Safety

Before installing, operating, or maintaining this equipment, all hazards and preventive measures must be fully understood. Refer to Section 1.6 Safety in this Operation Guide for general warnings about safety that pertain to the CombiFlash NextGen system.

While specific hazards may vary according to location and application, take heed in the following general warning:

⚠️ 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.

You are urged to read and follow the general warnings contained in the CombiFlash NextGen Important Information document (P/N 69-5253-086). This document is shipped with your CombiFlash NextGen system and can be downloaded from https://www.teledyneisisco.com/chromatography/combiflash.

Potential hazards covered by the CombiFlash NextGen Important Information document include, but are not limited to:

- **Chemical.** Chemicals used with the CombiFlash NextGen system may be classified as carcinogenic, bio-hazardous, flammable, or radioactive.
- **Laboratory.** Chemical vapors may be flammable or otherwise hazardous.
- **Equipment.** Use of the CombiFlash NextGen instrument in any way not specified in the manual, may impair the protection provided by the instrument.
- **Electrical.** Spark sources and static electricity pose a potential hazard.

In all cases, use good laboratory practices and standard safety procedures, and follow all applicable regulations.
## 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.

<table>
<thead>
<tr>
<th>Hazard Symbols</th>
<th>The equipment and this manual use symbols used to warn of hazards. The symbols are explained in the table below.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Warnings and Cautions</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Exclamation Point" /></td>
<td>The exclamation point within the triangle is a warning sign alerting you of important instructions in the instrument’s technical reference manual.</td>
</tr>
<tr>
<td><img src="image" alt="Lightning Flash and Arrowhead" /></td>
<td>The lightning flash and arrowhead within the triangle is a warning sign alerting you of “dangerous voltage” inside the product.</td>
</tr>
<tr>
<td><strong>Symboles de sécurité</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Exclamation Point" /></td>
<td>Ce symbole signale l’existence d’instructions importantes relatives au produit dans ce manuel.</td>
</tr>
<tr>
<td><img src="image" alt="Lightning Flash and Arrowhead" /></td>
<td>Ce symbole signale la présence d’un danger d’électocution.</td>
</tr>
<tr>
<td><strong>Warnungen und Vorsichtshinweise</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Exclamation Point" /></td>
<td>Das Ausrufezeichen in Dreieck ist ein Warnzeichen, das Sie darauf aufmerksam macht, daß wichtige Anleitungen zu diesem Handbuch gehören.</td>
</tr>
<tr>
<td><img src="image" alt="Lightning Flash and Arrowhead" /></td>
<td>Der gepfeilte Blitz im Dreieck ist ein Warnzeichen, das Sie vor “gefährlichen Spannungen” im Inneren des Produkts warnt.</td>
</tr>
<tr>
<td><strong>Advertencias y Precauciones</strong></td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Exclamation Point" /></td>
<td>Esta señal le advierte sobre la importancia de las instrucciones del manual que acompañan a este producto.</td>
</tr>
<tr>
<td><img src="image" alt="Lightning Flash and Arrowhead" /></td>
<td>Esta señal alerta sobre la presencia de alto voltaje en el interior del producto.</td>
</tr>
</tbody>
</table>
Technical assistance for the Teledyne ISCO CombiFlash NextGen can be obtained from:

Teledyne ISCO
4700 Superior St.
Lincoln NE 68504
Phone: (800) 775-2965 or (402) 853-5340
Fax: (402) 465-3001
E-mail: IscoService@teledyne.com
CombiFlash NextGen Systems

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1.1 Overview

This Installation Guide provides:

- Safety information
- Unpacking instructions
- Installation instructions, including connections with Windows and iOS operating systems
- Basic user operation
- Software overview
- Certification and warranty information

1.2 Product Overview

The Teledyne ISCO CombiFlash NextGen flash chromatography system gives you high-productivity automation, programmable gradients, UV detection and peak separation, and automatic detection of columns and collection tube racks (system dependent). Its small size makes it a great “personal system” and well-suited for operation within chemical hoods and other limited indoor spaces. An optional fraction collection enclosure allows usage on an open bench in some environments.

⚠️ WARNING

Avoid hazardous practices! If you use this instrument in any way not specified in this manual, the protection provided by the instrument may be impaired; this may increase your risk of injury.
The CombiFlash NextGen systems are available in different configurations: NextGen 100, NextGen 300, and NextGen 300+.

