does not limit the injection amount that can be programmed. This warning can be ignored at your discretion.

**Column Position 1, 2, etc. –** Maps a column to a specific position on the column selection valve.

- Each number matches a column definition to a corresponding column position in the ACCQ*Prep* SFC system as indicated by the labels on the lines that connect the columns. Defining these columns is described in Section 2.6.5 *Defining a Column*.
- Only defined columns are shown on the MAIN screen, where runs are started. If a column position is not plumbed, define the position as NOT INSTALLED to prevent accidental use of the position is advised.

### 2.6.1 Installing a Loop

Installing a loop is similar to installing a column (Section 2.6.4); however, you need a 1/4" wrench to connect the loop directly to the injection valve in the back of the oven box drawer.

*Figure 2-6 The sample injection valve*

### 2.6.2 Removing a Column from the System

Ensure that the column is stored under appropriate solvent storage conditions, as described by the column manufacturer.

1. Open TOOLS > MANUAL CONTROL.
   a. If the pumps are running, select STOP FLOW. (If the pumps aren't currently running, this button will not be visible.)
   b. Select the column position you wish to replace.
   c. Vent system pressure.
2. Open the oven box drawer.


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3. Gently loosen the outlet fitting on the column (or union) to release any pressure still on the column.

**⚠ CAUTION**

**Flowpath Under Pressure**

Before removing any component in the high-pressure flowline, such as tubing, the sample loop, in-line filter, column, etc. stop the pump and confirm the system pressure has fallen to zero. Depending on the position in the flow path, the system may need to be vented via Manual Control to release the pressure completely. Removing any of these parts while high pressure remains could cause the mobile phase and the clogging particles to eject rapidly and injury could occur.

4. After the column pressure is fully relieved from the outlet, the inlet fitting may be removed.
5. Connect the tubing from the column selection valve via a union.

### 2.6.3 Priming the Cosolvent Pump

Be sure that the cosolvent pump is properly primed before use, after changing or refilling solvents, and when directed. Otherwise, $CO_2$ can accumulate in the cosolvent fluid path and enter the sample injection flow path, which could lead to sample loss.

1. Make sure that adequate amounts of the desired cosolvents for priming are available to the solvent inlet lines.
2. Turn the manual prime valve control (Figure 2-7) to PRIME. The control is located to the right at the front of the pump module. This opens a PeakTrak solvent priming options window automatically (Figure 2-8).

*Figure 2-7 The manual prime valve control*

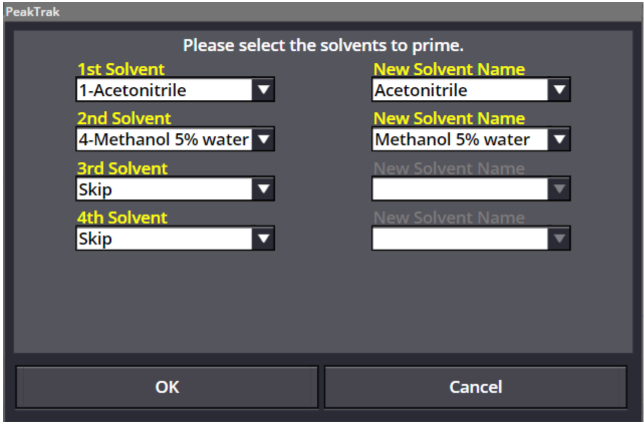


*Figure 2-8 The PeakTrak solvent priming options window*

3. Using the dropdown lists on the PeakTrak window, choose solvents for the priming process.
   - Solvents are run sequentially starting with the 1ST SOLVENT in the list and proceeding to the 2ND, etc.
   - The NEW SOLVENT name is the solvent that will run when priming with the corresponding solvent in the left-side list is complete.
   - Choose the last solvent that you plan to use for your next separation as the last solvent in the list.
   - Make sure that the solvents are input in an order in which they will be miscible.
4. Select OK. The system primes with the list of solvents entered.
   - A window that reports the expected completion time of the priming run in seconds appears for each solvent's priming cycle.
   - A "priming complete" window announces the completion of the priming process.
5. Turn the manual prime valve control to RUN.

### 2.6.4 Loading a Column onto the System

**Note**

Follow column manufacturer guidelines for removal of columns and column storage conditions.

The COLUMN POSITION numbers on the CONFIGURATION window correspond to column positions in the ACCQ*Prep* SFC system, as indicated by the labels on the lines that connect the columns.

To connect a column to the system:

1. Open TOOLS > MANUAL CONTROL.


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a. If the pumps are running, select STOP FLOW. (If the pumps aren't currently running, this button will not be visible.)

b. Select the column position you wish to replace.

c. Vent system pressure.

2. Open the oven box drawer.
3. Ensure the column position you want to use is fully vented. (Refer to 2.6.2 *Removing a Column from the System*.)
4. Locate a pair of lines having the same label number for both sides of the column. Take note of the number; you will need it when you define a column (Section 2.6.5).
5. Connect the lines located in Step 4 to the column. Make sure that the column is oriented so that its arrow label matches the direction of arrows on the connecting lines. One end of each line should be connected to the column. The other end of each line should remain connected to the column select valve at the rear of the oven drawer.

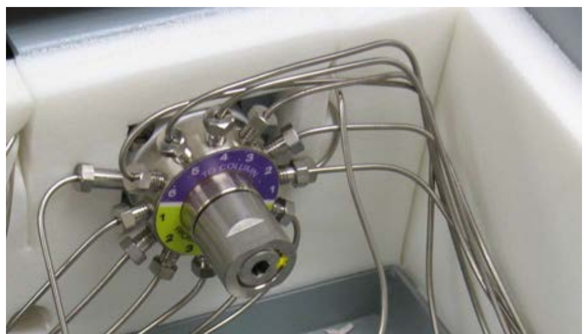

*Figure 2-9 The column select valve*

6. While the oven drawer is open, set the flow rate to 30 mL/min, choose 5% cosolvent, and select PUMP 5% COSOLVENT. Monitor the oven box and column fittings for any leaks. After you confirm that there are no leaks, you may close the oven drawer and use the column.

*Configuration > PrepSFC (tab) > Calibrate External SFC (button)*

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

To calibrate the external SFC, run universal test mix on both the external SFC and the ACCQ*Prep* with matching chemistry columns. Refer to Teledyne LABS *Chromatography Technical Note CHR-TN2410* for that procedure. After the parameters are determined, enter them into the ACCQPrep *SFC*:

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

4. Select OK to dismiss CONFIGURATION and to reveal the MAIN screen.

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

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

*The Calibrate External SFC window*

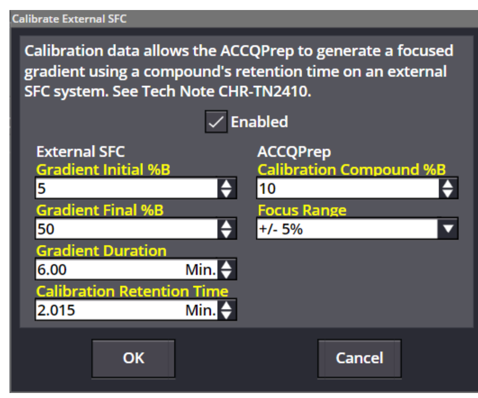


*Figure 2-10 The Calibrate External SFC window*

**ENABLED** – Provides gradient and calibration options used after calibrating an analytical SFC system to the preparative SFC; it also displays this option in the column method choices.

**External SFC options**

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

**ACCQ*Prep* SFC options**

- CALIBRATION COMPOUND %B – The %B that causes the calibration compound to elute at the correct retention time.
- FOCUS RANGE – This is commonly ±5%.


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### 2.6.5 Defining a Column

Accurate column settings are important to protecting your column, as many columns have pressure limits below the system pressure limit of the ACCQ*Prep* SFC system. PeakTrak automatically monitors the unique pressure limits of each column to prevent damage to columns from over-pressure events.

Additionally, column size and type parameters are important to update for the system to work optimally. The calculated column volume from these parameters are used in several key features of the system, including the Focus Gradient Generator and peak alignment.

Follow these steps to define a column:

1. Create a new column:
   a. On the MAIN screen, select TOOLS > CONFIGURATION. The CONFIGURATION window opens. On the PREP SFC tab, select the NEW button next to the COLUMN NAME menu. A window opens requesting a column name.
   b. Enter a name for the new column into the field. This name can describe the column or, for example, it can identify it exactly using the column serial number.
2. After the name is entered, select ENTER.
3. To complete the column definition, enter the MEDIA SIZE and column dimensions.
4. Select the INSIDE DIAMETER of the column. A flow rate for a default method is selected on the basis of the scaled-up linear velocity of a 4.6 mL analytical column running at 1 mL/min.
5. Select the LENGTH of the column. This provides the suggested default method 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 a typical limit for preparative SFC columns, but this can be changed for each column to a suitable pressure not exceeding the system pressure limit of 6000 psi. Refer to literature included with the column when entering the PRESSURE LIMIT.
7. SAVE the default method associated with the column.

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

Once the column definition is created, you can use it by assigning it to a column position on this window corresponding to a matching installed column; that is, to COLUMN POSITION 1, COLUMN POSITION 2, etc.

### 2.6.6 Resetting Column History

If you are replacing a column in a position with an identical column, you needn't create a new column definition, Instead, you can reuse the existing one by choosing its COLUMN NAME and

selecting RESET COLUMN HISTORY. That history includes the number of runs performed with the column and the solvents used for those runs.

### 2.6.7 Deleting a Column

To delete a column at CONFIGURATION > PREP HPLC:

1. Make sure that the to-be-deleted-column is not selected in a COLUMN POSITION field.
2. Select the column you would like to delete from the COLUMN NAME list.
3. Select DELETE.
4. If an error message states the method is currently in use, leave the CONFIGURATION window and go the to RUN screen. From there, select a different column.

### 2.6.8 Creating Other Methods

Methods created in the PREP SFC tab are associated with a particular column when created. If multiple methods are associated with a single column, these methods can be easily accessed whenever a particular column is selected. These methods are shown on the MAIN screen and the METHOD EDITOR in the column selection lists in the order that the methods were created.

Follow these steps to create the new method for a column:

1. Select TOOLS > CONFIGURATION. The CONFIGURATION window opens.
2. On the PREP SFC tab, select the COLUMN NAME that you want to associate with the new method.
3. Select DEFINE METHODS on the PREP SFC tab (Figure 2-11). The tab then shows options to define methods for your selected column (Figure 2-12).

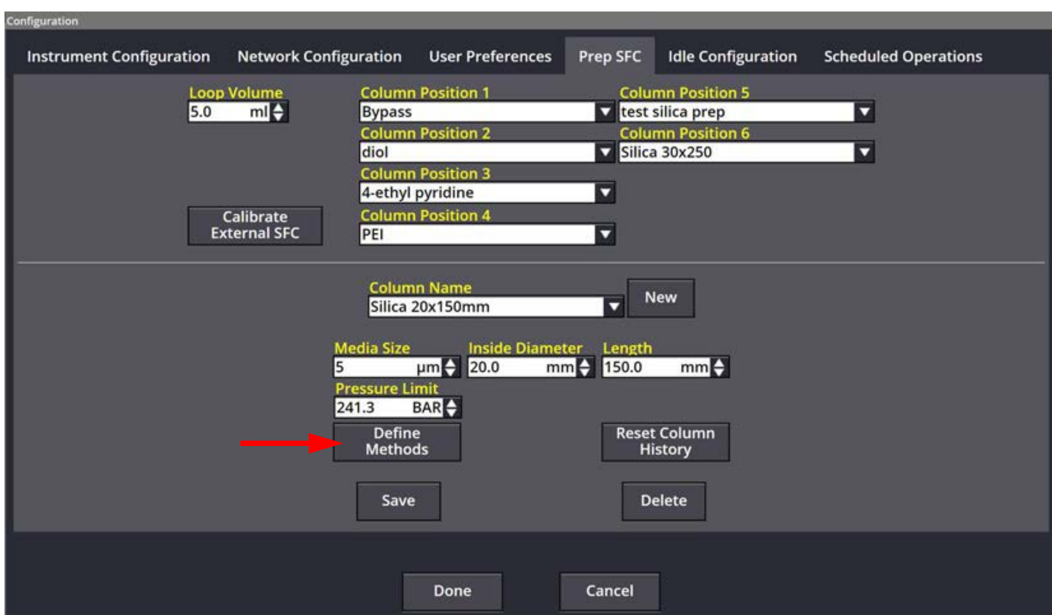


*Figure 2-11 The Define Methods button*


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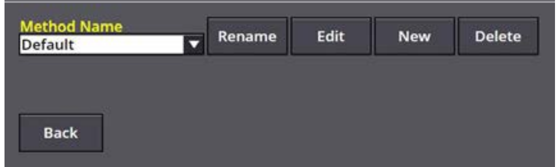


*Figure 2-12 Define Methods options on the Prep SFC tab (detail)*

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

### 2.6.9 Creating Scouting and Focused Gradients

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

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

1. Select TOOLS > CONFIGURATION. The Configuration window opens.
2. On the PREP SFC tab, select the COLUMN NAME that you want to associate with a scouting gradient (Figure 2-13).

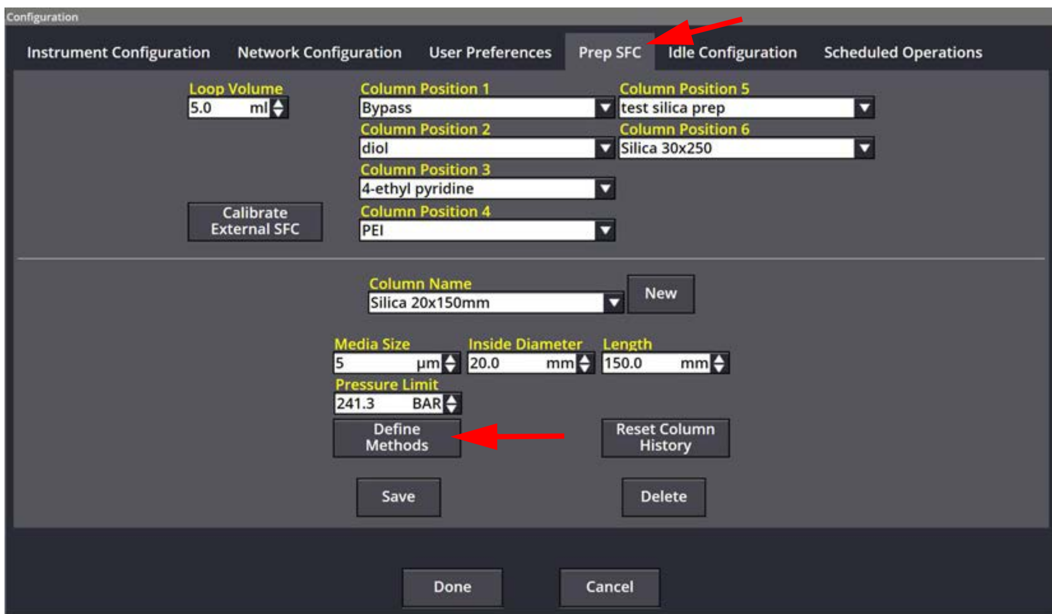


*Figure 2-13 Column Configuration*

3. Select DEFINE METHODS on the PREP SFC tab. The tab then shows options to define methods for your selected column.
4. Select NEW SCOUTING METHOD. This opens the SCOUTING METHOD FOR FOCUSED GRADIENT window (Figure 2-14).
5. Define the FLOW RATE, the INITIAL %B, and the FOCUS RANGE, and assign a METHOD NAME to the scouting method. This name will then be listed among the methods that can be selected to run on the column that you selected.

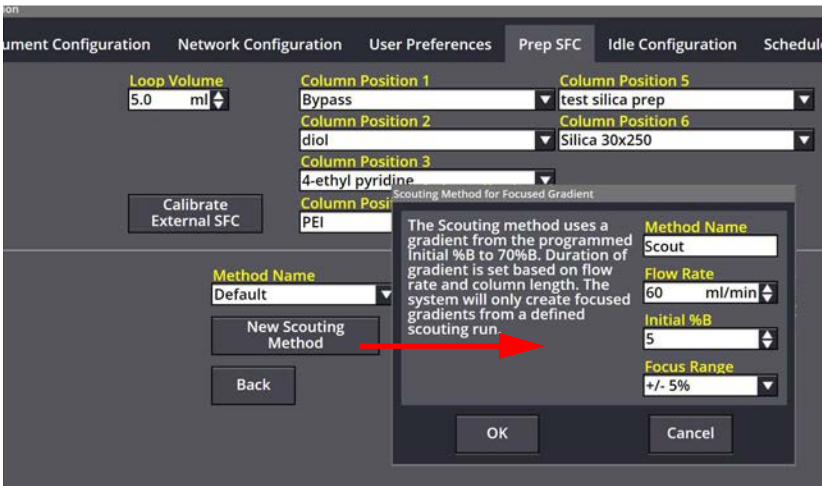


*Figure 2-14 Scouting Method definition window*

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


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7. In the MAIN screen, select the column that you want to use for the run.
8. Select the scouting method you previously defined for that column (Figure 2-15).

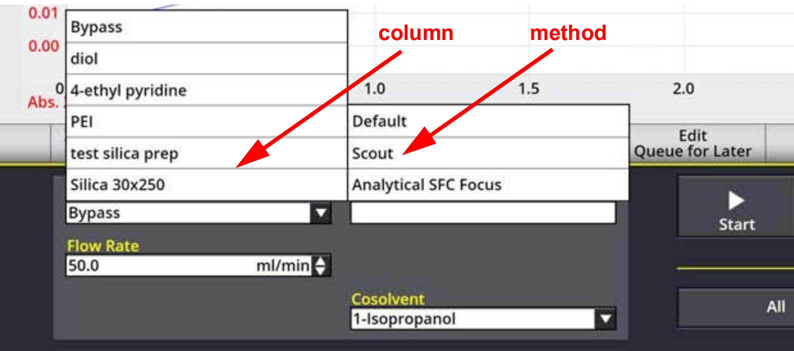


*Figure 2-15 Loading the Scouting method*

9. After the column and method are selected, select START.
10. Input your injection parameters as usual, and let the run proceed (Figure 2-16).

*Figure 2-16 A Scouting Run*

A RUN window appears upon completion of the run:

*Figure 2-17 Run file window at end of run.*

11. Define the focused gradient:

    a. Select the FOCUS GRADIENT button from the bottom right of the Run File window (Figure 2-17). The FOCUSED GRADIENT DEFINITION window appears (Figure 2-18).

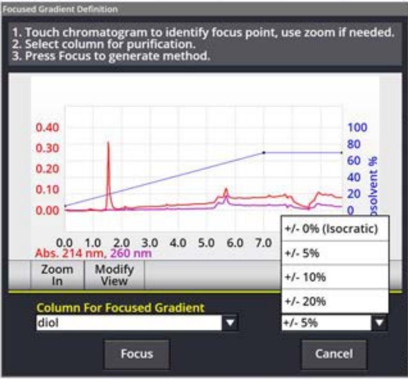


*Figure 2-18 Focused Gradient Definition window*

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

    c. Select the column for which to generate the optimized focused gradient from the list at the bottom of the window.

    d. Select a FOCUS RANGE (±0% to ±20%).

12. Select FOCUS. This generates an optimized method centered on the selected peak.

    A possibility: a dialog warns you that the target compound will eluted have too early or too late, so the generated


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method may not be truly optimal. Possibly, early elution could be remedied by using another solvent system or column. See 3.5 *Operation Troubleshooting* (*Peaks elute too early* and *Peaks elute too late*) and the Teledyne's Technical Note *Focused Gradients— What Do You Mean the Compound Eluted Too Early or Too Late?!* (TN61).

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

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

## 2.7 Idle Configuration

Configuration controls are provided to schedule and time operation of the heaters and the pump. Setting these controls provides a way to balance convenience with energy and solvent savings.

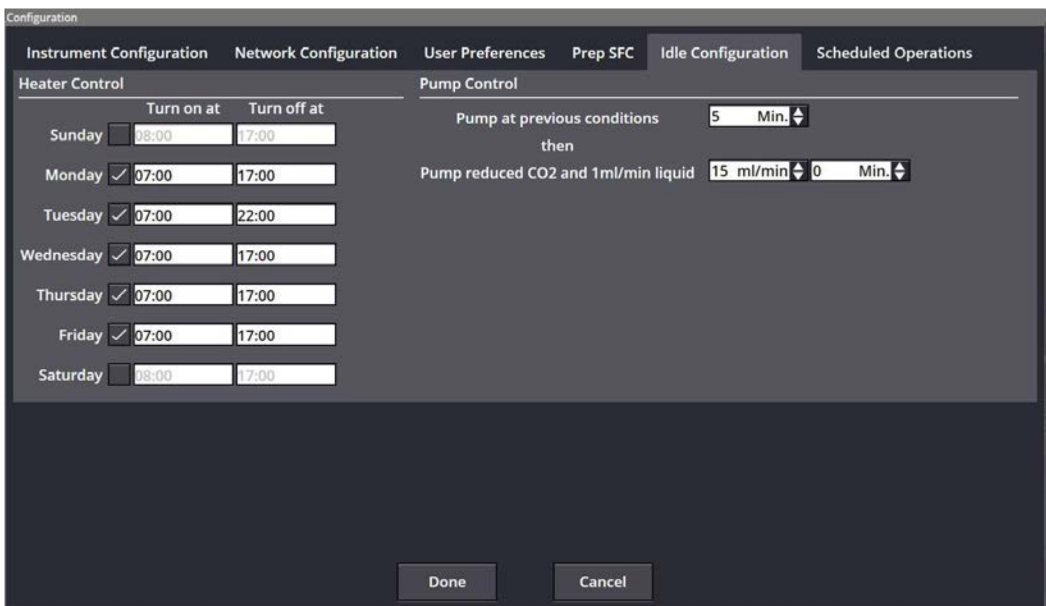


*Figure 2-19 The Idle Configuration tab*

The following options are found under TOOLS > CONFIGURATION > the IDLE CONFIGURATION tab:

**Heater Control** — Heaters can be scheduled to run on certain days and at certain times. This allows the heaters to be prewarmed in advance while using less energy than leaving the heaters on continuously. Without prewarming, the heaters could take from several minutes to a half an hour to warm up.

- SUNDAY, MONDAY, etc. — Selects days and schedules times at which heaters automatically turn on and off. The instrument can still be used on days that are not selected, but it will then be necessary to wait as the heaters warm up.

**Pump Control** — When the pumps start up, they take time

to pressurize the system with $CO_2$ and stabilize the flow. Pump Control allows the pumps to remain running after an operation is stopped. This provides for immediate use of the system, while reducing the use of solvent by limiting how long it is pumped. Such operations include performing a run, and operating the pumps in manual control.

- PUMP AT PREVIOUS CONDITIONS — Sets the length of time the pumps continue to run without any user input with the same flow rate and concentration before stopping. This results in the fastest start time, since the column's condition is left unchanged.
- PUMP REDUCED CO2 AND 1ML/MIN LIQUID — After the PUMP AT PREVIOUS CONDITIONS time expires, the PUMP REDUCED option allows the system to slow down the pump while keeping it running. This changes the concentration on the column, but allows running without waiting for the pumps to start up.

  Choose the flow rate at which the $CO_2$ pump runs and the number of minutes this flow rate will be used before the pumps are stopped. As noted on the CONFIGURATION window, the liquid always pumps at 1 mL/minute in this operating mode.

  - To pump at the previous rate only, set the PUMP REDUCED rate timing 0 minutes.
  - To pump at the reduced rate only, set PUMP AT PREVIOUS CONDITIONS to 0 minutes, then set the PUMP REDUCED rate timing to 1 minute or greater.

If times greater than 0 minutes are set for both pump options, the pump will operate at the previous rate for the specified time and then pump at the reduced rate for the specified time.

## 2.8 Scheduled Operations

Scheduled Operations can be found under: TOOLS > CONFIGURATION > SCHEDULED OPERATIONS (Figure 2-3).

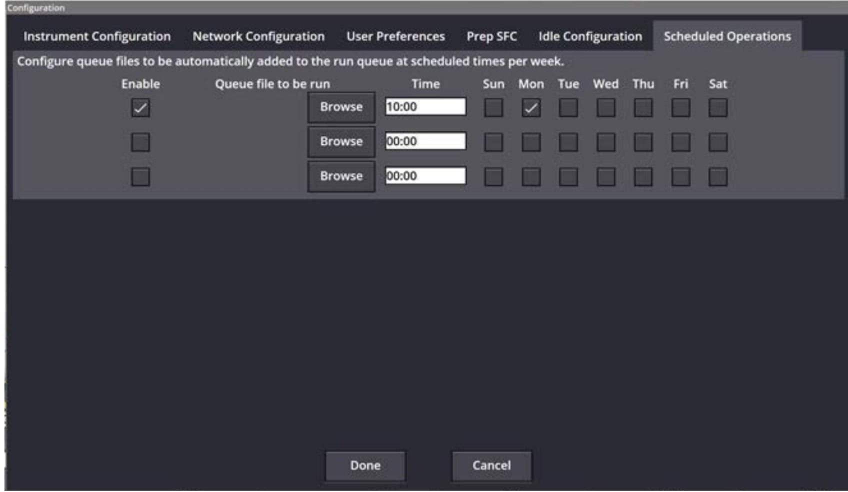


*Figure 2-20 Scheduled Operations*


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Here, routine operations can be scheduled to be run as needed. For example, you can create entries to

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

The operations to be run must be saved to queue files. When ENABLE is selected, BROWSE to select a queue file, then select a TIME and a day. The TIME must be entered in a 24:00 format. The TIME ZONE set in CONFIGURATION (Section 2.3) is used by the scheduler.

Entries are added to the active queue and are run after queued samples.

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# Section 3 Operation

## 3.1 Introduction

The ACCQ*Prep* SFC system is unique to the chromatography market. It provides several methods of operation that you can select to fit your needs.

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

One of the unique features of the ACCQ*Prep* SFC system is that a defined method file is not required for operation. You can load and modify a default or existing method for the current separation without needing to save the newly modified method.

In addition, any of the method parameters can be modified during the separation. None of these changes 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 that no information is lost.

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

## 3.2 Method Selection

There is more than one way to select a method:

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

If the method selected is adequate, select START to continue. If the method is not exactly as needed, it can be edited. Edit the common parameters of any method 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.

## 3.3 Starting a Separation

To separate a single sample, select START to start a separation. The MINIMUM RUN REQUIREMENTS and SAMPLE OPTIONS windows are displayed. There, you can select an injection technique (using the AutoSampler or the AutoInjector with the external sample loading probe) and select where the fractions will be collected (that is, into the fractionation valve or into the selected starting rack and tube position on the open-bed fraction collector on the AutoSampler module). The system projects the amount of solvent required and the separation duration.

**☑ Note**

It is important to use the supplied external sample loading probe or AutoSampler sample probe without modification because the system uses the known volumes of these probes in the sample loading process to ensure accurate sample injection.

*For an ELSD*

If an ELSD is installed on the system, MINIMUM RUN REQUIREMENTS provides the option to use the ELSD or disable it. Typically, ELSD method parameters are determined by the solvents used and are therefore somewhat universal when doing preparative separations of nonvolatile samples. Semi-volatile compounds may require optimization of the ELSD parameters.

**☑ Note**

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

*For a PurIon*

If a PurIon mass spectrometer is connected, the MINIMUM RUN REQUIREMENTS window 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.)

PurIon detection options can also be accessed from the METHOD EDITOR. Further information about these options can be found in 4.3.2 *Method Editor* (under *Peak Detection*).

### 3.3.1 Separation with an AutoInjector

1. Select START to start a separation. The MINIMUM RUN REQUIREMENTS window opens and, in front of it, the SAMPLE OPTIONS window.


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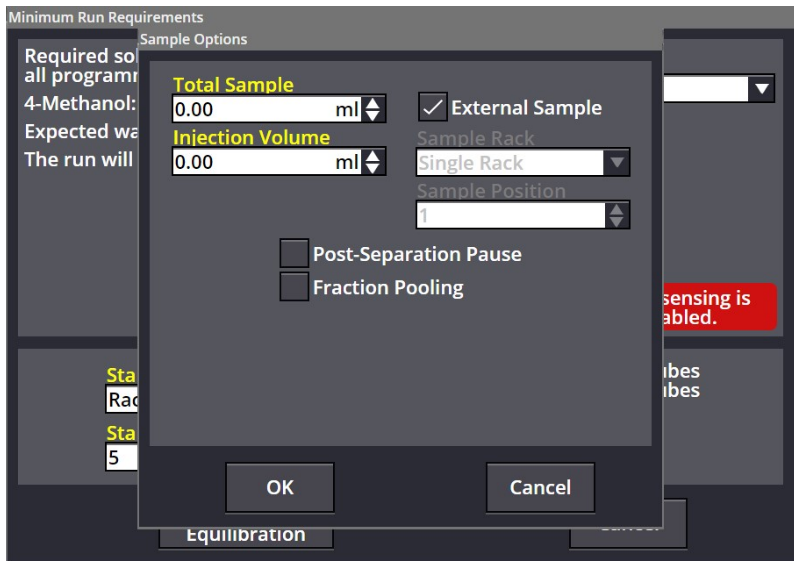


*Figure 3-1 Selecting the sample, injection volume, and parameters*

2. On the SAMPLE OPTIONS window, enter the TOTAL SAMPLE volume and the INJECTION VOLUME for each. Be aware that PeakTrak divides the total volume by the injection volume to determine the number of injections for each separation. The number of injections that these two volumes will yield is listed on the window.

> **Note**
> If you change loops on the system, it is important to enter the correct LOOP VOLUME (TOOLS > CONFIGURATION > the PREP SFC tab; see Section 2.6). This ensures that the samples entered do not greatly exceed the loop volume amount per injection.

During the programmed separation sequence, any changes made to the method are included on any of the remaining injections.

3. When no AutoSampler is installed or the FRACTIONATION VALVE is chosen for collection (Section 3.4), the COLLECTION DEFINITION window opens as well.
   a. Select a START and an END POSITION.
   b. Select a CONTAINER CAPACITY.
   c. When using a fractionation valve, FLUSH FRACTIONATION VALVE TUBING AFTER SEPARATION flushes the lines used during the run. The wash removes any sample that might be left inside the lines to improve sample recovery and to prepare the system for the next purification. See 3.4.1 *SFC Fractionation Valve Line Wash* for details.

**Note**

If Wash Fractionation Valve Tubing by default is checked in the Tools > Configuration window, Flush Fractionation Valve will be checked on the Collection Definition window by default.

d. Dismiss the COLLECTION DEFINITION window.

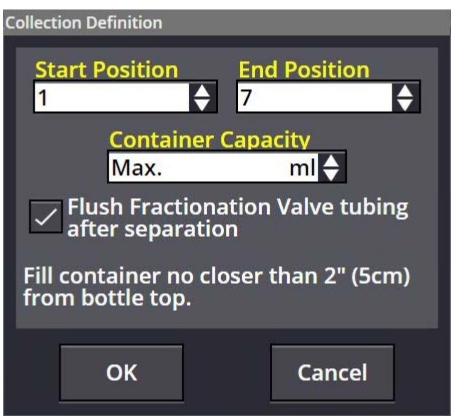


*Figure 3-2 The Collection Definition window*

4. Otherwise, if collecting fractions on the AutoSampler, choose the rack and starting tube position on the MINIMUM RUN REQUIREMENTS window as needed.

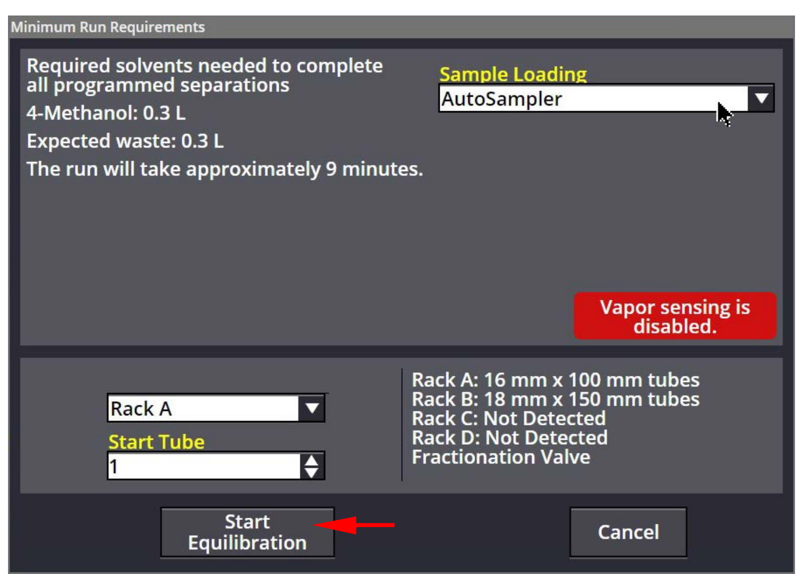


*Figure 3-3 Starting the equilibration from the Minimum Run Requirements window*

5. After you START EQUILIBRATION, the system prompts you to place the sample probe into your sample container (or wash solvent container to prime the probe). Then, the system prompts you to either load the sample or prime the probe.


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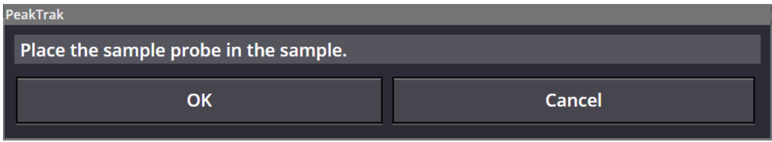


*Figure 3-4 Sample probe prompt*

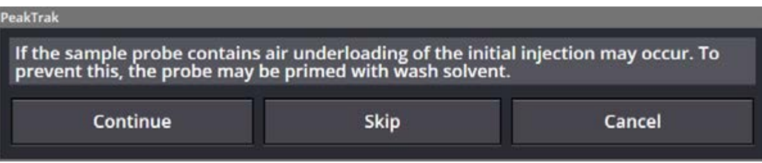


*Figure 3-5 Sample probe prime prompt*

a. In most instances, you should prime the probe.

b. After the priming sequence is complete, the system presents the same two options and instructs you to lift the sample probe to insert a small air gap between the sample and priming solvent. Doing so prevents mixing during loading and ensures higher injection accuracy for the first few injections.

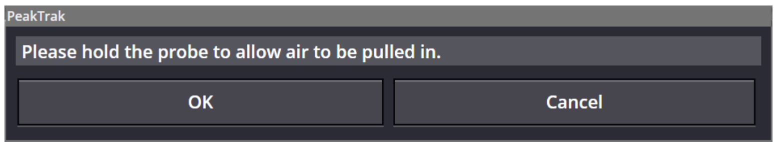


*Figure 3-6 The probe air pull prompt*

c. After the air gap is inserted, place the probe in the sample container and select LOAD SAMPLE.

**☑ Note**

Ensure the sample container is secured and the sample probe is secured ensuring it is at the bottom of the sample container to ensure as much sample as possible is loaded.

**☑ Note**

Note: The system begins any equilibration in the background but the run won't begin (and the equilibration will continue with the pumps running) until you acknowledge that the sample probe is in the sample container.

- If the sample probe is ever lost or damaged, it should be replaced with the AutoInjector Needle Assembly. (Visit https://store.teledyneisco.com for parts for your ACCQ*Prep* SFC system, or contact your local Teledyne LABS representative.)

6. After the last injection is performed, a sample probe wash dialog is shown, prompting you to wash the probe with a strong solvent. Perform these duties as requested.
7. If FLUSH FRACTIONATION VALVE TUBING AFTER SEPARATION was configured (step 3c), an SFC fractionation valve line wash takes place. Messages are displayed during and after the fractionation line wash:

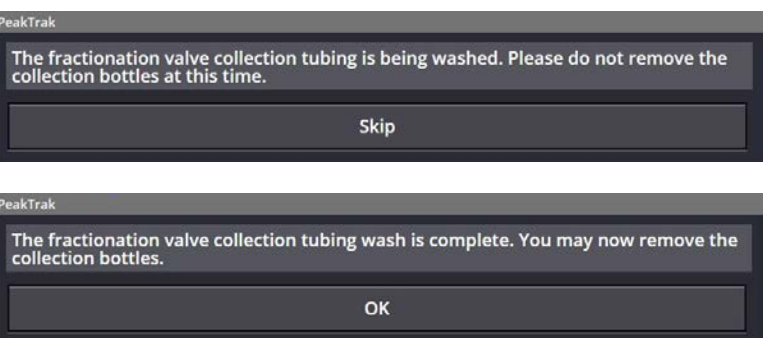


*Figure 3-7 Messages during and after line washing*

8. At the completion of the sequence, the final separation results are displayed.

*Viewing previous separations*

To view previous separations, select RESET to return to the HOME screen:

- 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 is named A01-I1, the second is A01-I2, and the third is named A01-I3, etc.

9. Select the file you want to display. The RUN FILE window opens.
10. The remaining injections can be immediately viewed by selecting the left (<) or right (>) arrows at the lower left and right of the file viewer window 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.



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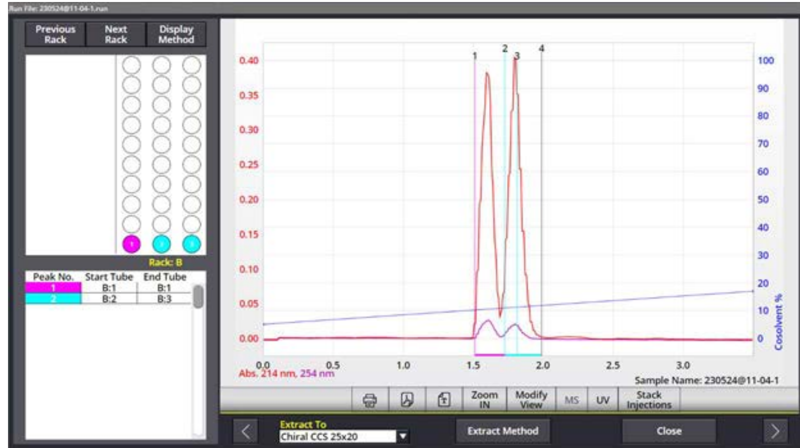
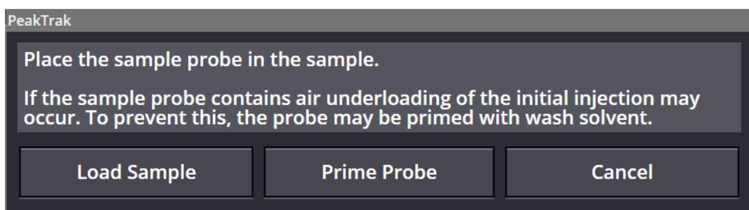



*Figure 3-8 Viewing injections*

### 3.3.2 AutoInjection Operating Steps

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

1. A prompt appears, allowing you to either prime the sample probe with solvent or to place the sample probe into the sample container before the separation begins. Select PRIME PROBE and go to step 2; otherwise, proceed to step 3.




2. You are prompted to place the sample probe into the sample container before the separation begins.
3. During column equilibrium, the sample loop is first placed into the run position. This position passes the cosolvent through the loop to wash out any leftover fluid from a previous separation and fills the loop with the cosolvent from the current separation. This prevents any strong solvent remaining in the loop from affecting the current separation.
4. Near the end of the equilibration, the injection valve moves to the sample load position.
5. The AutoInjector Module aspirates enough sample to fill the loop with the programmed amount and to compensate for the volume of the sample probe.
6. After the equilibration has completed, the injection valve moves to the separation position and the separation process continues.

7. After the first separation has completed, the next separation begins with the equilibration as described in steps 3–6. During this process, the AutoInjector Module continues to aspirate the programmed injection volume.
8. When the sample is aspirated for the final injection of the sequence, the total volume of all the sample injections matches the programmed amount.

- If the sample container held less sample than programmed, the final injection aspirates a small amount of air. Small amounts of air will not damage the 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, programming the injection sequence with about 0.5 mL more sample volume than the actual sample amount provided in the sample container is recommended. This ensures all of your sample is processed.
- If some sample remains in the sample probe (probe volume is approximately 43 mL), you can recover it by loosening the sample probe fitting at the injection valve to allow the sample to drain back into the sample container.

After completion of the final injection, the system washes the sample probe to prevent sample carryover during the next separation. To perform the wash, the system prompts you to place the probe in a strong wash solvent. This solvent should be capable of completely washing the sample from the probe. Then, 10 mL of strong solvent is aspirated through the probe to wash away any remaining sample from the probe.