**CombiFlash NextGen 100** – For general purpose Flash chromatography needs, this configuration delivers flow rates from 1 to 100 mL/min with a maximum pressure limit of 150 psi. The system delivers solvent gradients formed from the 2 solvent inlet lines. This configuration has an easily accessible fluid path that supports liquid sample introduction, and a variable wavelength PDA UV detector with optional UV-vis, Evaporative Light Scattering Detection (ELSD) or PurIon Mass Spectrometer detection.
CombiFlash NextGen 300 – For more advanced needs, the CombiFlash NextGen 300 is a 1–300 mL/min, 300 psi pressure limit (with injection valve option, otherwise 150 psi limit) system that forms a binary gradient by selecting two solvents from four inlets and also allows for the addition of a third solvent modifier. The NextGen 300 offers the choice of UV (200–400 nm) or full-spectrum UV-vis (200–800 nm) detection along with optional ELSD or PurIon Mass Spectrometer detection. This configuration allows for the choice of optional features, including

- an automatic, self-cleaning injection valve for sample introduction,
- RFID support for column and fraction collection rack identification,
- an internal air pump is used for post-run column air purging,
- active solvent level sensing,
- waste full bottle detection, and
- a choice of either a 12 inch or 15 inch touchscreen display.

Figure 1-2 CombiFlash NextGen 300 system with 12-inch display, 1–300 mL/min, 300 psi (with automatic injection valve)
**CombiFlash NextGen 300+** – For more options, the CombiFlash NextGen 300+ is a 1–300 mL/min, 300 psi pressure limit system that forms a binary gradient by selecting two solvents from four inlets and also allows for the addition of a third solvent modifier. This configuration has

- an automatic, self-cleaning injection valve for sample introduction,
- RFID support for column and fraction collection rack identification,
- an internal air pump for post-run column air purging,
- active solvent level sensing,
- waste full bottle detection,
- a choice of detectors: either UV (200–400 nm) or full spectrum UV-vis (200–800 nm) detection, along with optional ELSD or PurIon Mass Spectrometer detection, and
- a choice of either a 12 inch or 15 inch touchscreen display.

![CombiFlash NextGen 300+ system with 15-inch display, 1–300 mL/min, 300 psi](image)

**Figure 1-3** CombiFlash NextGen 300+ system with 15-inch display, 1–300 mL/min, 300 psi
**CombiFlash PurIon S or L** – This optional mass spectrometer detector has a detection range of 10 to 1200 Daltons (Da) (PurIon S) or 10-2000 Da (PurIon L). During purification, this detector can be combined with the UV or UV-vis detector to isolate UV and visible light absorbing compounds, as well as compounds with specific molecular weights or mass ranges.

All CombiFlash NextGen configurations are optimized for use with Teledyne ISCO’s RediSep® or RediSep Gold® columns, which are pre-packed with a variety of media. For example, the CombiFlash NextGen can purify samples from 4 milligrams to more than 33 grams using columns packed with 4 to 330 grams of silica gel. Larger columns, up to 3 kg, can be used with an optional external column mount (P/N: 60-5394-551).

Applications include purification of organic compounds for drug discovery, as well as research in agrochemicals, petrochemicals, natural products, polymers, and catalysts.

![Figure 1-4 CombiFlash NextGen 300+ with 15-inch display and PurIon](image-url)
1.3 Operating Overview

1.3.1 System Control Possibilities

The CombiFlash NextGen system is equipped with a capacitive touch screen display for local control.

The system also supports TCP/IP communication to allow direct or network control of the system by a computer using a modern web browser such as Microsoft Edge, Google Chrome, or Mozilla Firefox.

**Note**

Teledyne ISCO recommends that you obtain assistance from your Information Technology department before attempting direct or network connections.