### 3.3.3 Separation using an AutoSampler Module

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

To begin a separation sequence with the AutoSampler Module, do any of the following:

- **Create a sample queue entry**.
- **Select START to start with a single sample.** Selecting START displays the MINIMUM RUN REQUIREMENTS window, from which you can perform multiple injections on a single sample from the AutoSampler Module. A queue is created automatically.
- **Access the RUN tab.** This results in a screen and operation much like the standard ACCQ*Prep* SFC system without an installed AutoSampler Module.
- **Select the QUEUE button.** This allows you to set up a separation sequence for multiple samples.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### 3.3.4 Creating AutoSampler Queues

A queue can be created for use with the AutoSampler Module:

1. Install a sample vial rack before opening the QUEUE screen. This allows the software to limit sample programming to the positions available for sample vials.
2. Select the EDIT QUEUE button below Gradient Plot Area. The QUEUE screen opens (3.3.5 *The Queue Screen*). Select a row to edit its column entries. (You can also populate the screen with entries that you have saved to a file earlier using IMPORT SAMPLE LIST INTO QUEUE.) See Section 3.3.5 for a discussion of the QUEUE screen's column options.

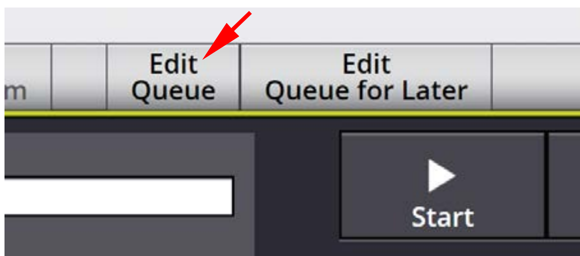


*Figure 3-9 The Edit Queue button*

3. After the queue is complete, either select the RUN window and select the START or select the menu (≡) on the first unfinished run and select START RUN. In either case, the RUN REQUIREMENTS window appears. Accept or modify the starting rack or tube for fraction collection.
4. While a separation is in process, you can access the QUEUE screen to add samples to the queue. Samples that have been completed cannot be edited. They can be viewed, however, 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 displays a prompt to determine if the rack contains empty tubes. If you respond that the tubes are empty, PeakTrak considers this rack available for future separations. Otherwise, PeakTrak continues to mark this rack as full of samples and not available for fraction use. This feature allows you to remove a rack to obtain a sample for verification while leaving the rack in the instrument for convenience and later removal.
6. After the entire queue has completed, the RESULT window is displayed with the last separation. To easily view previous separations, select the separation on the QUEUE window or select FILE > OPEN to view previous separations.

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

For example:

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

Steps 1-6 show you how to create a queue from scratch. However, you can also export such a list to a plain text file (.txt) for future use by selecting EXPORT COLUMN/METHOD LIST. Later, you can import that list to populate the QUEUE screen by selecting IMPORT SAMPLE LIST INTO QUEUE.

### 3.3.5 The Queue Screen

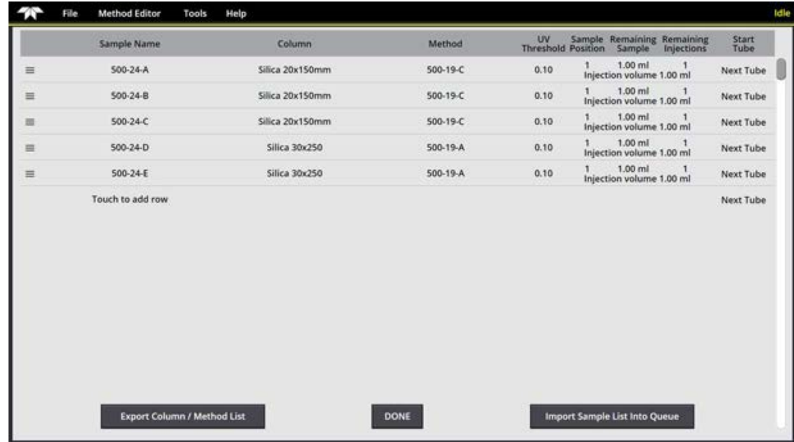


*Figure 3-10 Automation Queue screen*

Create new entries on the QUEUE screen by touching to add a row, then by editing the fields under each column.

**☑ Note**

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

**≡ (menu icon)** – Provides commands to start the separation (START RUN) from the QUEUE window, to remove a single row of an existing queue (REMOVE ROW), to ENABLE... or DISABLE POST-SEPARATION PAUSE, or to MOVE it to the QUEUE FOR LATER (Section 3.3.6).

- ENABLE POST-SEPARATION PAUSE — Halts the system after the first separation is completed for the sample. This allows you to adjust the separation parameters on the basis of 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
  - change the injection volume or otherwise modify the method as needed and disable the pause for the remaining injections, or
  - leave it enabled to allow a second condition scouting separation.

  Samples with pauses are easily found on the QUEUE window: their rows are highlighted.

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

**Column** – Lists a separation column and an associated method for use.

**Method** – After a column has been selected, the default method created for that column is automatically loaded. To change this method, touch the method name. A list of methods associated with the column appears. These methods are listed in the order they were created. Alternatively, select CUSTOMIZE CURRENT or BROWSE to populate this field.

Select CUSTOMIZE CURRENT from the list of methods 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” is 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** – The location of the sample for this separation. If the sample size is too large for a single vial, create a second line in the queue and access the second vial.

**Sample Volume** – The amount of sample to be separated. Typically, the amount you enter is slightly greater than the amount of sample to ensure that 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.

**Injection Volume** – Determines the number of injections in conjunction with the SAMPLE VOLUME column.

*The Sample Options Window*

Selecting a row under the sample or injection columns opens the SAMPLE OPTIONS window that configures them. (Also, a version of this window may open when you start a separation.)

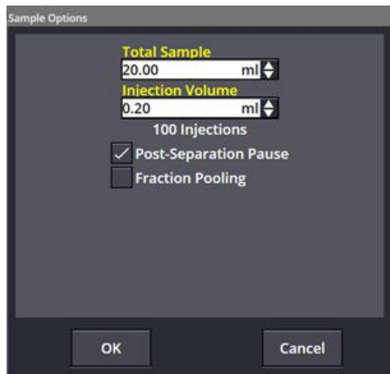


*Figure 3-11 The Sample Options window*

- TOTAL SAMPLE — The volume of the sample, in milliliters, selected from a list.
- INJECTION VOLUME — The volume of each injection, selected from a list. The number of injections (i.e., TOTAL SAMPLE ÷ INJECTION VOLUME) are listed below these settings.
- POST-SEPARATION PAUSE — Pauses the system between each injection. This allows fine-tuning of the gradient method, including INJECTION VOLUME, if desired. (An example of this is described in Section 3.3.9.)
- FRACTION POOLING — Collects peaks (compounds) in the same container. This reduces the number of containers used and combines the fractions containing the same compound from multiple injections. Works with both stacked and multiple injections. The TUBE ADVANCE button is available when this is selected.

**Start Tube** – Optimizes rack usage by sharing racks for multiple samples or optimizes work flow by placing each sample's fraction into individual racks to support multiple users.

**Export Column / Method List** – Save the column / method list on the QUEUE screen to a text file for later use.

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

### 3.3.6 Queue for Later

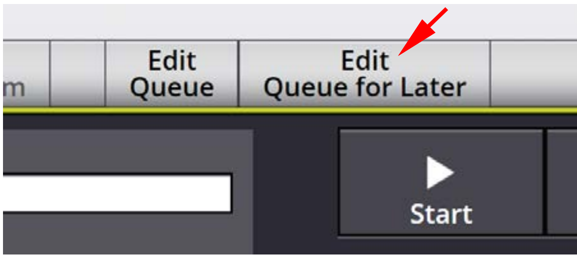


*Figure 3-12 Edit Queue for Later*



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This is similar to the QUEUE tab (Section 3.3.6). This allows you to add samples to the system without running. You can then get methods ready and run them when the sample is ready. As on the QUEUE tab, these queues can be saved (FILE > SAVE QUEUE AS).

### 3.3.7 AutoSampler Injection Techniques

This section describes operating protocols for the AutoSampler Module.

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

*Injection Sequence*

The AutoSampler default technique injects samples and washes the sample probe using the wash station solvent.

For reference, the sample injection steps are listed below:

1. During column equilibrium, the sample loop is first placed into the run position. This position passes the cosolvent through the loop to clear out any air or $CO_2$ that may have entered the loop. It also allows the wash solvent that is drawn up from the AutoSampler to be sent to waste instead of going into the loop.

> ☑ **Note**
>
> If the wash station solvent doesn't match the cosolvent, retention time could be affected.

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 for 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 brings sample up to the injection valve, compensating for the volume of the sample probe. Since the loop is still in the separation position, the wash solvent is sent to waste.
8. The loop is switched to the load position.

9. The remaining portion of the programmed injection volume is aspirated into the sample probe and loop. After the programmed amount of sample is aspirated into the probe, the probe is lifted out of the sample vial.
10. The probe is moved up and down over the sample tube to shake off any excess liquid to prevent the contamination of other samples or collection tubes.
11. The probe is dipped into the wash station to pull in a little solvent.
12. The probe is lifted, then it pulls in 50 µL air before being dipped into the wash station to pull in a small amount of solvent. This solvent chases the sample through the probe, ensuring that all of the sample reaches the sample loop for injection.
13. Air is drawn into the sample probe to draw the remaining sample into the loop without leaving any sample in the probe.
14. The sample is now loaded and the separation begins.
15. The next injection of the sample is accomplished by repeating the process described above.

*Probe washing sequence*

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

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

### 3.3.8 Fraction Collection with the AutoSampler

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

### 3.3.9 Stacked Injections

Injections can be “stacked;” that is, multiple injections can be injected after each other during the same isocratic run. Stacked injections save time and solvent by eliminating the equilibration and dead time for each injection when running multiple injections. Therefore, they are useful for rapidly purifying a large


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quantity of compound. PeakTrak provides both a means to set up multiple runs and a Stacked Injections Wizard from which you can fine-tune time windows.

*Method Development*

The Focus Gradient Generator can be used to calculate an isocratic method. The Stacked Injections Wizard can then be used to determine the cycle time and the time windows for peak collection. These are used to run stacked injections.

*Setup for Multiple Runs*

Follow the steps below to set up the system for multiple runs.

1. Develop a method for an isocratic run, or use the Focus Gradient Generator to calculate an isocratic method from it. (See 2.6.9 *Creating Scouting and Focused Gradients*.)

> ☑ **Note**
>
> A previous run could be loaded and used as the basis for a new run method by making edits to parameters such as INJECTION VOLUME, FLOW RATE, % COSOLVENT, and WAVELENGTH. However, it is best to confirm that the changes give suitable separation before using the new method for a stacked injection run.

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

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

   b. Select the FOCUS GRADIENT button on a RUN VIEWER window to open the Focus Gradient Generator window.

   c. Calculate the purification method:
      - Select a column.
      - Select the peak of interest.
      - Set the FOCUS RANGE to +/- 0% Isocratic.

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

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

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

2. Create the collection time windows

   a. Select EXTERNAL SAMPLE on the SAMPLE OPTIONS window.
      - For systems equipped with an AutoSampler, disconnect the sample probe at the injection port and replace it with the AutoInjector probe (PN 60-5234-657).

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

b. Select POST-SEPARATION PAUSE between injections so that the system is paused between each injection to allow the gradient method—including injection volumn—can be fine-tuned without configuring the system for an entirely new run.

**☑ Note**

The POST-SEPARATION PAUSE is the only time at which run parameters can be modified during a run.

c. Enter the TOTAL SAMPLE volume and INJECTION VOLUME for each run. To initially fine-tune the method, enter a small injection volume such as 0.2 mL (see Figure 3-11), as you will be able to change it later.

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

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

f. Select PEAKS on the RUN VIEWER window. Detected peaks are be used to determine the initial time windows to use for the stacked injections. (Do not worry that there are so many injections, as this number will decrease when the sample volume is increased.) Peak collection is discussed in Sections 4.3 and 4.3.2.

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

h. Select CONTINUE to run the method with the same or with changed parameters.

i. Once you are satisfied with the results of the single injection, select STACK INJECTIONS on the RUN VIEWER window. The STACKED INJECTION window opens. The system cdisplays a simulated stacked injection based on the isocratic run. The time windows displayed are based on the isocratic peak collection.

j. Adjust STACKED INJECTION settings as required. If no peaks were collected during the isocratic run, time windows are not displayed, but you can add them there manually. See *The Stacked Injection* window below for more information.

3. Prepare to run the stacked injections.

a. Verify that there is enough solvent (as listed on the MINIMUM RUN REQUIREMENTS window).

b. Verify that the waste container is empty.

c. Confirm that the sample is in place.


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d. Run the stacked injections by selecting OK on the STACKED INJECTIONS window.

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

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

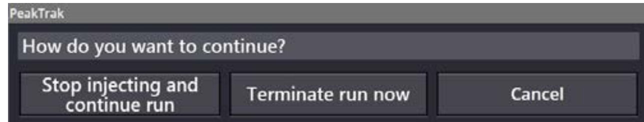


*Figure 3-13 Stopping stacked injections*

*Example Run*

An example run is shown in Figure 3-14, where there was a lot of resolution between the peaks. The high resolution allowed more sample to be loaded.

*Figure 3-14 Chromatogram showing High Resolution at the end of a run*

Features are provided to make further adjustments as necessary:

EDIT INJECTION - Allows the injection volume to be changed. In our example, the injection volume was changed to 2.0 mL for the next injection because the run suggested that there was sufficient resolution for a larger injection.

CONTINUE WITH PAUSE is found on a PEAKTRAK window (Figure 3-15); selecting it pauses the run after the next injection for further adjustments and also allows a stacked injection to be performed if that injection is suitable to be stacked.

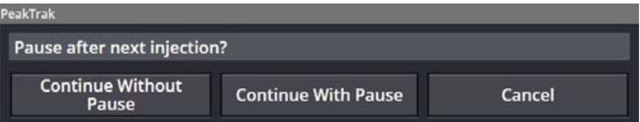


*Figure 3-15 The PeakTrak window.*

*Setting up the stacked injection*

Once the run looks good, stacked injections can be run (Figure 3-16).

*Figure 3-16 Higher sample loading, ready for stacked injections*

Selecting the STACK INJECTIONS button just below the chromatogram (shown circled in Figure 3-16) starts the Stacked Injections Wizard and opens the STACKED INJECTIONS window (Figures 3-17, 3-18, 3-19, and 3-20).

*The Stacked Injection window*

Figure 3-17 shows a simulation of three stacked injections. The various time windows were determined from the fraction collection and were used to determine the cycle time. The up and down arrows near the START and END settings allow fine tuning of the time window without entering values. The different colors


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(green and brown) represent a different stacked injection. The crosshatch patterns represent different time windows within an injection.

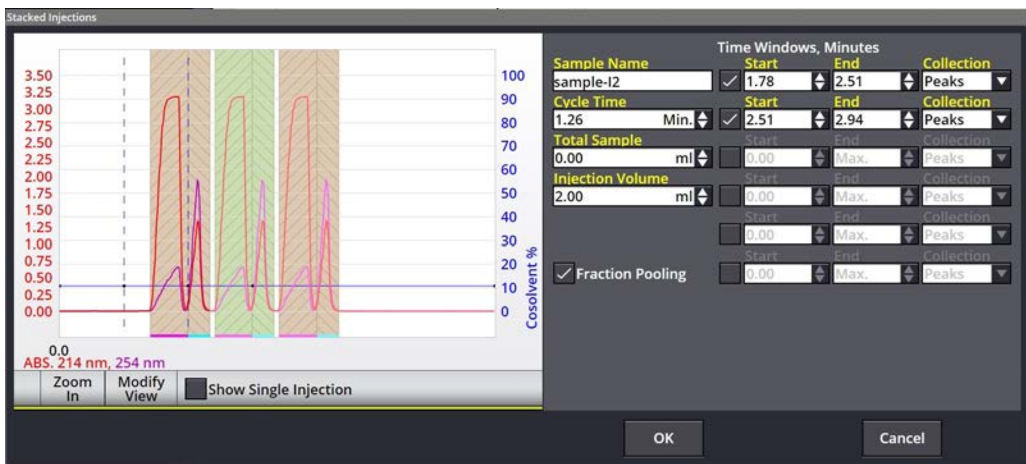


*Figure 3-17 Simulated stacked injections for the run in Figure 3-16*

Depending on the complexity of the chromatogram, it may be easier to adjust the time windows within the context of a single injection. Checking SHOW SINGLE INJECTION limits the view to one injection (Figure 3-18).

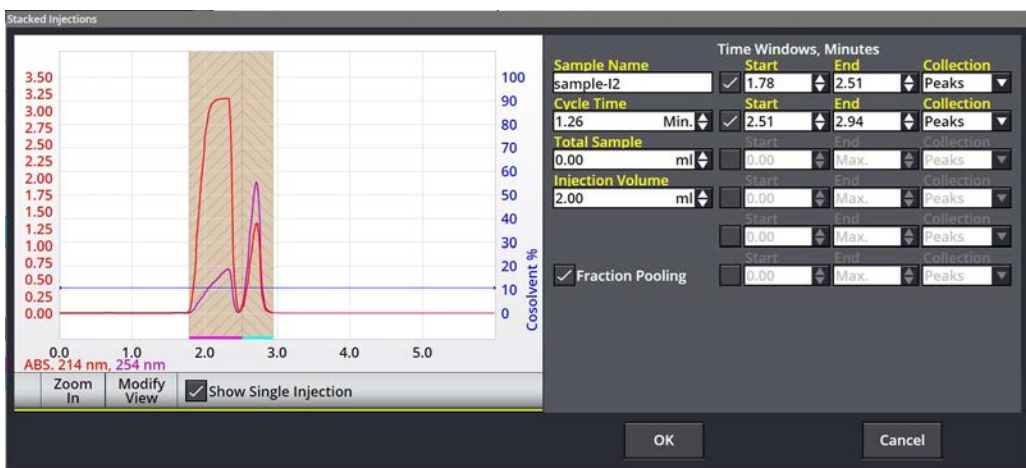


*Figure 3-18 Stacked Injections window in a Single Injection view*

Using the single injection view, it is easy to add a third time window if there is concern about mixing the two compounds (Figure 3-19). This time window can be added at the end of the list, and the time windows can be adjusted to accommodate the new one.

If no fractions were collected during the previous steps, SHOW SINGLE INJECTION also permits easier manual time window settings.

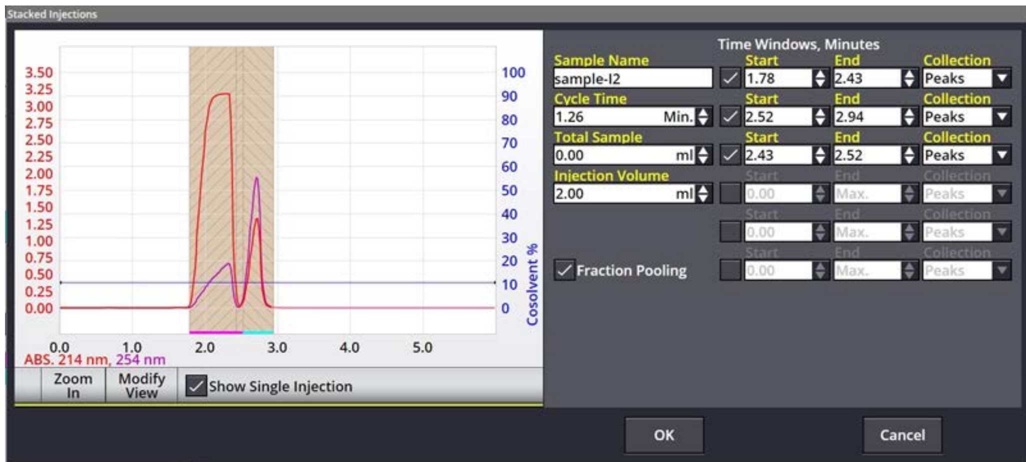


*Figure 3-19 Adding a Third Time Window*

Deselecting SHOW SINGLE INJECTION shows a simulation of three injections with the third collection window. The effects of changing the CYCLE TIME can be most easily viewed in this mode. Although the system remembers the injection volume, enter the total volume of sample before selecting OK.