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

1.3.2 Data Storage

To support operation from a variety of direct and network connections, the software and all files are stored in the CombiFlash NextGen unit on an internal hard drive. 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 Specification

| Table 1-1 CombiFlash NextGen System Specificationsa |
|------------------------------------------|-----------------|-----------------|
| Overall Dimensions (H × W × D)          | NextGen 100 Systems | NextGen 300/300+ Systems |
|                                         | 66 x 36 x 43 cm (26 x 14.1 x 17”) | 27.7 kg (61 lbs) |
| Weight                                  | 27.7 kg (61 lbs) | 33.6 kg (74 lbs) with Optional ELSD |
|                                        | 33.6 kg (74 lbs) with Optional ELSD | 33.6 kg (74 lbs) with Optional ELSD |
| Power Options                           | Input voltage range from 100 to 240 VAC, 50/60 Hz, 2.0 Amps maximum. | Line cord is the disconnect device. |
|                                        | Line Frequency 50/60 Hz | |
| Ambient Temperature                     | 20 to 40 °C (maximum temperature must be at least 15 °C below the boiling point of the solvent) | |
| Humidity (when connected to power)      | 95% relative humidity maximum at 20 to 40 °C | |
| Flow Rate Range                         | 1 to 100 mL/min | 1 to 300 mL/min |
| Flow Rate Accuracy (tested with water at 138 kPa or 20 psi) | ±10% | ±5% (5-200 mL/min) |
| Pressure Limit                           | 1035 kPa (150 psi) | Up to 2070 kPa (300 psi) |
|                                        | Up to 1035 kPa (150 psi) (if not equipped with injection valve) | |
Table 1-1  *CombiFlash* NextGen System Specifications\(^{a}\)

<table>
<thead>
<tr>
<th>Specification</th>
<th>Accuracy/Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure Display Accuracy</td>
<td>5% of full scale</td>
</tr>
<tr>
<td>Gradient Formation</td>
<td>Binary gradient from 1 and 2 solvent inlets.</td>
</tr>
<tr>
<td>Gradient Accuracy</td>
<td>±2% of full scale, typical; ±5% maximum</td>
</tr>
<tr>
<td>Peak Detection Modes</td>
<td>Slope or threshold</td>
</tr>
<tr>
<td>Flow Cell Pathlength</td>
<td>0.1 mm, ±25%</td>
</tr>
<tr>
<td>UV Detection Wavelength</td>
<td>200 to 400 nm, optional 200 to 800 nm UV-vis</td>
</tr>
<tr>
<td>Wavelength Accuracy</td>
<td>±1 nm</td>
</tr>
<tr>
<td>Fraction Accuracy</td>
<td>± [2mL + (flow rate ÷ 60)]</td>
</tr>
<tr>
<td>Optional ELSD</td>
<td></td>
</tr>
<tr>
<td>Gas Inlet Pressure</td>
<td>60 to 70 psig (414 to 483 kPa)</td>
</tr>
<tr>
<td>Gas Consumption</td>
<td>&lt;2.5 SLPM</td>
</tr>
<tr>
<td>Spray Chamber Temperature</td>
<td>Setting range: 10 to 60 °C Minimum guaranteed temperature is 5 °C below ambient</td>
</tr>
<tr>
<td>Drift Tube Temperature</td>
<td>Setting range: 30 to 90 °C Must be 5 °C above spray chamber temperature Maximum temperature is 60 °C above ambient</td>
</tr>
<tr>
<td>Split Flow Rate</td>
<td>0.75 mL/min, ±10%</td>
</tr>
<tr>
<td>Flow Rate Range</td>
<td>Minimum flow rate 5 ml/min. Lower flow rates will result in excessive detector signal delay.</td>
</tr>
</tbody>
</table>

**Electrical Safety per EN 61010-1**

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollution Degree</td>
<td>2</td>
</tr>
<tr>
<td>Installation Category</td>
<td>II</td>
</tr>
<tr>
<td>Maximum Altitude</td>
<td>2000 meters</td>
</tr>
</tbody>
</table>

\(^{a}\) All specifications are subject to change.

b. For safety, maximum pressure limits are limited by each column RFID-defined pressure limit. If the column is not RFID recognized, see Section 1.6.5. Maximum pressure limit is also set at 200 psi for methods using a solid-load cartridge.

c. Solvent supply containers can be located up to 0.9 m below *CombiFlash* NextGen systems except when using dichloromethane. Dichloromethane containers must be at the same level as the NextGen or above.

d. The syringe pumps on the NextGen 300 have a pressure limit of 300 PSI up to 200 ml/min. Above 200 ml/min the pressure limit drops linearly to 250 PSI. Keep in mind that other factors such as the flow path and column size may cause the maximum pressure limit to be reduced.

e. NextGen systems may also exhibit excessive pressure at maximum flow rates with some viscous solvents due to the tubing diameter used in the system.
Table 1-2 CombiFlash NextGen with Purlon System\textsuperscript{a,b}

| Dimensions (H x W x D) | Mass Spectrometer 26 x 11 x 22 in (66 x 28 x 56 cm)  
Roughing Pump 10 x 9 x 18 in (26 x 23 x 46 cm) |
|------------------------|--------------------------------------------------------------------------------------------------|
| Mass Spectrometry Detection | 10 – 1200 Dalton or 10-2000 Dalton, 1 Dalton Resolution  
Electrospray Ionization (ESI) or Atmospheric Pressure Chemical Ionization (APCI)  
Positive or Negative Ionization Mode |
| Gas Consumption | 4 SLPM |
| Split flow rates | 1.47, 6, and 20 μL/min |

\textsuperscript{a} All specifications are subject to change.

\textsuperscript{b} CombiFlash NextGen 300 or 300+ systems with Purlon detector are not be capable of operation at flow rates greater than 200 ml/min with some solvent systems due to excessive back pressure in the flow splitter valve.

Table 1-3 Component Materials List

<table>
<thead>
<tr>
<th>Teledyne ISCO RediSep Cartridge</th>
<th>Virgin Polypropylene, silica-based media (alumina columns also available), polyethylene (HDPE) frits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process Tubing</td>
<td>Fluoropolymer, Carbon impregnated fluoropolymer tubing and 316 stainless steel tubing</td>
</tr>
<tr>
<td>Drain Tubing</td>
<td>Vinyl with FEP liner</td>
</tr>
<tr>
<td>Process Valves</td>
<td>PEEK, FFKM (NextGen 300, 300+) (NextGen includes PTFE)</td>
</tr>
<tr>
<td>Sample Loading Cap</td>
<td>316 stainless steel</td>
</tr>
<tr>
<td>Sample Loading Cap Seal</td>
<td>PTFE, Elgiloy®</td>
</tr>
<tr>
<td>Injection Valve</td>
<td>PPS, 316 Stainless Steel</td>
</tr>
<tr>
<td>Flow Cell</td>
<td>303 SST, Type ES Quartz, FFKM (SIMRIZ SZ485)</td>
</tr>
<tr>
<td>Pump</td>
<td>316 stainless steel, PTFE, Elgiloy®</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Elgiloy is a registered trademark of Combined Metals of Chicago, L.L.C.
1.5 Controls, Indicators, and Features of CombiFlash NextGen Systems

Figures 1-5 through 1-8 illustrate the controls on the CombiFlash NextGen unit.

1. **Touch Panel LCD display** – Large 12 or 15 inch diagonal High Definition display for system monitoring and control.

2. **USB Port** – Convenient, front panel port that accepts USB flash memory drive. A flash drive may be inserted into this port for transferring files, importing and exporting methods, and performing system software updates.

3. **Adjustable Flash Column Mount** – The injection valve assembly and upper column mount slide along this mount so the system can accept a variety of column sizes.

4. **On/Standby Switch** – push button ON/STANDBY. Press and hold for system shut down.

5. **Fraction Collector Arm and Drop Former** – The arm and drop former move to deposit liquid in the collection tubes.

6. **Racks and Collection Tubes** – Racks hold the fraction collection tubes for all CombiFlash systems. Racks include an RFID tag which the system uses to read the rack type and collection tube size. The NextGen 100 system, however, cannot read the RFID tag.

7. **Sample Injection Port** – Luer-type fitting to accept the sample though either a solid load sample cartridge (shown) or a liquid injection using a syringe or similar device.

8. **Solid Load Cartridge Cap (SLCC) Ring Support** – Supports the Solid Load Cartridge (optional).
9. **SLCC Storage Bracket** – Stores the solid load cartridge cap (optional) when not in use.

10. **Lower Flash Column Mount** – Secures the column.

11. **Top Shelf** – Allows storage of accessories and solvent bottles.

12. **Vapor Enclosure** – Encloses the fraction collection area, allowing for hook up to external exhaust for use outside of hood (optional).

![CombiFlash NextGen features (sides)](image)
13. **Upper Drain Tube** – Liquids spilled on the top shelf are carried away through this tube to a user-supplied container.

14. **Ethernet Port** – An 8P8C jack for a network connection using a standard CAT5 cable, or for a direct connection to a computer.

15. **USB Port** – For control of peripheral equipment such as the PurIon mass spectrometer detector. This can also be used for an external keyboard, or mouse.

16. **Cooling Fan** – Cools the internal electronic assemblies.