After OK is selected, the system starts to run stacked injections.

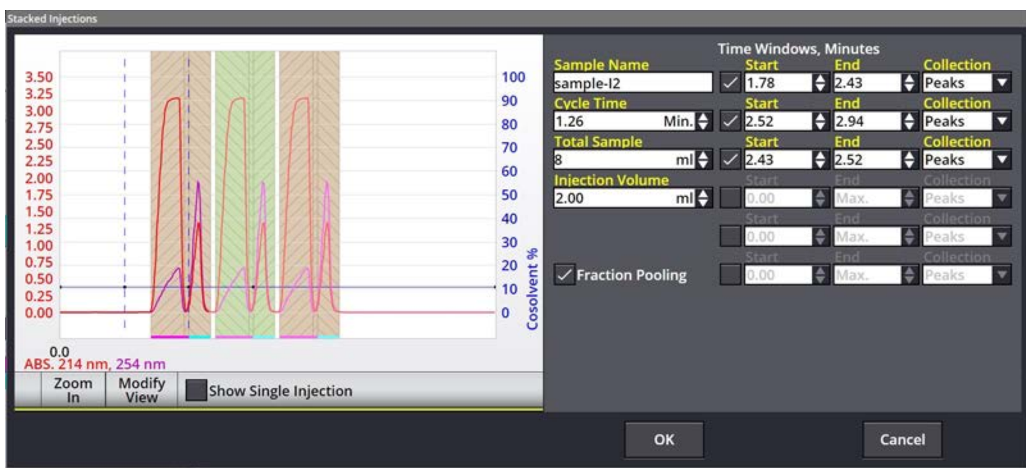


*Figure 3-20 Three Injections with a Third Time Window*

*Monitoring the injections*

During the run, selecting STACK INJECTIONS allows all the parameters for stacked injections to be adjusted during the run as needed.

*Figure 3-21 Four Stacked Injections with Time Windows Adjusted*

*Stacked Injections window options*

As on the MAIN screen, you can ZOOM IN and MODIFY VIEW.

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

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

**Total Sample –** A sample volume in mL.

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

**Fraction Pooling –** Collects corresponding peaks in the same container. This is the default. The largest possible containers should be used to minimize the number of fractions. FRACTION POOLING interacts with COLLECTION to determine peak collection behavior, as summarized in Table 3-1. The TUBE ADVANCE button is available when this is selected.

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

**Start –** The start of the time window in minutes.

**End –** The start of the time window in minutes.

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

| Table 3-1 Time window collection | | |
|---|---|---|
| | Fraction Pooling on | Fraction Pooling off |
| Peaks | Only the first detected peak is collected. Everything else diverted to waste. | Detected peaks collected in different containers. Non-peak eluant diverted to waste. |
| All | No peak detection. Everything within the time window collected into the same container. | Peak detection is enabled. Peaks in different containers. Non-peak eluant collected as fractions. |

## 3.4 Fraction Collection with Fractionation Valve

The fractionation valve is an alternate method to the AutoSampler fraction collector to collect peaks. A larger volume can be collected into individual fractions compared to the containers on the AutoSampler. However, the fractionation valve is limited to eight fractions.

To use the fractionation valve, select START, then follow the procedure described in Section 3.3, but choose these settings:

1. On the MINIMUM RUN REQUIREMENTS window, choose the fractionation valve instead of one of the racks (Figure 3-22).

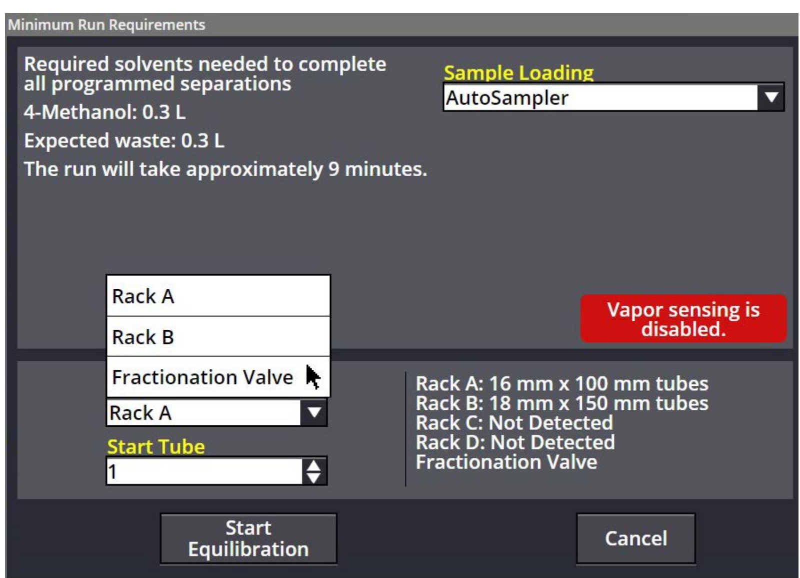


*Figure 3-22 The Minimum Run Requirements window*

2. On the COLLECTION DEFINITION window
   a. Enter the maximum fill volume for the containers used with the fractionation valve. “Max” is the value stored in the system configuration, but other values up to 5000 mL may be entered.
   b. Optionally, check FLUSH FRACTIONATION VALVE TUBING.


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c. Dismiss the window.

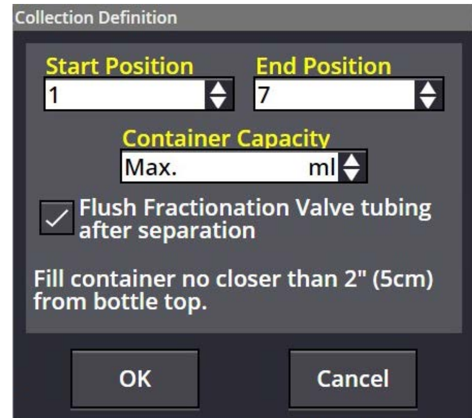


*Figure 3-23 Collection options*

**Note**

If Wash Fractionation Valve Tubing by default is checked in the Tools > Configuration window, Flush Fractionation Valve will be checked on the Collection Definition window by default.

*Fractionation bottle status area*

Selecting START EQUILIBRATION from the MINIMUM RUN REQUIREMENTS window adds bottle icons below the COLUMN, FLOW RATE, and COSOLVENT options on the MAIN screen. These icons show the fill status of fractionation valve containers.

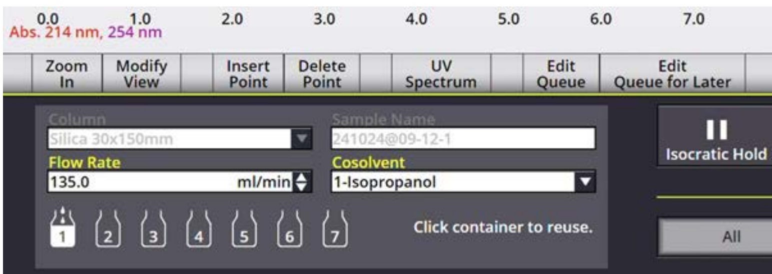


*Figure 3-24 Fraction bottle status area*

*Figure 3-25 Fraction bottle status icons*

You can reuse a fractionation valve container by selecting a full bottle icon after filling has stopped and the valve has advanced to the next empty container. PeakTrak will pause filling and prompt you to empty the container before continuing.

### 3.4.1 SFC Fractionation Valve Line Wash

The SFC fractionation valve line wash flushes the lines used during the run. It removes any sample that might be left inside the lines to improve sample recovery and to prepare the system for the next purification. A line wash can be requested for some or all separations:

- The procedure can be enabled or disabled for each individual run by checking FLUSH FRACTIONATION VALVE TUBING AFTER SEPARATION on the COLLECTION DEFINITION window.
- The procedure can be enabled by default for all purifications using the fractionation valve by checking TOOLS > CONFIGURATION > the USER PREFERENCES tab > WASH FRACTIONATION VALVE TUBING BY DEFAULT.

*How does it work?*

At the end of a run, the eluate composition at the end of the run is used to flush any lines used during the purification. Because approximately 5 mL of co-solvent is used for each line, the time required for the flush depends on the flow rate, % composition, and number of bottles used.

If the run is RESET, their will be no line wash. Use TERMINATE (the “fast-forward” button) to end the run early, which allows all the instrument cleaning procedures to be run.

## 3.5 Operation Troubleshooting

**Injection Valve Leak** – Refer to Section 5.5.2 *Injection Valve Rotor*. Recommendations:

- 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
- Formic acid modifiers can result in reduced injection valve seal lifetime. If possible, consider other modifiers such as TFA.

**Unexpected retention time or variable retention times for repeated injections –**

- Temperature or back pressure variation. Variations of only a few degrees can cause a visible retention time shift.
- Variable flow rates of $CO_2$ or the co-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 cosolvent 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.


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- Solvent supply fitting may be loose allowing small amounts of air into system, causing minor flow rate errors of $CO_2$ or the co-solvent.
- Strong solvent used for injection (if the repeated injection is a larger volume).

- Solvent bottle refilled with incorrect solvent.

**No peaks on chromatogram –**

- No sample injected:
  - Sample vial empty.
  - Sample probe plugged, injection loop plugged, or probe or injection pump fitting loose.
    - Perform a dummy injection and watch 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.
- Co-solvent not being delivered. Check solvent level, connections, check valves, confirm solvent lines in correct bottles, and 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. Those failures have corresponding error messages.

**Peaks elute too early –**

- Carbon dioxide not being delivered properly. Check solvent level, inspect or clean pump A check valves, confirm solvent lines in correct solvents, and 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.
- Incorrect method.

**Peaks elute too late –**

- Co-solvent not being delivered properly. Check solvent level, inspect or clean co-solvent pump check valves, confirm solvent lines in correct solvents, and look for leaks of air into the lines.
- Improper flow rate for one or more solvents. Check the flow rates 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. Possible causes and their remedies:

- The column requires washing. Wash the column with strong solvent.
- A compound is left from a prior injection. Make sure that the gradient goes to 70% co-solvent 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 –**


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- 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 slowly washing out of the column. Wash the column with strong solvent to eliminate compounds from prior separations.

**ELSD detection issues –**

- No peaks detected
  - If the compounds are volatile, they may not be detected by ELSD or may need a lower drift tube temperature. Refer to the discussion on ELSD operation in Section 4.3.2 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. Refer to the discussion on ELSD operation in Section 4.3.2 before adjusting the ELSD operating temperatures.
  - Increase ELSD sensitivity and gain in the method editor to see if the peaks are simply too small to be easily seen.
  - Internal tubing that splits flow from the system to the ELSD may be blocked. 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 4.3.2 before adjusting the ELSD operating temperatures.
- 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 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 the method development window, 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 –**

- Improper connections:
  - Verify that the column is connected properly to the Column Select Valve. (If the column output goes to the wrong port, it is dead-ended.)
- Column gets clogged:

d. Remove guard column (if installed) and check back pressure. If this corrects the problem, replace the guard column or follow the washing procedure below.

e. Remove preparative column and check back pressure.

f. If the column is clogged, use a non-SFC system to 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.

g. Reverse the column connections and flush.



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h. 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.

i. Filter samples in the future to prevent problems.

**Fractions not correct volume –**

- Air trapped in pump head:
  - Reprime and test again.
  - Some cosolvent may be lost to evaporation from the expansion of $CO_2$ during fraction collection.
- 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.
- Replace the rotor. The Accessory Kit (PN 60-5269-011) includes the rotor. See Section 5.5.2 *Injection Valve Rotor* for the replacement procedure.

Parts for ACCQ*Prep* SFC systems are available online at https://store.teledyneisco.com.
Or, contact your local Teledyne LABS representative.

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# ACCQ*Prep*® SFC

## *Section 4 PeakTrak*

### 4.1 Overview

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

#### 4.1.1 PeakTrak Window Elements

The MAIN screen displays the current METHOD FILE. From MAIN, you can access many of PeakTrak's features, view the system status, and view or edit the method file settings. The MAIN screen can be divided into the following regions:

**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 just below the menu. Status messages may indicate

- the system mode,
- the current position in a run, expressed in time or column volumes,
- the current %co-solvent, and
- the flow rate.

**Main Region –** Here, you can view or modify method file settings. Frequently used method settings are displayed on the MAIN screen, while advanced method settings are displayed on the METHOD EDITOR.

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

*Figure 4-1 The Main screen*

***Note***

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

### 4.1.2 Method 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 turned on, it associates a default method in the system with the column selected as a default in the CONFIGURATION window. In a single column system, this reflects the column mounted on the system. This default method is stored on the system hard drive, and a copy of the method is automatically loaded into a temporary use area.

If the default method meets your needs, select START to start a separation. On the other hand, if you want to customize the method for use on this separation only, edit the method. (Editing the gradient by dragging a gradient point is a common 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 then name the method. If you didn't save the method, but you later want to reuse it, open a data file that used the method and extract it for reuse. The system uses the method file in the temporary area to direct the system operation after you select START.


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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. Method files have a .pmtd file name extension.

*Default Methods*

When PeakTrak is started, the system loads a Default Method. A Default Method contains Teledyne-recommended basic and advanced settings for the use of a default column (as selected on the CONFIGURATION window). There can be a Default Method for each size and type of column defined in the CONFIGURATION window.

Default Methods provide a starting point for your separation or purification. From these initial settings, you can perform a purification run, or you can modify the settings for your next run. Subsequent runs will use the settings in the active (temporary) window. If you have modified the settings, you can save the Method file for future use.

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

### 4.1.3 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, rack and tube information, a pressure trace of system pressure, spectral data, a chromatogram containing information from each of the detectors, and (optionally) mass spectrometer spectral data (if one is installed). The saved method parameters do not include reference to the method name, as that method could have subsequently been edited to different parameters.

You can open and review the run files stored by the system. To open a Run File, use FILE > OPEN. Run files have the .run filename extension. For more information about viewing runs, see Section 4.4.7 *Viewing Runs*.

## 4.2 PeakTrak Menu Options

PeakTrak main menu options:

- FILE
- METHOD EDITOR
- MS
- TOOLS
- HELP

### 4.2.1 File

The FILE menu provides commands to perform file operations, print, and log out of the system:

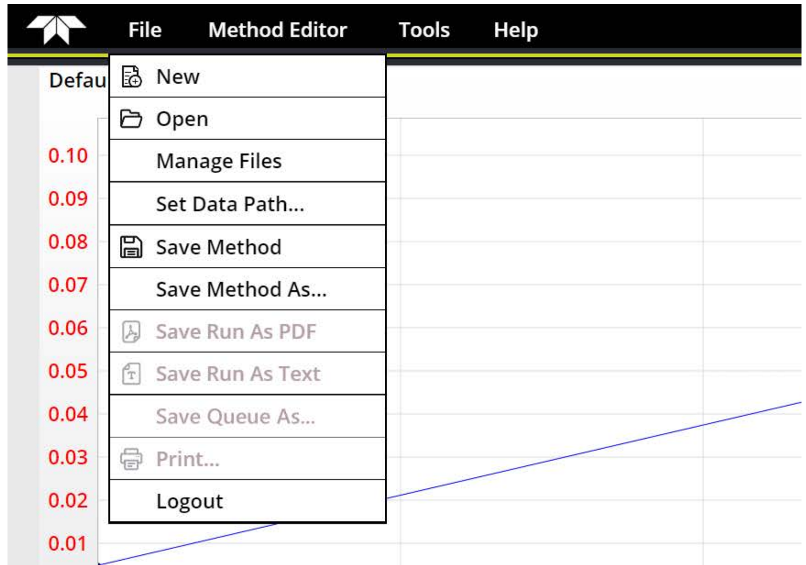


*Figure 4-2 The File menu*

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

**Open –** Opens a Method file (.pmtd) or a Run file (.run) stored on the system's internal hard drive.

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

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

**Note**

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

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

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


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**Note**

DEFAULT METHODS are an exception and cannot be overwritten by SAVE METHOD. If a DEFAULT METHOD is open and you attempt to save any modifications to that method, the FILES window appears, so you can rename the method file. This preserves the default method. The DEFAULT METHOD must be edited in the Prep SFC tab on the CONFIGURATION window.

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

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

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

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

**Print –** Prints the completed run on the network printer if selected from the touchscreen. If selected from a remotely connected computer, PeakTrak displays the printer window. From this window, you can select and configure the printer of your choice. After configuring the settings, PRINT the completed run.

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

### 4.2.2 Method Editor (menu item)

Selecting the METHOD EDITOR menu command opens the METHOD EDITOR window. Use this window to view and modify basic and advanced METHOD FILE settings. (See Section 4.3.2 *Method Editor* for detailed information about the Method Editor window.)

### 4.2.3 MS

The MS menu item is only available when a PurIon Mass Spectrometer is added to the ACCQ*Prep* system. Furthermore, Method Development, Manual Control, and Ionization Settings are not available from a remotely connected computer via a web browser.

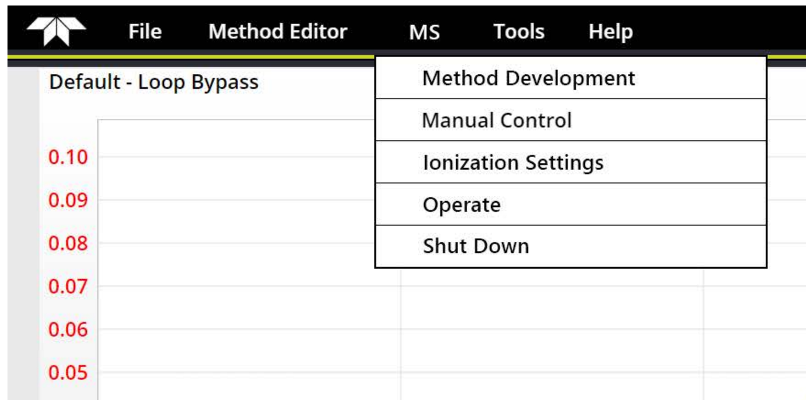


*Figure 4-3 The MS menu*

**Method Development** – Opens the MS METHOD DEVELOPMENT window to verify ionization conditions for the compounds to be purified and to provide access to the Ion Finder (see Section 4.3.7). METHOD DEVELOPMENT is not available from a remotely connected computer via a web browser.

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

**Ionization Settings** – 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** – 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 replaces OPERATE.

**Standby** – 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. Additionally, it sets the inlet capillary heater temperature to 50 °C, turns the remaining heaters off, and lowers the gas flow rate to about 0.2 liters/minute. When in standby mode, OPERATE is used to return the PurIon to the operational state (i.e., high voltages on, heaters on, and gas flow at the proper rate of about 4 liters/min). When the mass spectrometer is already in standby mode, STANDBY is


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unavailable; the OPERATE button displays instead.

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

⚠ **WARNING**

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

### 4.2.4 Tools

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

*Figure 4-4 The Tools menu*

**Manual Control** – Opens 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. Options on the MANUAL CONTROL window are covered in detail in Section 4.3.10 *Manual Control*.

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

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

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

**Set Administrator Password** – Opens the SET ADMINISTRATOR PASSWORD window.

Passwords prevent unauthorized modifications to:

- User management
- System date and time

- System password

If password protection is enabled, you must enter a password before any of these settings can be accessed.

The default password set on the system is “accqprep”. Refer to Section 4.3.12 *Set Administrator Password* to change it. Password protection can also be disabled so that all users can modify the entry.

**User Management –** Opens the USER MANAGEMENT window. You can use this window to add or remove users from the system.

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

After users are created, they can be assigned access levels for the system (Figure 4-5; this table is available when you select Help on the USER MANAGEMENT window).

| 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 solvent 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 |

*Figure 4-5 Table of user level access*

**Vent System Pressure –** Opens a dialog from which you can confirm that you want to vent the system.

**Recovery Run –** Continues a previous separation after an error to recover sample from the column


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### 4.2.5 Help

The HELP menu provides options to service your ACCQ*Prep* SFC system and to report information about it.

*Figure 4-6 The Help menu*

**Service –** Allows access to the system's service functions by qualified service personnel. These service functions are password protected to restrict their use to only trained personnel. Access to these functions requires the administrator password.

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

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

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

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

**About ISCO –** Displays contact information for Teledyne ISCO, a unit of Teledyne. You may contact Teledyne LABS at www.teledynelabs.com.