**WARNING**

Do not touch the fan.

17. **Solvent and Waste Level Sensing Outlet Ports (optional on some systems)** – uses air or user-supplied gas to measure the hydrostatic pressure of the solvent above the weighted tubing outlet.

18. **Solvent and Waste Level Ports (optional on some systems)** – Supports use of sealed or almost sealed supply bottle caps while maintaining solvent level sensing capability.
19. Solvent Inlet Ports – A and B solvent supply. The CombiFlash NextGen system allows only a single solvent each for A and B (weak and strong solvent), while the remaining models support up to 4 solvents which can be used as either the weak or strong solvent.

20. Optional External Gas Port – An inlet for an internal pump which supplies pressurized air or user-supplied gas for solvent level sensing, waste container full detection, and system/column purges. The external gas should be regulated to 2–5 psig before connecting the source to this port. The port is present only on system equipped with solvent level sensing.

21. Fraction Collection Exhaust Port – Outlet with a 2" O.D. fitting to connect the vapor enclosure to an external exhaust.

22. Mains Power – Connects the system to AC line voltage.

23. Mains Circuit Breaker – This is a voltage-independent circuit breaker which also removes power from all active components inside the instrument.

24. Waste Port – Fluid not directed to the fraction collector exits to the waste container here.

25. Lower Drain Tube – This tube carries away liquids spilled onto the rack area.


27. ELSD Exhaust Port – (ELSD equipped models only) Vents the carrier gas and vaporized solvents.

28. Nitrogen Inlet – (ELSD equipped models only) Connects to the carrier gas supply (60 to 70 psi).

29. P-trap Drain – (ELSD equipped models only) During operation, condensate from the Thermo-split™ process drains from the system. An internal P-trap prevents aerosol particles from escaping through this drain.
30. **Pump Drain Outlet** – (ELSD equipped models only) An internal drip tray is located below the splitter for the ELSD. Should the pump or its fittings leak, liquids will drain from this port to protect internal components.

31. **P-Trap Drain Vent Assembly** – Fixture to allow P-trap to drain. Vent portion should be aligned vertically.

<table>
<thead>
<tr>
<th>CAUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discontinue use of the CombiFlash NextGen system if liquid is present at the Pump Drain. Contact Teledyne ISCO technical service for assistance with correcting the leak.</td>
</tr>
</tbody>
</table>

### 1.6 Safety

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

<table>
<thead>
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</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>WARNING</th>
</tr>
</thead>
<tbody>
<tr>
<td>If you are using flammable solvents or chemicals with this system, vapor concentration levels may exceed the maximum exposure levels as recommended by OSHA Guide 1910.1000. To reduce those levels to a safe exposure, Teledyne ISCO recommends that you place the system in a laboratory hood designed for the purpose of ventilation. This hood should be constructed and operated in accordance with federal state and local regulations. In the event of a solvent or chemical spill, your organization should have a plan to deal with these mishaps. In all cases, use good laboratory practices and standard safety procedures.</td>
</tr>
</tbody>
</table>
WARNING
The CombiFlash NextGen system has redundant safety devices to limit pressure to less than 300 psi (2068 kPa), 150 psi (1034 kPa) for systems without an automated injection valve. RediSep columns smaller than 100 g are CE certified using standard IEC61010-1 for use on the CombiFlash NextGen. RediSep columns 100 g or larger. 100 g meet Pressure Vessel Directive 97/23/EC.

1.6.1 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.

1.6.2 Hazard Symbols
The equipment and this manual use symbols used to warn of hazards. The symbols are explained in Table 1-4.

<table>
<thead>
<tr>
<th>Table 1-4 Hazard Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Warnings and Cautions</strong></td>
</tr>
<tr>
<td>![⚠️] The exclamation point within the triangle is a warning sign alerting you of important instructions in the instrument's technical reference manual.</td>
</tr>
<tr>
<td>![⚡️] The lightning flash and arrowhead within the triangle is a warning sign alerting you of &quot;dangerous voltage&quot; inside the product.</td>
</tr>
<tr>
<td>![⚠️] The pinch point symbol warns you that your fingers or hands will be seriously injured if you place them between the moving parts of the mechanism near these symbols.</td>
</tr>
</tbody>
</table>

**Symboles de sécurité**
### 1.6.3 Additional Information

Technical assistance for the CombiFlash NextGen system 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

### 1.6.4 System Pressure Management

CombiFlash NextGen series flash systems use a variety of techniques to ensure user safety due to potential compound crashes and the resulting pressure increase. The systems include

<table>
<thead>
<tr>
<th>Table 1-4 Hazard Symbols (Continued)</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Warning] Ce symbole signale l’existence d’instructions importantes relatives au produit dans ce manuel.</td>
</tr>
<tr>
<td><img src="warning.png" alt="" /> Ce symbole signale la présence d’un danger d’électocution.</td>
</tr>
<tr>
<td>![Warning] Risque de pincement. Ces symboles vous avertit que les mains ou les doigts seront blessés sérieusement si vous les mettez entre les éléments en mouvement du mécanisme près de ces symboles.</td>
</tr>
<tr>
<td>![Warning] Warnungen und Vorsichtshinweise</td>
</tr>
<tr>
<td>Das Ausrufezeichen in Dreieck ist ein Warnzeichen, das Sie darauf aufmerksam macht, daß wichtige Anleitungen zu diesem Handbuch gehören.</td>
</tr>
<tr>
<td>![Warning] Der gepfeilte Blitz im Dreieck ist ein Warnzeichen, das Sie vor “gefährlichen Spannungen” im Inneren des Produkts warnt.</td>
</tr>
<tr>
<td>![Warning] Vorsicht Quetschgefahr! Dieses Symbol warnt vor einer unmittelbar drohenden Verletzungsgefahr für Finger und Hände, wenn diese zwischen die beweglichen Teile des gekennzeichneten Gerätes geraten.</td>
</tr>
<tr>
<td>![Warning] Advertencias y Precauciones</td>
</tr>
<tr>
<td>Esta señal le advierte sobre la importancia de las instrucciones del manual que acompañan a este producto.</td>
</tr>
<tr>
<td>![Warning] Esta señal alerta sobre la presencia de alto voltaje en el interior del producto.</td>
</tr>
<tr>
<td>![Warning] Punto del machacamiento. Sus dedos o manos seriously serán dañados si usted los coloca entre las piezas móviles cerca de estos símbolos.</td>
</tr>
</tbody>
</table>
redundant pressure measuring devices to maintain safe operation in the event of component failure. If the two pressure readings aren’t within 15 psi of each other, it would indicate that one of the transducers may have failed. An error message is displayed and system operation is halted until the problem is resolved.

In addition, if both transducers readings match perfectly and exhibit no change with time, it is an indication that the system may have failed to update the pressure reading, which is also a fault and will result in an error message.

1.6.5 Pressure Limits

Teledyne ISCO RediSep columns have the pressure limit of the column coded onto an RFID (Radio Frequency IDentification) tag embedded in the column label. The encoded pressure limit is half the average burst pressure for the column size in use. This information is read by the CombiFlash NextGen system (if equipped with the RFID option) and used to establish the maximum operating conditions for the separation.

Note

4 g silica columns are an exception; they have no RFID tag and the system assumes a pressure limit of 200 psi which is less than half their average burst pressure.

If a column without a functional RFID tag is used, the NextGen systems assume that if a 4 g column is being used, the maximum acceptable pressure is 200 psi (150 psi for the NextGen system only). All other column sizes default to a 100 psi pressure limit. These limits are set to allow safe operation with most commercially available flash columns.

If the NextGen system owner is confident of the quality of columns procured, they can override the default (non-RFID tagged column) pressure limits.

To access this override:

1. Select the HELP drop down and choose the SERVICE screen option.
2. Entering the SERVICE screen requires using the administrative password. The default password on all new systems is “combiflash”.
3. In the SERVICE screens, select PUMPS tab and the NON-RFID COLUMN PRESSURE LIMITS option to program the desired pressure limits for each column size.
4. Exit the SERVICE screen and allow the system to reboot.

Note

To allow a system administrator to preserve these settings, the system administrator can change the password from the default under the TOOLS drop down.
Note

If the administrator password is changed, it is highly recommended that the new password be saved in a secure location.

Note

The pressure limit is 200 psi, if using the solid-load cartridge loading method.

1.6.6 Overpressure Conditions

If the pressure transducers are properly functioning and an overpressure condition is detected, the system will respond depending on the severity of overpressure.