### 4.2.6 Backup / Restore Procedures

To back up instrument settings and data:

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

To restore instrument settings and data from a backup:

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

## 4.3 PeakTrak Windows

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

- *Main screen*
- *Method Editor*
- *Column Data*
- *Files*
- *Set Data Path*
- *MS Method Development*
- *MS Manual Control*
- *MS Ionization Settings*
- *Manual Control*
- *Set Administrator Password*

### 4.3.1 Main screen

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


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*Figure 4-7 The Gradient Plot area*

**Gradient Plot –** 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 co-solvent. By default, PeakTrak automatically scales the left Y-axis to best display the detector trace. PeakTrak also sets the upper limit of the right Y-axis to 100%. You can override the Y-axes scales by selecting the MODIFY VIEW button and setting the values you want.

To zoom into a portion of the graphic area, “pinch zoom” and “swipe”. To pinch zoom, place two fingers on the touchscreen in the graphics area, then move them apart to zoom in. Moving both fingers together (swiping) pans the display area. (A single finger touch is interpreted as a selection rather than as a pan function.) Alternatively, you can also use the ZOOM controls discussed below

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

The following controls appear in the Gradient Plot Area:

- INSERT POINT — Enables the gradient point insert mode. When this mode is active, select a place on the gradient curve to add a single point. You can then drag the new point to any position. Select INSERT once for each gradient point that must be added to the plot area.

- DELETE POINT — Enables the gradient point delete mode. When this mode is active, the point nearest to the next point selected on the gradient curve is deleted. Select DELETE once for each point to be deleted from the plot area.

MS - Displays the current mass spectrum on PurIon systems. This spectrum hides the flow rate and solvent selection controls. Selecting the MS control again causes the spectrum to disappear. When the spectrum is displayed, touching the chromatogram at any point prior to the current elution time displays a spectrum at that point in time. The elution time corresponding to the spectrum is displayed under the spectrum.

- LIVE DATA shows the current spectrum.
- 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. Up to four spectra can be saved in a run report. The button having a triangle pointing down cycles through saved spectra.
- The ± button displays the positive or negative ion spectrum (PurIon S and PurIon L systems only). A dot in the button indicates whether the positive or negative ionization spectrum is displayed.
- UV SPECTRUM — UV (UV-Vis on ACCQ*Prep* SFC systems with a UV-Vis detector) displays the UV spectrum on systems. This spectrum hides the flow rate and solvent selection controls. Selecting 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.
  - LIVE DATA shows the current spectrum.
  - ADD SPECTRUM saves the spectrum at a point for inclusion in a run report screen. Up to four spectra can be saved in a run report.
  - The button having a triangle pointing down cycles through saved spectra.
- EDIT QUEUE — Opens the QUEUE screen, which configures queues for use with the AutoSampler. See Section 3.3.5 *The Queue Screen* for more information.
- RUN LENGTH — Sets the length of the run. Type or select a length.
- SYSTEM STATUS BAR — Shows temperature and system pressure status:
  - OVEN TEMPERATURE
  - MEASURED BPR PRESSURE
  - the SYSTEM PRESSURE GAUGE, which includes


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- the Measured System Pressure

- the current System Pressure Limit.

*System Status Bar*

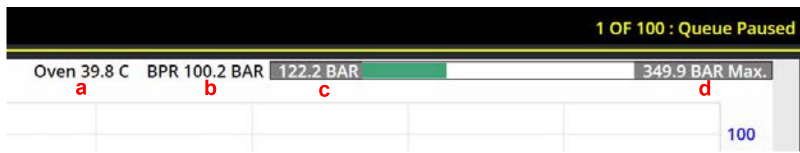


*Figure 4-8 The System Status Bar*

The **oven temperature** is shown at far left beside the measured BPR pressure.

- In Figure 4-8 (**a**) it is 39.8 °C.

The **measured BPR pressure** shown to the left of the gauge.

- In Figure 4-8 (**b**) it is 100.2 BAR.

The **system pressure** is shown at the left end of the pressure gauge. It includes the measured BPR pressure.

- In Figure 4-8 (**c**) it is 122.2 BAR.

The **system pressure limit** is derived.

- This limit equals the entered column Pressure Limit + the measured BPR Pressure
- Exceeding the system pressure limit results in a hard stop of the system.

**Note**

When setting the column parameters, the Pressure Limit entered in the Prep SFC tab of the Configuration window should be the pressure limit specified by the literature included with the column.

The **actual maximum system pressure limit**

- In Figure 4-8 (**d**) it is 349.9 BAR.
- It is a *dynamic* limit, as it depends on the measured BPR pressure at any moment.
- It is the lesser of these:
  - the *maximum-rated system pressure limit* (6000 psi (414 BAR))
  - the setting to the column Pressure Limit + the *measured BPR pressure.*

Example

column Pressure Limit of 240 BAR
+ measured BPR pressure of 100.2 BAR
= actual maximum system pressure limit of 340.2 BAR

340.2 BAR is less than 414 BAR, so it becomes the maximum system pressure limit.

The system calculates maximum pressure using *measured BPR pressure* and not the METHOD EDITOR > BPR PRESSURE setting because these may differ. By using the actual measured BPR and making the limit dynamic based on the basis of the BPR value, the system prevents damage to the column while the BPR is still being set.

The **actual pressure at column** is a derived figure. It is the system pressure minus the *measured BPR pressure*.

**Run Settings –**

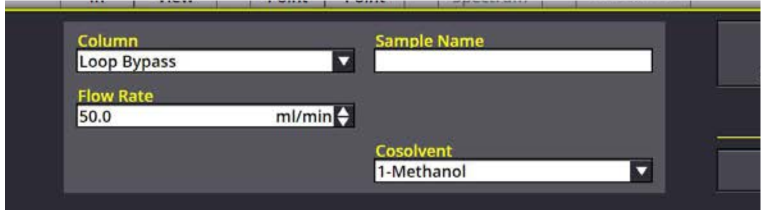


*Figure 4-9 Main: Run Settings*

- COLUMN — The column used on the system. Other installed columns to use for purification can be selected from the list. Methods that are already associated with the selected column can be selected from the second level of the list.
- 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 select START to begin the run.
- FLOW RATE — The flow rate for the run. You can type it or select it from the list.
- COSOLVENT — The four co-solvents that have been configured via TOOLS > CONFIGURATION > INSTRUMENT CONFIGURATION (see Section 2.1 *Configuration of the ACCQPrep SFC*). Select one of the four from the list.

**Run Control Buttons –** Figure 4-10 shows the buttons as they appear when a run is not underway.

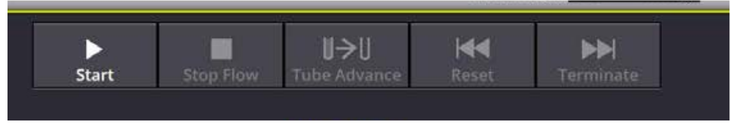


*Figure 4-10 Main: Run Control buttons*


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- START/CONTINUE — Starts or resumes the run. After a run has started, this is replaced by the ISOCRATIC HOLD button.
- ISOCRATIC HOLD (the "pause" symbol) — Holds the %co-solvent at the current value while the system continues to operate. This is only present after the run has started; otherwise, the START button replaces it.

**☑ Note**

Pausing the run extends the run length. While the run is in the paused state, you can resume the gradient by selecting START, or stop the run by selecting STOP FLOW. If you resume the run, the system continues the gradient curve from the %co-sovlent when the system was paused.

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

  If you want to terminate the separation prematurely, TERMINATE is recommended. Unlike REWIND, TERMINATE does not modify the current method's run length setting.
- TERMINATE (the "fast forward" symbol) — Allows you to jump to the next step of a run. After you have started a run, the system performs several steps. The first step is to deliver solvents using the programmed gradient for the entire run length. When this step is complete, the system enters the sample probe cleaning step. Selecting TERMINATE instructs 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 screen.

*Figure 4-11 Main: Peak Collection buttons*

- ALL — Collects all eluant in the fraction collection tubes. Detected peaks will advance to the next tube automatically to maximize peak concentration and purity
- PEAKS — Collects only eluted peaks in the fraction collection tubes.
- NONE — Diverts all eluant 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 & 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 (Section 4.4.7 *Viewing Runs*). Selecting a test tube with the mass spectrum window displayed displays the mass spectrum for compounds collected into that tube (PurIon systems only). Selecting adjacent tubes allows you to determine which fractions may potentially contain impurities.
The peak collection data is displayed on the left side of the MAIN window:

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

### 4.3.2 Method Editor

The Method Editor provides the means to create and edit methods for chromatography runs. In the Editor, you can:

- Select a column to use.
- Set oven and preheater temperatures.
- Set flow rate and back pressure.
- Create time windows to limit the fraction collection to times during the run that you specify.
- Select where eluant will be collected.


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- View and edit the current gradient.
- Set detector settings.
- Save methods to Run Files for later retrieval.

To open the window, select METHOD EDITOR from the Peak Trak menu at the top of the screen.

*Figure 4-12 The Method Editor window*

**Button bar –** Provides buttons to work with method files and display column data.

*Figure 4-13 Method Editor button bar*

- NEW (icon) — Opens a new method file using the default method settings for the selected column.
- OPEN (icon) — Opens a method file stored on the system's internal hard drive.
- SAVE (icon) — Saves any modifications to the current method file. If you attempt to save modifications to a default method file, the FILES window opens instead and allows you to rename the file to preserve the default method.
- SAVE AS — Opens the FILES window. From this window you can rename the current method and save it on the system's internal storage.

- EXIT — Closes the METHOD EDITOR and return to the MAIN WINDOW.
- COLUMN DATA — Opens the COLUMN DATA window. This window reports information about the column selected on the MAIN screen. The COLUMN DATA button is found at the top right corner of the window.

**Run Settings –**

*Figure 4-14 Method Editor: Run Settings*

- 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 generates a date/time stamp for the name when you select START to begin the run.
- FLOW RATE — The flow rate for the run. You can type it or select it from the list.When you use the Teledyne LABS 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.
- PREHEATER — Controls the oven and preheater temperature. This cannot be changed once a method is in use.
- BPR PRESSURE — Sets a pressure limit (between 100 and 160 BAR) for the back pressure regulator (BPR).
  - Controlling the pressure of the mobile phase—including back pressure—is important because pressure affects the phase's density, which in turn affects the retention factor, peak resolution, and consistency between runs.
  - The BPR PRESSURE limit, when combined with the column PRESSURE LIMIT (Section 2.6.5), sets the system pressure limit. Were the system to exceed its pressure limit, a hard stop would occur.


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- The entry to BPR PRESSURE does not necessarily dictate at-column pressure because, at any given moment, the actual back pressure may not have yet reached the BPR PRESSURE limit.
- The BPR PRESSURE setting to a method cannot be changed while the method is in use.

- INITIAL WASTE & TIME WINDOWS — Opens TIME WINDOWS, a window from which you can view and modify waste and define time windows. Time windows can limit the fraction collection to specific time durations of the run. See Section 4.4.1 *Editing a Method* for more information.

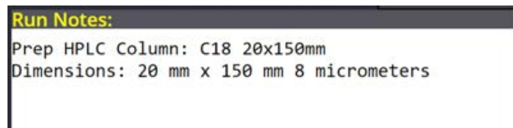
**Time Windows –**

- INITIAL WASTE - The volume of eluant 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. This feature conserves collection tubes by diverting all fluids until the first peak is expected.

  **Time Windows Section -** All times are relative to start of the run, just following the sample injection. To use time windows, check a box to activate a row, then enter START and END times. You can define up to six time windows.

- PEAK COLLECTION - Describes how peak states limit when fluid is collected.
  - Peaks - Instructs the module to collect fluid only when the time window is active and a peak is present.
  - All - Instructs the module to collect all fluid during the time window. Detected peaks will still trigger tube advances. During stacked injections, all fluid is collected during the time window regardless of peaks.

RUN NOTES — Provides a text entry box into which you can enter comments or notes for the run. These comments are saved with the run and appear in TXT and PDF reports. Column identification information is automatically placed in this box.




**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 co-solvent.

The gradient plot area also provides a convenient method to edit the gradient curve. You can select and drag any of the points to change the shape of the curve, or use the buttons at the bottom of the plot area to insert and delete points. To zoom into a portion of the graphic area, use “pinch zoom” and “swipe.” Pinch zoom by placing two fingers on the touchscreen in the graphics area, then move 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 as a pan function.)

The following controls appear in the Gradient Plot Area:

- INSERT POINT — Enables the gradient point insert mode. When this mode is active, select a place on the gradient curve to add a single point. You can then drag the new point to any position. Select INSERT once for each gradient point that must be added to the plot area.
- DELETE POINT — Enables the gradient point delete mode. When this mode is active, the system deletes the point nearest the next point selected on the gradient curve. Select DELETE once for each point that must be deleted from the plot area.
- RUN LENGTH — Sets the length of the run. Type or select a length.

**Note**

Changing the run length changes the scale of the X-axis on the gradient. Points that define the gradient are automatically scaled to fit the new run length.

The RUN VIEWER also has a plot area with its own controls. See Section 4.4.7 *Viewing Runs* for a discussion of these controls.

**Solvent and Gradient Options –**

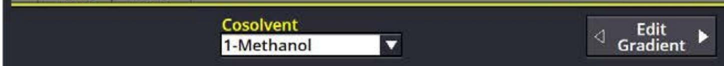



*Figure 4-15 Method Editor: Solvent and Gradient options*

- COSOLVENT — The solvents listed are those defined by the CONFIGURATION settings. You can program mid-run co-solvent changes by selecting EDIT GRADIENT to open the GRADIENT TABLE.
- EDIT GRADIENT — This table changes the FLOW RATE at the time points on the gradient plot area. Select EDIT GRADIENT to toggle the gradient table open or closed. After the table is opened, add or delete time points, or select a field to highlight it and make the row editable. Changes that you make to the table are reflected in the gradient plot area.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For complete instructions on defining gradient curves, see Section 4.4.3 *Defining a Gradient*.

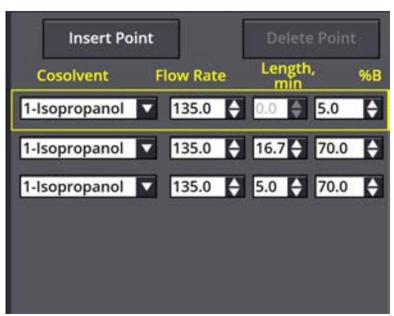


*Figure 4-16 Gradient Table*

- INSERT POINT — Adds a time point before the point whose row is highlighted on the table. The new point appears at the same location as the highlighted row's point. You can move it along the X-axis by changing the LENGTH entry.
- DELETE POINT – Removes the highlighted row and its associated point from the table. You cannot delete the initial point. There must be at least two points to define a gradient. The system automatically updates the RUN LENGTH setting or scales the other points when you change the number of points and their duration on the curve. To close the GRADIENT TABLE, select the EDIT GRADIENT button. See Section 4.4.3 *Defining a Gradient*.
- COSOLVENT – A cosolvent that have been entered on the CONFIGURATION window > INSTRUMENT CONFIGURATION tab.
- FLOW RATE – The flow rate in mL per minute. A change in flow rate at a point is shown in the gradient plot area as a vertical line through and a callout at the previous point.

**☑ Note**

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

- LENGTH, MIN – The number of minutes or column volumes from the previous time point, as viewed along the X-axis of the gradient plot area.
- %B – The percentage of solvent B. Adjusting a value on a row also moves its point along the Y-axis.

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

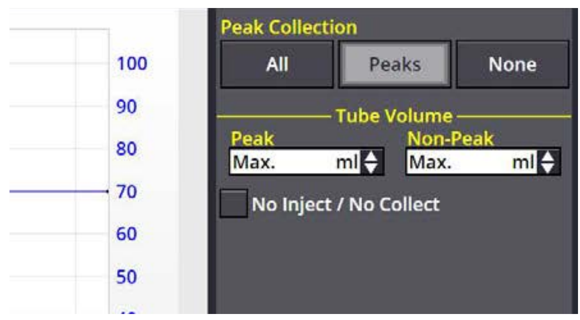


*Figure 4-17 Method Editor: Peak Collection and Tube Volume*

- ALL — Collects all fluids in the fraction collection tubes during a run.
- PEAKS — Collects only eluted peaks in the fraction collection tubes during a run.
- NONE — Diverts all fluids during a run to the waste port.

See Section 4.4.1 *Editing a Method* for a more detailed discussion of peak collection.

**Tube Volume** – 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 select TUBE ADVANCE button.

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

**Peak Detection** – This section of METHOD EDITOR provides peak detection options. When a detection method is selected, its DETAILS button opens a window (Figure 4-18) on which you can modify that detection method's settings.


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**☑ Note**

You can select up to four peak detection options on the ACCQ*Prep* systems,. If more than one option is selected, such as $\lambda_1$ with $\lambda_2$, the system considers a peak to be present when an option is true (a logical OR).

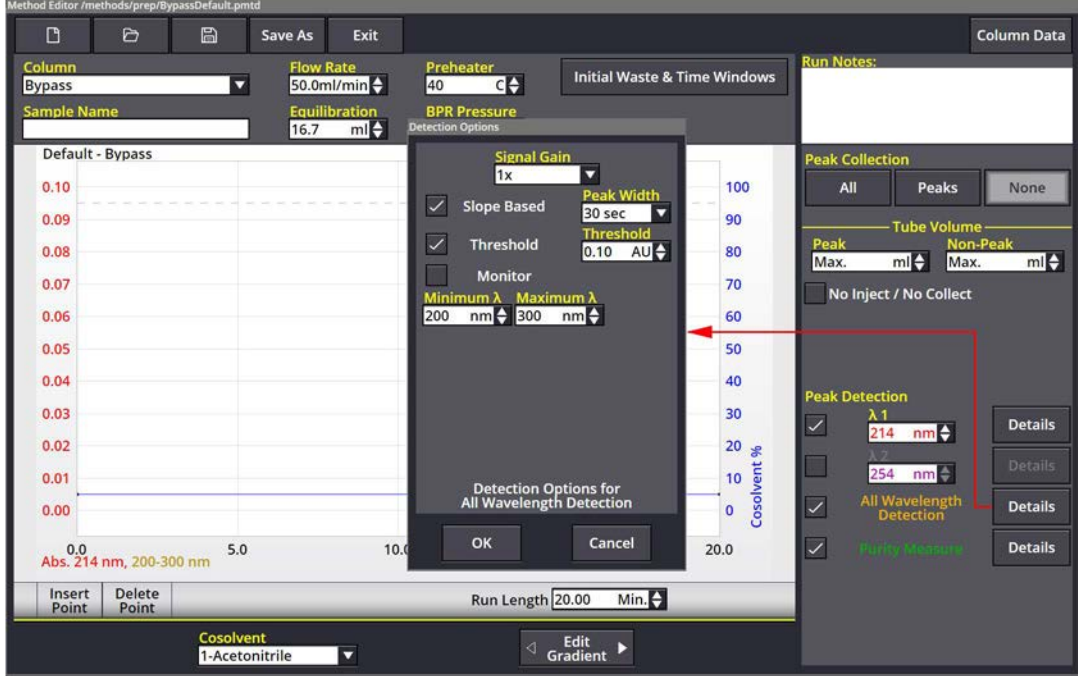


*Figure 4-18A Peak Detection settings window*

All enabled detection methods are displayed on the chromatogram. Options and settings for the ACCQ*Prep* SFC system are described below.

*Wavelength Peak Detection*

- $\lambda_1$ and $\lambda_2$ (wavelength 1 and 2) — Instruct the system to use and allow you to configure primary and secondary wavelength detection. $\lambda_2$ is set to monitor (displayed but not used for peak detection and cutting) by default. When checked, type or select the peak detection wavelength in nanometers, then select DETAILS to configure additional settings:

*Wavelength Peak Detection Details*

  - SIGNAL GAIN – Modifies the gain. Use this to scale the detector signal.
  - SLOPE BASED — When selected, enables slope-based peak detection. Peaks are recorded if the slope algorithm detects a peak similar to the average PEAK WIDTH. Select the average peak width setting. Peak widths are measured at the baseline. The slope detector typically detects peak widths ranging from about 0.2 to 2 times the peak width setting. For example, if you entered a peak width of 1 minute, the range would be 12 seconds to 2 minutes. For best

operation, the peak width should be set to just over the average peak width being separated. For instance, if the average peak width is 45 seconds, enter a peak width of 1 minute. 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. For most SFC, 1 minute is a good starting point for a peak width.