- If the blockage is severe and the pressure reading is 75 psi over the pressure limit, the system stops immediately.
- If the pressure exceeds the limit by less than 75 psi, the system drops the flow rate to 50% of the programmed flow rate.
- If after 5 seconds the limit is still exceeded, the flow is reduced by another 50%.
  - In either of these conditions, the system will continue the separation.
- If the blockage resolves due to increased gradient strength and the pressure drops below 30% of the pressure limit, the flow rate will increase stepwise to the original flow rate.
- If the two flow rate reductions of 50% are insufficient to resolve an overpressure condition, the system will stop the separation to allow the user to take steps to resolve the blockage.
CombiFlash NextGen Systems

Section 2 Preparation

This section provides instructions for unpacking and installing the CombiFlash NextGen system. The PurIon Mass Spectrometer has supplementary instructions included for installation of the PurIon in sections 2.1 through 2.16.

**Note**
CombiFlash PurIon system installation is not covered by this manual. Instead, refer to instruction sheet 60-5233-638 (included with the PurIon) for the installation procedures.

**Note**
Section 2.16 contains an Installation Qualification checklist. If required, sign off the checklist entries as you successfully complete the following sections.

### 2.1 Unpacking the System

The CombiFlash NextGen system is shipped in a single carton. Carefully unpack the shipment and inspect the contents.

**WARNING**
The system is heavy. Use a two-person lift to prevent injury.

**CAUTION**
Do not lift the system by the fraction collector arm. Use the lifting handles located on the instrument side panels. The left side has a flush mount shipping handle, while the right side can be lifted by gripping the side wall above the fraction collection area.

If there is any damage to the shipping carton or any components, contact the shipping agent and Teledyne ISCO (or its authorized representative) immediately.

**WARNING**
If there is any evidence that the system has been damaged in shipping, do not plug it into AC power. Contact Teledyne ISCO or its authorized representative for advice.
Compare the contents of the boxes with the enclosed packing slips. If there are any shortages, contact Teledyne ISCO immediately.

The fraction collector arm is stowed to prevent damage during shipping. Perform the following steps to release the arm after shipment.

1. Loosen the screw retaining the arm to the right side of the fraction collection area (Figure 2-1).

2. Pull off the front cover of the unit by gripping the edges of the cover and pulling forward off the instrument.

3. Store the screw by screwing it into the screw storage (Figure 2-2).

4. Replace the front cover by pressing back onto the instrument.

5. The screw should be reinstalled if the system must be shipped again.
2.2 Instrument Location

The CombiFlash NextGen systems have a relatively small footprint, requiring about 1550 square centimeters (240 in²) of level bench space. Ensure that the system has at least 3 cm (1.25 in) of air space behind it for ventilation. Additional space may be required for solvent and waste containers.

Refer to Table 1-1 for environmental conditions and power requirements.

---

**WARNING**

The system is heavy. Use a two-person lift to prevent injury.

Before making any connections to the NextGen system, place it on the bench or in the fume hood where it will be operated. Temporarily position the system so you can access the back panels to complete the connections.

2.3 Connect Power

Ensure that the mains breaker switch is in the OFF position. Then, use the supplied IEC 60320-1 C13 power cord to connect the system to mains power.

---

**WARNING**

Mains power must meet the voltage, frequency, and amperage requirements listed on the serial number label.

---

**WARNING**

As long as the AC mains power cord is connected, power is inside the unit. The mains power cord is the disconnect device.

Position the 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.

2.4 Plumbing Connections

---

**WARNING**

Risk of fire or equipment damage. Failure to connect waste port tubing may allow organic solvents to pool in unsafe areas, possibly creating dangerous levels of flammable vapors.

---

**WARNING**

Risk of fire ignited by electrostatic discharges. Never substitute the black tubing on CombiFlash systems. The black tubing (P/N 023-0503-06) is conductive. This tubing is required to dissipate static electricity.
![CAUTION]

Elevated flammable vapor levels are possible. Ensure that the waste container is adequately ventilated, preferably by placing it in a fume hood.

The CombiFlash NextGen instrument comes with the solvent inlet lines and waste lines pre-assembled to it. To install the unit:

1. Remove the 2 screws and shipping clamps identified in Figure 2-3. This releases the tubing lines for placement in the solvent supply and waste reservoirs.

![Figure 2-3 Location of the shipping clamps](image)

![Note]

For convenience, the inlet filter and inlet line tubing assemblies have been pre-assembled with a bottle cap that is typical for the region (GL 38 in the USA and GL 45 in the rest of the world). If these caps aren’t suitable for your containers, you will have to make modifications as needed.