- THRESHOLD — When selected, enables threshold peak detection. Peaks then start when the detector signal exceeds the programmed value. The peak ends 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 — When selected, instructs the system to only use the detection source as a monitor. The detection source is then displayed as a trace on the gradient plot area, but is not used to cut peaks.

*All Wavelength Peak Detection*

- ALL WAVELENGTH DETECTION — When selected, detects peaks within a user-selected range of wavelengths. Select its DETAILS button to configure additional settings. These settings include the SIGNAL GAIN, SLOPE BASED, and MONITOR options described earlier for $\lambda_1$ and $\lambda_2$. Other DETAILS settings are unique to ALL WAVELENGTH Detection:
  - PEAK WIDTH — Determines solvent absorbance suppression. Peaks that are twice the peak width are deemed to be solvent or other baseline drift, and their absorbance spectra are subtracted as assumed baseline spectra. Additionally, you can type or select the minimum and maximum wavelengths limits in nanometers. Doing so eliminates areas of little spectral information from the all wavelength detection signal, resulting in a stronger signal.

*All Wavelength Details*

  - MINIMUM (MAXIMUM) $\lambda$ – That is, the minimum and maximum wavelength detected. Set this to avoid detection of certain wavelengths—for example, those associated with unwanted compounds.

*ELS Peak Detector*

- ELS Detector — When selected, enables the evaporative light scattering (ELS) detector on an ACCQ*Prep* system. Select its DETAILS button to configure additional settings:


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*ELS Detector Details*

- SENSITIVITY - Can be selected as NORMAL to keep largest peaks on scale or HIGH to detect small peaks. The default setting is HIGH to ensure most compounds are detected.
- SIGNAL GAIN – Modifies the gain to scale the detector signal.
- SLOPE BASED AND THRESHOLD – Refer to these features described earlier for λ1 and λ2.
- DRIFT TUBE TEMPERATURE – The default settings is 60 °C for normal phase default methods. 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 TOOLS > MANUAL CONTROL > ELSD ON to turn on the flow of gas through the drift tube without liquid. Temperatures that are too high can cause semivolatile compounds to vaporize and become undetectable.

*Mass Spectrometer Peak Detection*

- MASS SPECTROMETER – When selected, monitors or detects compounds with a PurIon mass spectrometer system (PurIon systems only). Mass-directed peak detection can be set for up to 6 masses or 5 masses and 1 range.

  THRESHOLD – Selects the START and END signal intensity ratios for directing fraction collection. Selecting THRESHOLD deselects MONITOR.

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

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

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

- END threshold – The percentage of the maximum peak height of the current peak at which detection stops and collection ends. For example, entering 20% ends collection at 20% of the peak height. Setting a percentage of peak height can allow proper collection of both large and small peaks. Setting this to 0% instructs peak collection to use the START noise threshold.
- MONITOR – When selected, prevents fractionation based on the mass spectrometer signal.
- TERMINATE ON TARGET – When selected, stops the run after all mass spectrometer detection ions have been detected.
- DETECTION IONS – Sets 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.
- SPECTRUM START/END – (PurIon S and PurIon L systems only)
- POSITIVE IONIZATION – Selects positive ion spectrum (PurIon S and PurIon L systems only). Selecting POSITIVE IONIZATION deselects NEGATIVE IONIZATION.
- NEGATIVE IONIZATIOn – Selects positive ion spectrum (PurIon S and PurIon L systems only). Selecting NEGATIVE IONIZATION deselects POSITIVE IONIZATION.
- ION SETTINGS – Sets ionization parameters to enhance detection of molecular ion peaks.
- PURION LOADING – Sets the mass load (High, Medium, or Low); that is, the quantity of the carrier flow rate that is sent to the mass spectrometer (MS).

  High: 20 µl/min

  Medium: 6 µl/min

  Low: 1.467 µl/min

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

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

At a system flow rate of 20 ml/min, the split ratios are 1000:1, 3333:1, and 13633:1. (That is,
20 ml ÷ 20, 6, and 1.467 µl/min, respectively.)

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


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- MS Detection Start/End – Sets the start and end times at which the MRA valve is active during the method. This avoids salts and other contaminates at the start of a run and prevents unwanted compound from being sent to the mass spectrometer during a column wash.

**☑ Note**

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

*Purity Measure Peak Detection*

- PURITY MEASURE — When checked, displays a ratio of the selected wavelengths if two absorbance wavelengths are used. Select its DETAILS button to configure additional settings:

*Purity Measure Details*

- SHOW RATIO — Displays a ratio of the selected wavelengths when two absorbance wavelengths are used. The ratio trace is often a useful indicator of purity.
- SHOW SPECTRAL PURITY — Measures purity by using a comparison of the entire UV-spectra measured at differing times as a purity measurement. It also allows fractionation based on spectral purity.
- SPECTRAL PURITY DETECTION — Allows fractionation based on spectral purity.
- THRESHOLD — Works similarly to the THRESHOLD setting for $\lambda_1$ and $\lambda_2$. Checking SPECTRAL PURITY DETECTION makes this THRESHOLD option available.

### 4.3.3 Column Data

The COLUMN DATA window reports information about the column installed in the system. This information includes the “Number of times used,” “First Used On,” and “Last Used On” information, which can help you determine when the column should be replaced. The “Last Fluid Used” helps you determine if any solvent remaining in the column will be miscible with the solvent currently used in the system. To view the COLUMN DATA window, open the METHOD EDITOR, then select the COLUMN DATA button.

### 4.3.4 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 and selecting the OPEN and SAVE AS buttons open this window. Use FILES 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 updates. You can select the folder names to return to upper folder levels.
- File and Folder Operation buttons:

- COPY — Copies a highlighted file to the system's clipboard memory.
- PASTE — Pastes a file from the clipboard memory. If the file of the same name is already in the current folder, the system will ignore PASTE to prevent the original file from being overwritten.
- DELETE — Deletes a highlighted file or a folder and its contents.
- NEW FOLDER (icon) — Creates and names a new folder.
- UP (icon) — Allows you to browse the contents of the next-higher folder level.
- SEARCH — Finds matching file names when you enter a keyword and select SEARCH. Select CLEAR SEARCH to clear the results.

- File Management options —
  - Select COPY FILES TO FLASH DRIVE or MOVE FILES TO FLASH DRIVE to create archive copies (PDF or Text) of the files on a connected flash drive. Select DELETE BY AGE to specify an age limit beyond which to delete files from the internal hard drive.
  - FILE/FOLDER info scroll box — Lists the contents of the current folder. The contents can be sorted by selecting the column headings.
  - FILE TYPE — When shown, limits the display to a certain type of files. Select the file type from the list.
  - FILE NAME — A entry box used to identify the currently selected (highlighted) file or folder when browsing and opening files. When using SAVE AS, use this box to name the file.
  - OPEN/SAVE/DELETE — Performs the listed action.
  - LOAD PREVIOUS RUN FROM DETECTED RACK — Appears when using FILE > OPEN. Selecting this option causes the system to read the RFID tag on a single rack and displays the last RUN FILE collected on the detected rack on this instrument. This feature is useful you are unsure of the rack's contents.

☑ **Note**

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

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


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### 4.3.5 Set Data Path

FILE > SET DATA PATH 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 the directory you selected.

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

- CURRENT DATA PATH — The top-left corner of the window displays the path (current folder). As you browse through the files, the path updates.
- NEW — Adds and allows you to name a subfolder within the currently selected folder.
- DELETE — Deletes the selected folder.
- Folder selection box — Lists the available folders; select them by checking their boxes.
- OK — Saves your selection as the data path and closes the window.
- CANCEL — Closes the window without changing the data path.

### 4.3.6 MS Method Development

*MS > Method Development*

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

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

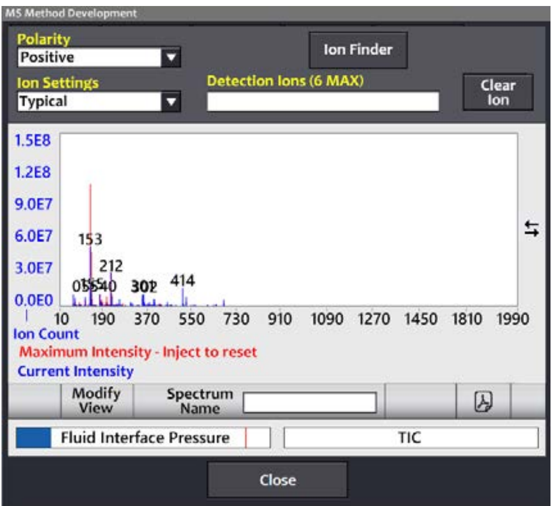


*Figure 4-19 Mass Spectrometer Method Development window*

- POLARITY — On the PurIon system, this 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—Opens the ION FINDER window. The Ion Finder identifies potential adducts, fragments, and multiply charged species of the compound of interest when the compound of interest does not show up in the spectrum graph. Such ions can then be used to detect the compound of interest. See Section 4.3.7 Ion Finder.
- DETECTION IONS—After a spectrum is collected, selecting 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 μg/mL.

- MODIFY VIEW—Sets the displayed range of the mass spectrum. The PurIon system still collects the entire mass range even when the range is set within than the minimum and maximum values allowed for the spectrometer. Values can range from 10 to 1200 Da (PurIon or PurIon S) or 2000 Da (PurIon L). Changing the mass range defined by these controls may cause the Y-axis (ion count) scaling to change on the basis of the tallest peak within the range. The display only labels the m/z for the 10 most prevalent ions currently displayed. Formatting the graph to a narrower range causes the system to relabel the ions to identify ions that may not have been intense enough to be labeled on the broader range graph.


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- SPECTRUM NAME— The file name for the mass spectrum is needed only if you want to save the mass spectrum to a file for later viewing.
- SAVE DATA— Saves the mass spectral data to the internal hard drive.
- SAVE AS PDF— Saves the displayed spectrum on either a USB drive or a remote computer's hard drive. The file name is the same as the spectrum name except for the .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, displayed in a ribbon gauge. If the pressure approaches the level of the red line, the system is clogged and should be cleaned to allow proper operation.

### 4.3.7 Ion Finder

*MS > MS Method Development > Ion Finder*

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

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

After entering a molecular weight, the Ion Finder algorithm looks for masses in the spectrum that match loss of various fragments, adducts, and multiply charged ion species. It can detect and report multimers, solvent adducts, and charge carriers that replace protons such as sodium and potassium. It can detect positive, negative, and multiply charged ions.

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

**☑ Note**

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

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

To use the Ion Finder:

1. Use the METHOD DEVELOPMENT window (MS > METHOD DEVELOPMENT) to inject a sample.
2. Select POLARITY and ION SETTINGS. Changing the ION SETTINGS changes the ions seen. Remember that ION SETTINGS choices do not transfer to the detection options for the mass spectrometer in the METHOD EDITOR—the ion settings that

gave a good result in the MS METHOD DEVELOPMENT window should be chosen.

3. Select ION FINDER. The ION FINDER window opens to display a list potential adducts and fragments detected in the compound of interest. Due to isotopes, multiple peaks near one another may be found. (For example, 546, 547, 818, and 819. See Figure 4-20.)
4. Optionally, select the checkbox next to a listed ion to add it to the ions to be detected.

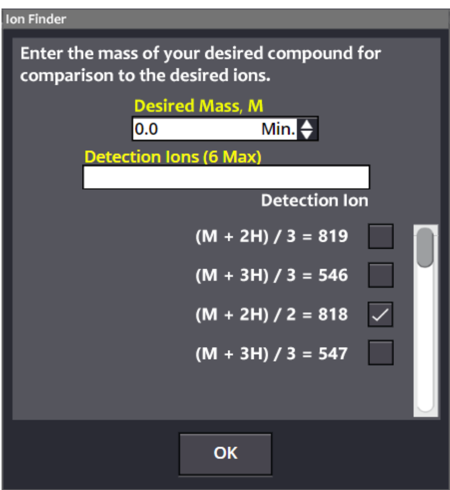


*Figure 4-20 The Ion Finder window*

DESIRED MASS – The molecular weight of your compound, rounded to the nearest integer.

DETECTED IONS – The list of ions to be detected.

DETECTION ION – The 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.

### 4.3.8 MS Manual Control

*MS > Manual Control*

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

MS MANUAL CONTROL 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 troubleshooting.


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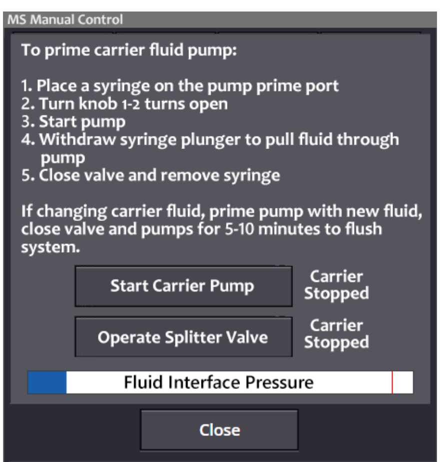


*Figure 4-21 Mass Spectrometer Manual Control window*

- START CARRIER PUMP — Turns the carrier pump on for priming or to replace one carrier solvent with another one. A purge is complete in ~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 — Displays the carrier solvent pressure in a ribbon gauge. If the pressure approaches the level of the red line, the system is clogged and should be cleaned to allow good response.
- CLOSE — Closes the window and stops the carrier pump if it is running.

### 4.3.9 MS Ionization Settings

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

*MS > Ionization Settings*

The MS IONIZATION SETTINGS improve the ionization of a particular compound by reducing fragmentation or adduct formation.

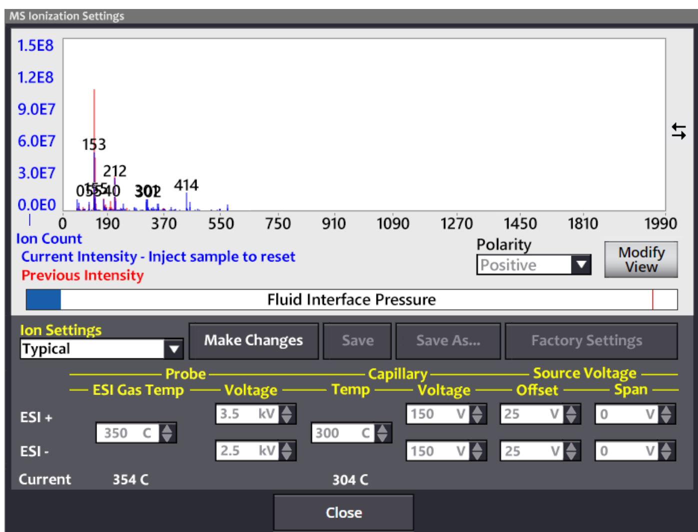


*Figure 4-22 Mass Spectrometer Ionization Settings window*

- MASS SPECTRUM GRAPH — Displays the mass spectrum. There are two mass spectra displayed. Blue displays the maximum of the current injection. Red displays the maximum of the previous injection for comparison. When a new injection is performed, the blue spectrum replaces the red spectrum, and a new blue spectrum is started. To inject a sample, move the PurIon injection valve to the "Inject Sample" position.

  Inject the sample (20 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 — Sets the displayed range of the mass spectrum. The PurIon system still collects the entire mass range even when the range is set within than the minimum and maximum values allowed for the spectrometer. Values can range from 50 to 1200 Da (PurIon and PurIon S) or 2000 Da (PurIon L). Changing


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the mass range defined by these controls may cause the Y-axis (ion count) scaling to change on the basis of the tallest peak within the range.

- FLUID INTERFACE PRESSURE — Displays the carrier solvent pressure 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 — Allows you to change the settings to improve the intensity of an ion.
- SAVE — Saves 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 changes the labels on the control to reflect the probe installed in the mass spectrometer.
- GAS TEMP — The temperature of the nebulization gas for the probe. Lower temperatures are used for more delicate, heat labile compounds. The temperature is set to quickly evaporate the carrier solvent. (Note: PurIon S and PurIon L systems display a single temperature for both positive and negative ionization).
- VOLTAGE (Current) — Displays a voltage setting (ESI probes) or current value (APCI probes). Lower values are used for more delicate compounds.

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

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

### 4.3.10 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.

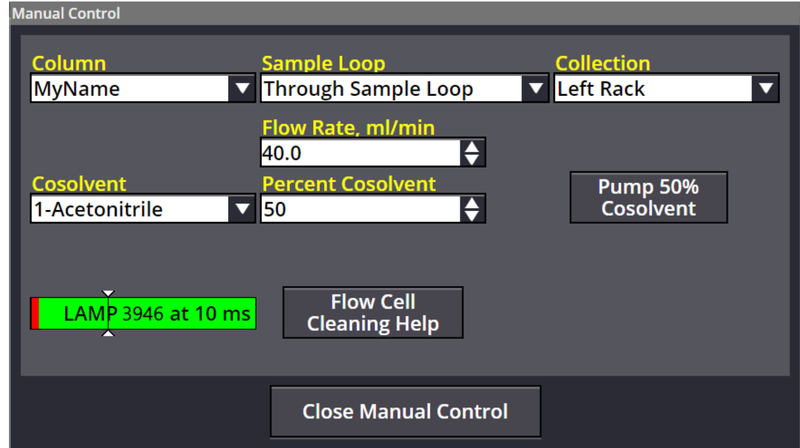


*Figure 4-23 Manual Control Settings - Quick Cleaning*

MANUAL CONTROL window options:

- COLUMN – The column that is in the flow path. There is always such a column. Choose one from the list box, making sure the co-solvent is compatible with the chosen column.
- SAMPLE LOOP – Settings made here allow fluid going through the injection valve to either BYPASS or go THROUGH (the) SAMPLE LOOP.
- COSOLVENT – Allows selection of the solvent that is pumped during manual pump operation.
- PERCENT COSOLVENT – The mixture percentage. Type it or select it from the list box.
- FLOW RATE – The pump's flow rate in mL/min. Type it or select it from the list box. The minimum flow rate available for manual control is lower than that on the METHOD EDITOR window. This is because some columns may need to be onboarded with that lower flow rate.
- COLLECTION – The destination for fluid. By default, fluid is pumped to the waste port during manual control. Alternatively, solvent can be collected in a fraction valve position or to a tube that you select from the list.

  This feature can be useful when trying to recover a compound that has precipitated or “crashed” somewhere in the


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fluid path. In that case, you can collect or flush the column in the MANUAL CONTROL window, then select a destination to send the eluate (Section 4.4.8). Afterwards, evaporate the eluant and rerun the sample.

- PERCENT XX% CO-SOLVENT – The percentage of co-solvent pumped with $CO_2$.
- STOP – Stops the pump. This button is only available while the pump is running.

The raw lamp energy display can be used to verify flow cell cleanliness. If the energy is low, it can be monitored while pumping a solvent to clean the flow cell (5.4 *Flow Cell Cleaning*). Select CLOSE MANUAL CONTROL to close the window.

### 4.3.11 Automation Manual Control

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

*Tools > Automation Wash Control*

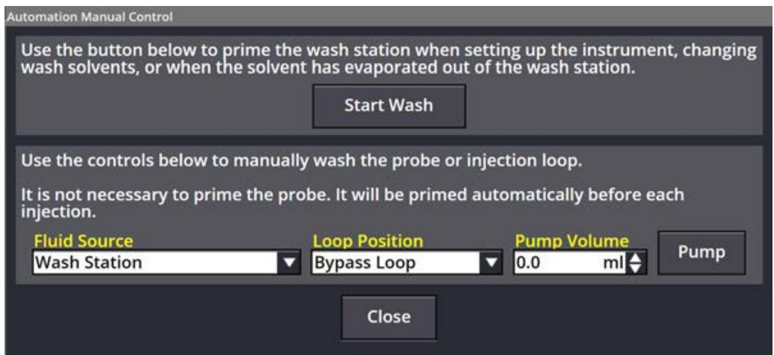


*Figure 4-24 The Automation Manual Control window*

To prime the wash station:

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

### 4.3.12 Set Administrator Password

This window, opened by selecting TOOLS > SET ADMINISTRATOR PASSWORD from the PeakTrak menu, 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. Make a record of the password and store it in a safe place.
5. Select OK to save your settings and close the window.

The following menu commands access administrative areas of PeakTrak and may require a password:

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

Password protection is disabled when the administrator password is left blank; then, all users can use the commands listed above. Furthermore, non-administrative users have their own passwords. Any user can have access to administrative areas if given administrative permissions in the USER MANAGEMENT window. (see Section 4.2.4 *Tools*: User Management).

## 4.4 Examples of PeakTrak actions

This section provides 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 ACCQ*Prep* SFC system

### 4.4.1 Editing a Method

Editing a method allows you to tailor the operation of the ACCQ*Prep* SFC 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 %co-solvent gradient mix, flow rate, solvents, and run length. These settings are part of the MAIN screen (Section 4.3.1), and are the most frequently changed settings when developing or improving purification methods.

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

The other settings are:

- FLOW RATE — This defaults to the value created when setting up the SFC column but can be changed for each separation as required. Note that the minimum flow rate available on the MANUAL CONTROL window may be lower than this rate to aid in loading certain columns.
- COSOLVENT — The solvents listed are those defined by the CONFIGURATION window.


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*Advanced Method Settings*

The METHOD EDITOR window (Section 4.2.2) gives you access to advanced settings such as EQUILIBRATION volume, INITIAL WASTE, PEAK DETECTION, and PEAK COLLECTION mode.

- 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:

1. On the METHOD EDITOR, select INITIAL WASTE AND TIME. The TIME WINDOWS window opens.
2. Enter a start time and end time. All times are relative to start of the run just following the sample injection.
3. Choose a peak collection mode. If you select Peaks, the module collects fluid only when the time window is active and a peak is present. If you select All, the module collects all fluid during the time window regardless of the peak state. Detected peaks still trigger tube advances.
4. Enter more time windows as needed. You can define up to six time windows.

- PEAK COLLECTION — There are three peak collection options: ALL, PEAKS, or NONE.
  - The ALL option collects peak and non-peak fluid in tubes.
  - The PEAKS option collects only peak fluid in tubes and diverts all other fluids to the waste port.
  - The NONE option diverts 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 eluant 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 and smaller fraction volumes of fluids of interest.

The fraction collector advances to the next tube whenever a peak is detected. Some notes about collector behavior:

- The system advances to the next tube if a new peak is detected before completing the last one (sometimes called a “double advance”).
- Tubes may also advance when using multiple detectors, depending on impurities or sensitivity to a given compound by each detector.
- 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.

**Tube Volume** – Options in this section allow you to 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 that is less than the capacity defined by the CONFIGURATION window.

**Peak Detection** – This section of the METHOD EDITOR 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 are displayed on the chromatogram.

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

See Section 4.3.2, *Wavelength Peak Detection* for more information about peak detection settings. Some useful method options are described below:

- $\lambda_1$ and $\lambda_2$ (wavelength 1 and 2) — Provides primary and secondary wavelength detection. Type or select the peak detection wavelength in nanometers, then select DETAILS to configure additional settings.

> ☑ ***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 - Select to only use the detection source as a monitor. When selected, the detection source is displayed as a trace on the gradient plot area but is not used to cut peaks.


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- ALL WAVELENGTH DETECTION — Detects peaks within a user-selected range of wavelengths. When enabled, select the DETAILS button to configure additional settings.
- ELS DETECTOR — The evaporative light scattering (ELS) detector on an ACCQ*Prep* system.
- MASS SPECTROMETER (PurIon systems only) — Monitors or detects compounds with a PurIon mass spectrometer system.
- ION SETTINGS – Sets ionization parameters to enhance detection of molecular ion peaks.
- PURITY MEASURE — Displays a ratio of the selected wavelengths if two absorbance wavelengths are used. The ratio trace is often a useful indicator of purity.

Selecting PEAK DETECTION DETAILS opens additional detector options for λ1 and λ2, ALL WAVELENGTH DETECTION, MASS SPECTROMETER, ELS DETECTION, and PURITY MEASURE peak detection. See Section 4.3.2 for detailed information about peak detection settings.

**Saving Changes to the Method File** – After you have edited the method file, you can save the changes for future use:

1. Select the SAVE AS. The FILES window opens.
2. Give the method file a descriptive name, then select SAVE.

The method file is stored by the ACCQ*Prep* system and will be available for future runs.

### 4.4.2 An Alternative Way to Create Method Files

Other than by editing a method file on the ACCQ*Prep* system, there is another way to create method files:

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

### 4.4.3 Defining a Gradient

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

The methods above work in both the MAIN window and METHOD EDITOR window. Alternatively, you can modify, add, insert, and delete points using a tabular view on the METHOD EDITOR window. Select the EDIT GRADIENT button to open this view, then use the controls to modify the settings. Select the EDIT GRADIENT button again to close the table view.

### 4.4.4 Real-time Gradient Editing

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

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

### 4.4.5 Stacked Injections

Stacked Injections save time and solvent when running multiple injections. Stacked injections options are found on the RUN REQUIREMENTS window that appears when you START a run. See Section 3.3.9 *Stacked Injections* for step-by-step instructions and information about settings.

### 4.4.6 Monitoring the Purity Measure

When using two wavelength detection options, you can also display a ratio of the two wavelengths, 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 is 1.33. Since the relationship of absorbance to concentration is not variable, the ratio remains steady while the concentration changes from the beginning to the end of the peak. During the duration of the peak, the ratio will be 1.33, and this constant value is displayed as a horizontal line.

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

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


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

### 4.4.7 Viewing Runs

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

1. From the MAIN window, select FILE > OPEN, or select the OPEN button. The FILES window is displayed.
2. Choose a Run file and select the OPEN button. The RUN VIEWER window opens.

Included on the PeakTrak RUN VIEWER:

- Rack and tube information — The left pane of the window lists the current rack, a map for that rack, and a table that lists the peaks and their corresponding tube numbers. If the window is currently displaying the collection parameters, select 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 selecting a tube.
- Method parameters — Select DISPLAY METHOD to view a summary of the peak detection and collection settings for the run.
- Chromatogram — Displayed on the right side and identified by the sample name in the window’s title bar.
- OPTION buttons — Quickly access frequently used commands while viewing a run:

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

  SAVE AS PDF — Saves the run summary as a PDF file. When viewing the run from a remote personal computer, you can save the summary 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 TEXT — Saves the run summary as an ASCII text file. When viewing the run from a remote personal computer, you can save the summary.

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 the touch panel, pinch zoom or pan to view the results screen in greater detail. Pan requires the use of two fingers to differentiate a pan action from a select action. You can also select ZOOM IN OR ZOOM OUT.
- MODIFY VIEW — Opens a window from which you can set the left and right Y-axis scales. These scales are controlled by the ABSORBANCE and %B UPPER LIMIT. Selecting SHOW THRESHOLD LEVEL shows the threshold level setting of all detectors except the mass spectrometer. The threshold levels are color-coded to the detector trace.
- REFERENCE CHROMATOGRAM - Allows you to pull up a previous run to compare to current run. Time scaling matches the longest run. If runs are of different length, the shorter run will be missing that part of the chromatogram. Also, zooming on one spectrum also scales the other spectrum similarly.
- MS - Displays a mass spectrum (PurIon systems only). Selecting in the Chromatogram window displays a mass spectrum at that point during the run. Selecting on a tube in the rack map shows the mass spectrum of the contents of that fraction.
- UV (is displayed as “vis” on UV-Vis equipped systems) - Displays a UV or UV-visible spectrum (UV-Vis systems only). Selection within the Chromatogram window display a mass spectrum at that point during the run.
- STACK INJECTIONS — Opens the STACKED INJECTIONS window, from which time windows can be adjusted. See Section 3.3.9 *Stacked Injections*.
- EXTRACT METHOD — Loads a new method file on the basis of the parameters for the run you are viewing.
- EXTRACT TO — Lists columns to extract to.
- CLOSE — Closes the run file view when you are done viewing it

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

If the run used multiple racks, the PREVIOUS and NEXT RACK buttons are active. Select either to scroll through the available racks. The currently displayed rack is identified



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by the letter 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. These may be visible if you are monitoring a second wavelength.
- Green traces. These are from the ELSD.
- The PurIon traces color coded with the selected masses below the graph Y-axis.
- The blue gradient curve that was used during the run. The % Co-solvent scale is shown in blue on the right Y-axis.
- The X-axis that depicts the run time, shown as minutes.
- Vertical lines that appear at intervals along the X-axis. These lines indicate collection tube changes. To prevent the plot area from being obscured by tube change marks, PeakTrak may limit the number of marks.

### 4.4.8 Manual Control of the ACCQ*Prep* SFC

Manual Control can assist with method development, maintenance of the system plumbing, and system troubleshooting. To manually control the ACCQ*Prep* SFC system, first open the MANUAL CONTROL window by selecting TOOL > MANUAL CONTROL.

*Pumping Solvents*

To pump a mixture and adjust the PERCENT COSOLVENT setting and then select PUMP% COSOLVENT. When finished, select STOP FLOW.

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 fraction valve position or to a collection tube on the AutoSampler. To do so, select the COLLECTION option and then select a fraction valve position tube ("next" or specific tube number).

*Raw Lamp Energy*

The left side of the MANUAL CONTROL window shows a Raw Lamp Energy gauge. It 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 desired compounds from being diverted to waste.

Depending on what you are doing with the MANUAL CONTROL functions, low lamp energy could indicate normal operation. 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. Or, abnormal operation could be indicated, such as a flow cell blocked by a compound that has precipitated or by a film built up on the flow cell.


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# Section 5 Maintenance

## 5.1 Introduction

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

### 5.1.1 Cleaning

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

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

**⚠ CAUTION**

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

### 5.1.2 Collection Rack and Tray Cleaning

**⚠ WARNING**

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

The collection tube racks and tray are made of conductive plastic. Dirt, film, or coatings might prevent their ability to dissipate static electricity. To avoid problems that 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.

## 5.2 System Standby and Shut Down

During extended periods of inactivity, you can place the system in STANDBY to conserve power. To do so, log off the system (FILE > LOGOUT) and press the power button ( ⏻ )on the *front* of the unit.

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

**⚠ WARNING**

**As long as the AC mains power cord is connected and rear circuit breakers are in the ON position, power is inside the unit. The mains power cord is the disconnect device. Position the ACCQ*Prep* 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 or turning off the rear circuit breakers 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 or turning off the rear circuit breakers.

### 5.2.1 Tubing Inspection

**⚠ WARNING**

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

Perform a tubing inspection monthly:

1. Visually inspect the solvent, waste, and drain tubing. The tubing must be free of any damage, kinks, or deterioration. Fittings must show no signs of leaks.
2. Check pumps at the front of the pump module for leaks, frosting, and condensation.
3. Visually inspect the back-side tubing for leaks, frosting, and condensation.

Correct any deficiencies before returning the instrument to operation.



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## 5.3 Preventive Maintenance

The system requires preventive maintenance for safe and reliable operation. A Preventive Maintenance Kit (PN 60-5267-020) is available from Teledyne LABS for this purpose.

Refer to the schedule below for the minimum periodic maintenance requirements.

**As Needed** – Perform these tasks as conditions require:

- Cleaning (Section 5.1.1).
- Quick flow cell cleaning when recommended by a system alert message (Section 5.4).
- Wipe the cone on PurIon system with a wipe soaked with methanol or water to remove visible residue near 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 co-solvent to flush residual compounds from the column, internal tubing, and flow cell. If performing stacked injections or multiple injections of same sample, a flush is also necessary. Refer to section 5.4.
- 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 the sample probe wash to run to completion to wash residual compounds from the sample line and from the ELSD (if installed) flow path.

**Weekly** – Perform these tasks at least weekly, and more frequently if conditions warrant:

- Make sure that the chiller liquid is at an adequate level.
  - Make sure the chiller liquid level is between 1.0"–2.6" from the top edge of the internal reservoir.
- Make sure that the chiller is cold.
  - Make sure that chiller is cooling the liquid to set temperature by checking the LCD display on the chiller.
- Make sure that the chiller has adequate flow.
  - Make sure that liquid is flowing through chiller tubing to the $CO_2$ pump heads in the SFC pump box.

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

- Tubing Inspection (Section 5.2.1).
- Collection rack and tray cleaning (Section 5.1.2).
- Monthly flow cell cleaning (Section 5.4.2).

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

- Lamp replacement.
- Lamp fan cleaning.

- Drop former alignment (if AutoSampler is installed).
- Fractionation valve rotor replacement.
- Change roughing pump oil (PurIon systems only).


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## 5.4 Flow Cell Cleaning

### 5.4.1 Post Separation

As a preventive measure, all default column methods finish the separation run with a high percentage of co-solvent (Figure 5-1). This brief time (one to six column volumes) of strong solvent flushes residual compounds from the column, flow cell, and internal tubing.

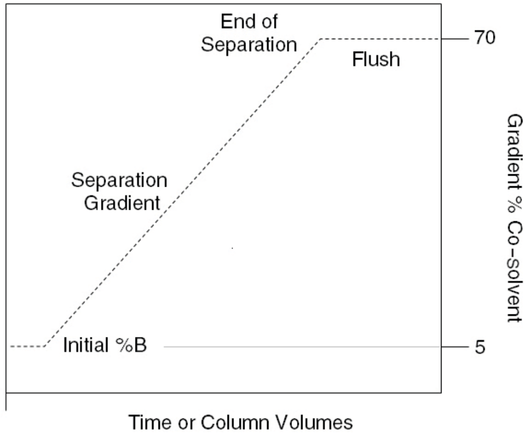


*Figure 5-1 Default post-separation column and flow cell flush*

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 5-2).

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

If stacked injections or multiple injection sequences were run, allow a post-separation flush to run.

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 ammonium hydroxide.

### 5.4.2 Flow Cell Cleaning when Recommended

The raw lamp energy gauge measures the cleanliness of the flow cell. A dirty flow cell may cause the detector signal to appear "noisy."

Perform *Flow Cell Cleaning when Recommended* (Section 5.4.2) once a month for preventive maintenance of the flow cell.

Selecting TOOLS > MANUAL CONTROL opens the MANUAL CONTROL window on which the raw lamp energy gauge is displayed. The gauge has two ranges: red and green (Figure 5-2).

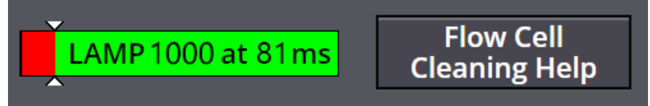


*Figure 5-2 Raw lamp energy gauge*

- **Red** – Indicates that 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** – Indicates that lamp energy is sufficient to detect peaks within typical system limits.

When the lamp energy is lower than normal (Figure 5-2), the system will recommend flow cell cleaning before starting a separation run.

When the system displays this message, you can:

- **Cancel Run** (recommended) – Select this to perform a quick cleaning described in the following steps.
- **Continue Collect All** – Select this 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** – Displays the flow cell cleaning on-line help topic.

To perform a quick cleaning after selecting the CANCEL RUN:

1. Install a union in one of the column positions. Correctly installed, this turns that position into a bypass.

**☑ Note**

You can rename the column position using TOOLS > CONFIGURATION > PREP SFC. Giving the COLUMN POSITION a descriptive name like “Bypass” will make it easier to find in step 5a.

2. Using the control on the front of the unit, prime the co-solvent inlet lines: first with methanol, then with acetone.
3. Open TOOLS > METHOD EDITOR and change
   - the PREHEATER temperature to 70° C, and
   - the BPR PRESSURE to 160 BAR (2321 psi).

   Save these settings by selecting the SAVE icon, then EXIT.
4. Select TOOLS > MANUAL CONTROL. This opens the MANUAL CONTROL window.

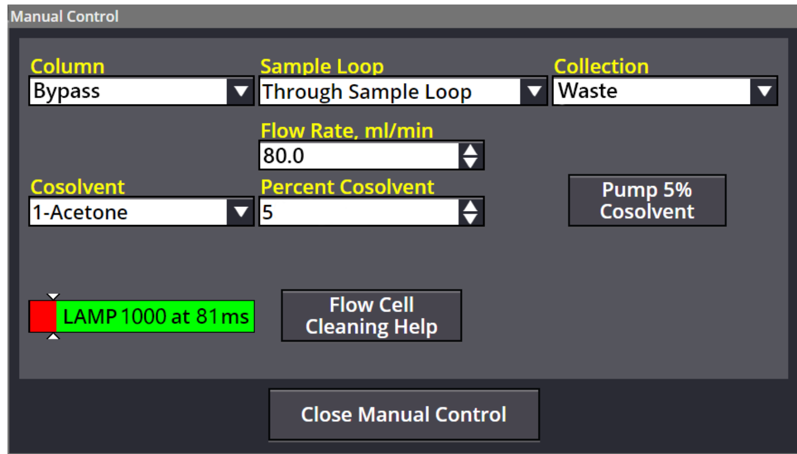


*Figure 5-3 Manual Control Settings - Quick Cleaning*

5. Make the following settings on the MANUAL CONTROL window (Figure 5-3):
   a. Verify that the COLUMN position that contains the union inserted in Step 1 is selected.
   b. Set the FLOW RATE to 80 mL/minute.
   c. Set SAMPLE LOOP to Through Sample Loop.
   d. Set COLLECTION to Waste.
   e. Verify that the COSOLVENT selected is the solvent inlet line submerged in the Acetone solvent supply container.

**⚠ CAUTION**

Choosing the wrong column position in Step 4a could cause the solvent mixture to be pumped through a column. If the mixture is incompatible with the column, it could be damaged.

6. Set the PERCENT COSOLVENT to 5% and select PUMP 5% COSOLVENT. Allow the pump to run for 2 minutes.
7. Change the PERCENT COSOLVENT to 30%. Run this for 2 minutes.
8. Change PERCENT COSOLVENT to 70%. Note that because acetone absorbs light at 254 nm, the lamp reading may remain in the red.
9. Stop the pump, then select the solvent line containing methanol. Run this in the same fashion as steps 5-7, with 30% and then 70% co-solvent for 2 minutes each.

After the acetone is washed from the system, the light intensity should read in the green on the lamp energy gauge.

If washing with methanol fails to restore the lamp energy out of the red, contact the Teledyne ISCO Technical service department.

**☑ *Note***

Over time, pumping co-solvent at a moderate flow rate (50 to 80 mL/min) will usually solubilize obstructions, including those resulting from the last run or left over from the cleaning procedure. Generally, the recommended solvent is the highest polarity solvent that you have recently used.

## 5.5 ACCQ*Prep* SFC Maintenance

### 5.5.1 Maintenance Tools and Instructions

ACCQ*Prep* SFC systems include an accessory kit containing spare rotors, parts, and other useful items for periodic maintenance.

Often, this requires gaining access to the pump compartment by gripping the edges of the front panel and pulling forward on both sides (Figure 5-4).


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*Figure 5-4 Front panel of the ACCQ*Prep *SFC system*

### 5.5.2 Injection Valve Rotor

Failure of the injection valve rotor requires its removal, cleaning, or (potentially) its replacement. Symptoms of failure are leaking, high pressure caused by clogging, and flow to undesired flow paths.

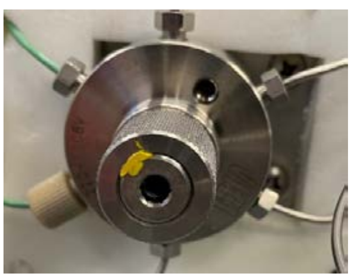

*Figure 5-5 The injection valve rotor assembly*

> **Note**
> The injection valve rotor is a common wear part. Since it is a sealing surface, it can be damaged by particulates in the sample. To prolong the life of the rotor, ensure that your sample is properly filtered (<20 µm) to prevent premature failure.

To replace to rotor, follow these steps:

*Removing the Injection Valve Rotor*

1. Have the Accessory Kit (PN 60-5269-011) on hand. This kit is included with the ACCQ*Prep* SFC system and includes the rotor for this procedure.

> **Note**
> For other parts for your ACCQ*Prep* SFC system, visit https://store.teledyneisco.com, or contact your local Teledyne LABS representative.

2. Do a system vent. Select TOOLS > MANUAL CONTROL > VENT SYSTEM PRESSURE). Allow at lease 15 minutes for the instrument to depressurize. You can monitor the System

and BPR pressure reading in the System Status Bar at the top left hand corner of the touchscreen.

3. Turn off the power to the system.
4. Access the column oven module. Remove the valve thumbscrew by hand. If the thumbscrew does not move by hand, gently use pliers or channel locks to loosen, then finish removal by hand.

**⚠ CAUTION**

Do not use an Allen wrench or other driver to loosen or tighten the valve. Turn it using the knurled outer portion only.

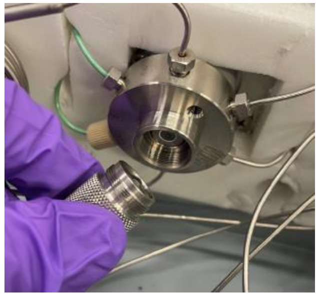

*Figure 5-6 Rotor thumbscrew removal*

5. Locate the rotor inside and slip out by using a magnet.

6. Make note of marking on rotor, as it needs to go back in the same orientation.


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- Use a marker to mark the front face of the rotor or make note of the embossed letter on the rear edge of the rotor, as it needs to go back in the same orientation.

7. Install new rotor by performing the removal steps in the reverse order. Installation is similar to removal. When inserting the rotor, ensure that it is oriented in the same direction as it was when removed.

### 5.5.3 Column Select Valve Rotor Replacement

Failure of the column select valve rotor requires its removal, cleaning, or (potentially) its replacement. Symptoms of failure are leaking to the exterior of the valve, high pressure caused by clogging, and leaking to an undesired column.

To replace to rotor, follow these steps:

1. Have the Accessory Kit (PN 60-5269-011) on hand. This kit is included with the ACCQ*Prep* SFC system and includes the rotor for this procedure.
2. Do a system vent. Select TOOLS > MANUAL CONTROL > VENT SYSTEM PRESSURE. Allow at lease 15 minutes for the instrument to depressurize. You can monitor the System and BPR pressure reading in the System Status Bar at the top left hand corner of the touchscreen.
3. Turn off the power to the system.
4. Access the column oven module. Using a 5/8" open-faced wrench (or adjustable open-faced wrench) to loosen the rotor cap.

*Figure 5-7 Loosening the rotor cap*

5. Finish removing the rotor cap by hand.

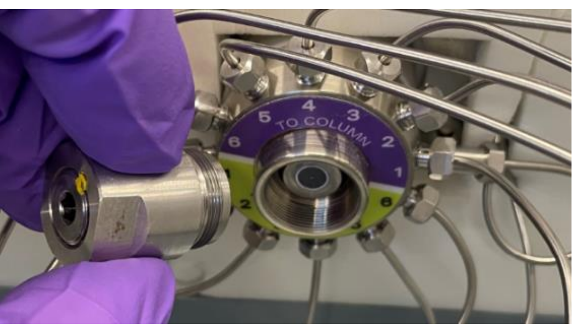


*Figure 5-8 Removing the rotor cap*

6. Pull out the rotor using a magnet to grab it.

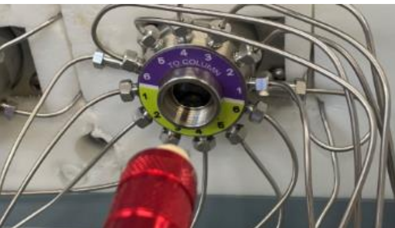

*Figure 5-9 Removing the rotor*

7. Install new rotor by performing the removal steps in the reverse order. Installing the new rotor is aided by using needle-nose pliers. Take care to not scratch the rotor.

*Figure 5-10 Tightening the new rotor*

8. Test new rotor by running the unit in Manual Control and verifying that there is flow to each column position without leaking or over-pressurization errors.

### 5.5.4 Fractionation Valve Rotor Replacement

Failure of the fractionation valve rotor requires its removal, cleaning, or (potentially) its replacement. Symptoms of failure are leaking, flow to undesired ports, overpressure, and fraction valve error messages.


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*Figure 5-11 The fractionation valve*

To replace to rotor, follow these steps:

1. Have the Accessory Kit (PN 60-5269-011) on hand. This kit is included with the ACCQ*Prep* SFC system and includes the rotor for this procedure.
2. Do a system vent. Select TOOLS > MANUAL CONTROL > VENT SYSTEM PRESSURE). Allow at lease 15 minutes for the instrument to depressurize. You can monitor the System and BPR pressure reading in the SYSTEM STATUS BAR at the top left hand corner of the touchscreen.
3. Turn off the power to the system. Disconnect any tubing lines connected to the fraction valve.
4. Using a Phillips screwdriver, remove the four screws securing the case top of the control module. Set the screws and the case aside.

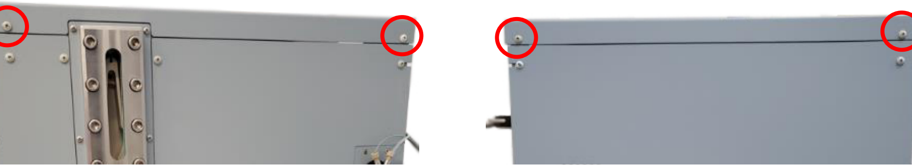

*Figure 5-12 Control module case top*

5. Remove fractionation valve bracket by removing the two nuts that secure its mounting bracket to the side of the chassis. Use a 5/16" nut driver or wrench. Be sure to unplug the USB and power connectors as well, noting their locations.

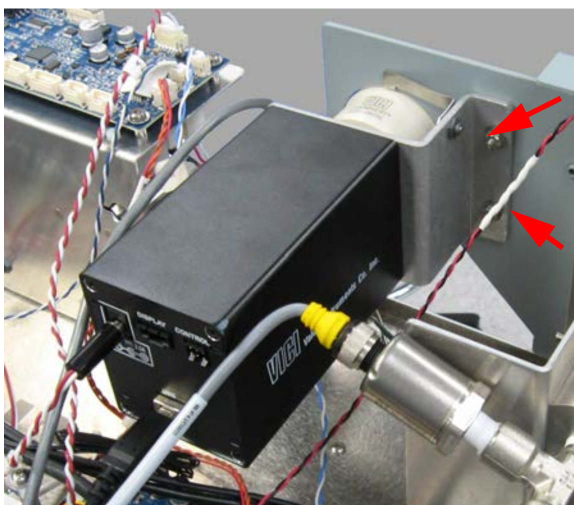

*Figure 5-13 The fractionation valve mounted (left) and removed (right)*

6. Locate and remove the Allen nuts using a 7/64" Allen wrench.

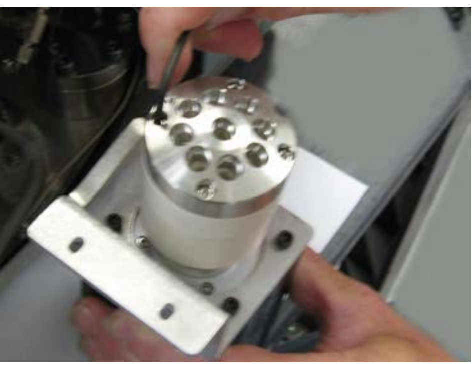

*Figure 5-14 Loosening of the fractionation valve stator*

7. With all mounting Allen nuts removed, pull the stator away from the valve body. It it not necessary to remove the retaining clip the holds the stator to the mounting bracket.


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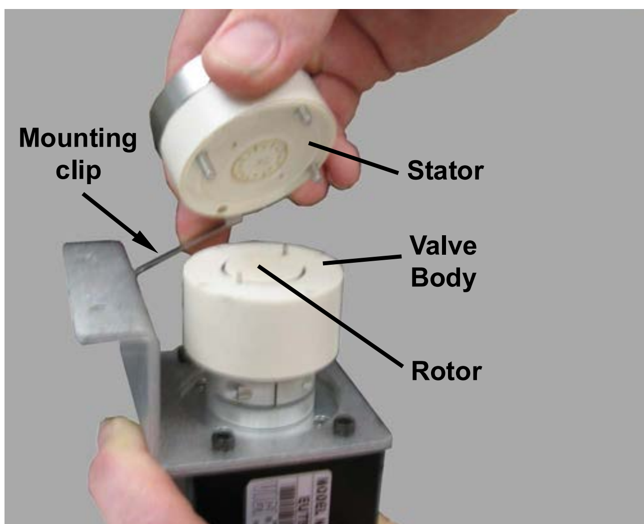
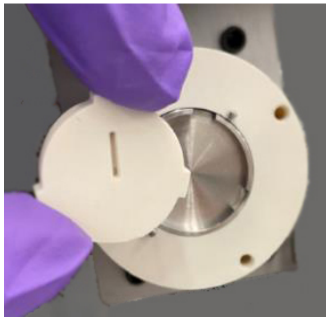



*Figure 5-15 Removal of the fractionation valve stator*

8. Slip out the rotor from the valve. This may require the use of needle-nose pliers or another tool, but take care not to scratch any surfaces of the valve.

*Figure 5-16 Removal of the fractionation valve rotor*

9. Install the new rotor with the flow channel facing towards the stator face.



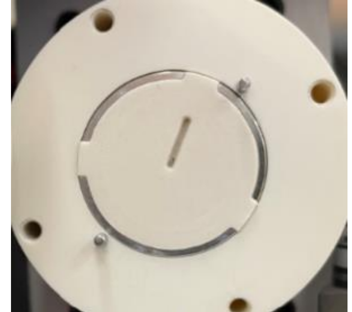


*Figure 5-17 Installation of the fractionation valve rotor*

10. Line up the stator face with the 1 port near the 12 o'clock position. The stator face will not go into position unless the offset mounting pins are aligned.

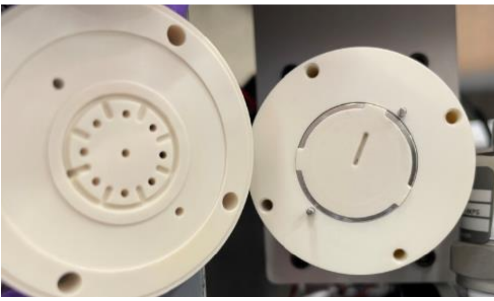
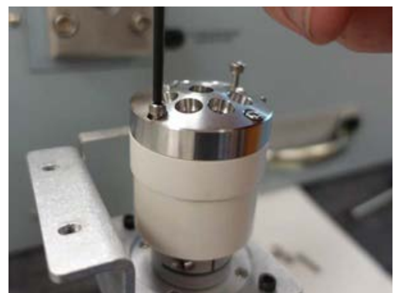



*Figure 5-18 Reinstallation of the fractionation valve stator*

11. Reinstall the mounting Allen head nuts in a criss-cross pattern as illustrated.

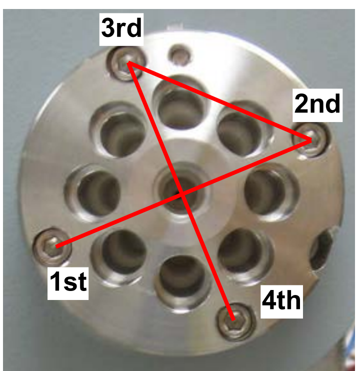



*Figure 5-19 Fastening the fractionation valve stator*

12. Reinstall the fractionation valve assembly in the ACCQ*Prep* SFC unit chassis, taking care to reattach the USB and power cables.
13. Test new rotor by running the unit in Manual Control through the fraction ports and to waste.

## 5.6 AutoSampler Maintenance

*Peristaltic Tubing Replacement*

For peristaltic tubing for your ACCQ*Prep* SFC system, visit https://store.teledyneisco.com.


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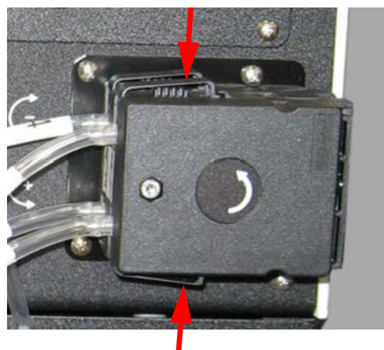
*Figure 5-20 The peristaltic pump at the back of the AutoSampler*

1. At the pump: push the levers at the top and bottom of each tube retaining clip, then pull the clips out (Figure 5-21).



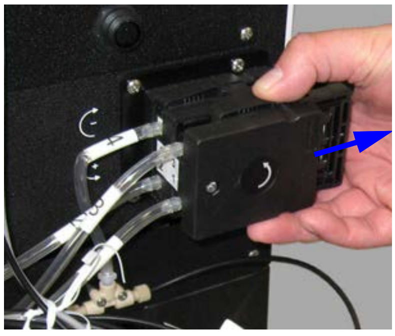


*Figure 5-21 Removing a retaining clip*

2. Disconnect the upper and lower clear tubing from each peristaltic tube (Figure 5-22).

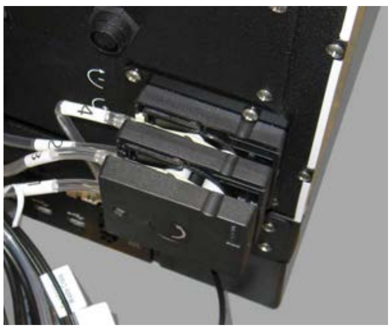


*Figure 5-22 Disconnecting the clear tubing*

3. Remove the peristaltic tubing. Clips on the tubing that secure the ends to the inner edge of the pump slide out (Figure 5-23).

*Figure 5-23 Removing the tubing*

4. Install the new peristaltic tubing with the thicker tubing towards the AutoSampler.
    - Install the new peristaltic tubing with the 3.2 mm ID tubing (thicker) towards the AutoSampler (ports labeled 2 and 4). 2.0 mm ID tubing (thinner) furthest away from the AutoSampler (ports labeled 1 and 3).
5. Reconnect the clear tubing. Be sure that the numbers on the tubes correspond to the numbers on the pump.
6. Replace the tubing retaining clips.

## 5.7 PurIon Maintenance

### 5.7.1 ESI and APCI Removal from PurIon S and PurIon L

Refer to Figure 5-24.

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

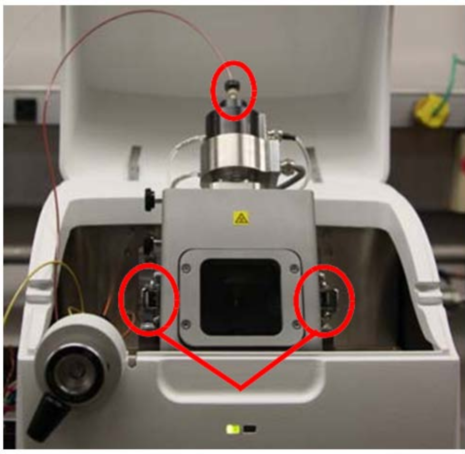


*Figure 5-24 ESI and APCI removal from PurIon S and PurIon L*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### 5.7.2 ESI and APCI Replacement PurIon S and PurIon L

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

### 5.7.3 Cleaning the Ionization Source Capillary

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

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

### 5.7.4 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 ‘heated desolvation’ on the side of the housing.

### 5.7.5 Overpressure Error

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

- If the error occurs during a run, select CONTINUE WITHOUT PurIon. The purification then continues without the PurIon signal or peak detection but relies 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.
- Select START CARRIER PUMP.
- Watch the pressure on the ribbon gauge.
- Loosen the fitting at the source inlet. If the pressure drops, then the source capillary is plugged.

- If there is still an error or the pressure remains high, then the plug is between that point and the splitter valve (or the valve itself). Continue to loosen fittings going back the to the splitter valve until the error is corrected.

### 5.7.6 PurIon Cone Cleaning

This error occurs if the capillary inlet to the PurIon vacuum region is restricted causing a higher vacuum reading than normal. PurIon S & L models compatible with the NextGen systems have an internal valve that allows removal of the capillary inlet without venting the vacuum system to allow easy cleaning. Even with the easy cleaning capability, Teledyne LABS recommends keeping a spare capillary inlet cone assembly (PN 25-0000-085) to allow rapid replacement to minimize downtime while cleaning the plugged capillary.

### 5.7.7 Capillary Inlet Cone Removal

1. Set the PurIon to standby.
2. Wait approximately 15 minutes for the cone to cool.
3. Remove the ion source assembly. See section 5.7.3.
4. Wearing gloves (typical lab gloves are usually sufficient), place your fingers on the top surface of the cone and turn counterclockwise to unscrew the capillary inlet cone. Often this is sufficient to remove the capillary. If you are unable to unscrew the part manually, use an adjustable wrench on the flats of the cone to unscrew them. The flats are not large, and they may be difficult to see while a wrench is seated on them.

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

### 5.7.8 Capillary Inlet Cone Cleaning

1. Remove the O-ring under the capillary base, then sonicate the capillary in a methonal:water (50:50) mixture for 30 minutes (Figure 5-20).

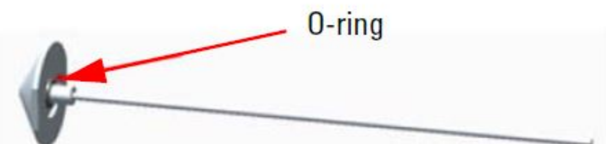


*Figure 5-25 Location of O-Ring*

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

### 5.7.9 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


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slight restriction in a downward motion as the part is almost completely inserted. This is the capillary opening the valve to the vacuum region.

3. Screw the capillary inlet cone into the inlet. Finger tight is sufficient as long as the part is fully seated.
4. Replace the ion source.
5. Place the PurIon in the operate mode.

#### 5.7.10 PurIon Troubleshooting

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

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

**Table 5-1 Common PurIon Error Codes and Resolutions**

| Error message | Resolution |
|---|---|
| PurIon temperatures are stabilizing.<br>[r] seconds remain. (where [r] is a number) | The PurIon has several areas with heaters. The software has a set timer to allow temperatures to come up to operating conditions. After that time, a separate error is thrown if the heaters are not within an acceptable band. The default time is 300 seconds after entering the operate condition.<br>During the standby condition most heaters are set to OFF except the inlet capillary with is set to 50 °C during standby. |
| The PurIon vacuum level is too low to operate. Please verify that the roughing pump is on and operating correctly.<br>Pirani Pressure: [s1] mbar. (where s1 is the vacuum reading). | The Pirani pressure must be below 5.5E-3 mbar before the PurIon turbo pump will operate. This message appears when placing the PurIon in operate without turning on the roughing pump,<br>This error generally occurs if the user forgets to turn the roughing pump back on after cleaning the capillary or changing the pump oil. |
| The PurIon has been shut down. It will be unavailable for use until the ACCQPrep has been rebooted. Be sure that the PurIon and fluid interface are both turned on before rebooting the ACCQPrep. | This message is displayed after the PurIon has been successfully shut down through the SHUTDOWN command. It serves as a reminder that an ACCQPrep reboot will be necessary for the PurIon to be enabled again by the system. |
| The splitter valve seals have exceeded their recommended life. The PurIon will continue to operate, but there is an increased possibility of leakage at the splitter valve and loss of PurIon detection during a separation. | The splitter valve supplier has stated that typical valve life exceeds 1,000,000 actuations. After that time, there is no method of determining when it will leak. The valve can be rebuilt using the valve rebuild kit (PN 60-5234-629) or continue to use and monitor for leakage. Leakage should not drip out the bottom of the fluid interface front cover. |
| No ion source is detected on the PurIon. Verify that the ion source is properly installed and connected. If the source is still not detected, contact a qualified service technician. Error 309 | The PurIon has reported that the ion source high voltage cable is not properly plugged into its socket. This is the cable with the round connector and is to the right and behind the ion source.<br>This generally occurs upon changing or cleaning probes. |
| A plug has occurred in the PurIon fluid lines. The separation can be continued without PurIon detection or continued if the plug is corrected. Error 310 | This error message is displayed if a plug is detected during a separation. |

**Table 5-1 Common PurIon Error Codes and Resolutions**

| | |
|---|---|
| The PurIon inlet cone may be plugged, which could prevent detection. Continued operation will not cause damage. Contact a qualified service technician to clean the cone; Pirani Pressure: [s1] mbar. Error 315 | During normal operation, the Pirani pressure should be >1.5E-3 mbar. Anything less is an indication of either partial or complete plugging of the capillary cone entrance to the vacuum area. See cleaning the cone capillary (5.7.3). |
| A plug has occurred in the PurIon fluid lines. The plug must be corrected to allow continued operation. Error 316. | This error occurs if the tubing is plugged while the method development screen is in use. See cleaning the probe capillary (5.7.3). |
| 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 PurIon systems (not PurIon S or PurIon L), the probe isn't seated properly. Loosen the thumbscrew on the front of the ion source housing, push down on the probe, then tighten the thumbscrew. |
| The fluid interface pressure is too low. Error 325 | This could be due to lack of carrier fluid, loss of pump prime, or leakage. |


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