2. Place the solvent lines in the appropriate solvent supply containers.

If the solvent lines are unable to reach the supply container (such as when placed below the work surface), the third clamp that retains the tubing above the inlet ports can be removed. In addition, solvent supply extension lines are available and have
been verified at flow rates up to 200 mL/min. If using dichloromethane (DCM) in a warm environment, flows may be limited to lower values. If equipped with solvent level-sensing feature installed and enabled, the system will stop and notify the user that the solvent bottle is empty when its remaining contents are within the range set at **TOOLS > CONFIGURATION > MINIMUM SOLVENT LEVEL.** The level is solvent density dependent.

3. Place the waste line in a suitable waste container. The waste line assembly has a level sense line installed into the cap. The system (if equipped with solvent level-sensing feature) will consider the waste full when the end of the sense line is submerged below the surface of the fluid (solvent density dependent).

4. Place the waste cap on the desired waste bottle.

5. If you use a dedicated waste container instead of a bottle, you may not be able to implement waste level sensing as designed. In these cases, work with your Environmental Health and Safety group to determine how to proceed. This may require disassembly of the supplied waste line to meet your needs.

**Note**

When using higher-density solvents such as dichloromethane (DCM), place the solvent container level with or above the CombiFlash NextGen system. Placing solvent containers below the level of the system can contribute to decreased flow due to the high vapor pressure of DCM. This problem becomes more pronounced as the ambient temperature increases.

### 2.5 External Gas

#### 2.5.1 CombiFlash NextGen

The CombiFlash NextGen systems use an internal air pump to provide compressed air for solvent and waste level sensing. Optionally, the system can deliver a compressed gas. Inert or other gases may be more suitable than air for some applications.

If ambient air is compatible with your solvents and waste, simply leave the optional external gas port open.

If using a gas instead of air, connect the user-supplied 1/8 inch I.D. tubing to the optional external gas port. Connect the other end to the user-supplied compressed gas. The external compressed gas should be dry, filtered, and regulated to 2–5 psi (0.14–0.34 atm, 13.8–34.5 KPa).
2.5.2 CombiFlash NextGen Equipped with Optional ELSD

ELSD operation requires a carrier gas. To connect the carrier gas to the CombiFlash NextGen unit:

1. Locate the 1/8 inch O.D. FEP tubing (P/N 023-0503-02) from the accessory package.

2. Push one end of the tubing into the NITROGEN INLET port on the back of the system. The tubing should be fully seated in the port.

3. Cut the tubing to length and connect the other end to the user-supplied carrier gas. An assortment of 1/8 inch adapters are supplied in the accessory kit to complete the connection to your gas source.

**Note**

Teledyne ISCO recommends >99% pure nitrogen from a source that can deliver 2.5 SLPM at 60 to 70 psi.
2.6 Connect and Route Drain Lines

⚠️ WARNING
Risk of fire or equipment damage. Failure to connect drain lines 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.

The CombiFlash NextGen system has drain tubes extending from its back panel. The tubes drain away any liquid spilled on the top shelf and the tray beneath the fraction collection racks.

1. Test the fraction collector drain by connecting a vacuum or air supply source to the outlet end of the drain tube. Then, verify the presence of such vacuum or air supply source on the drain (Figure 2-6).

2. Test the top shelf drain by connecting a vacuum or air supply source to the outlet end of the drain tube. Then, verify the presence of such vacuum or air supply source on the drain (Figure 2-7).

3. Route the end of both drain tubes to a suitable waste fluid collection container.

Figure 2-6 Fraction collection tray drain hole

Figure 2-7 Top shelf drain
**Note**

It may be necessary to extend the drain tube. If so, splice the tubing with user-supplied tubing. The user-supplied tubing should have an inside diameter no smaller than the existing drain tubing, and must be compatible with the solvents used by the system. Route this extension tubing to the waste collection vessel.

**CombiFlash with optional ELSD only** The CombiFlash ELSD system has additional drains that must be connected (Figure 2-8):

4. Locate the 1/4 inch I.D. silicone tubing and a nylon tubing clamp from the accessory kit. Connect one end of the tubing to the pump drain port and secure it with the clamp.

5. Install the ELSD P-trap vent assembly found in the accessory package by placing the short tube into the port on the rear panel, orienting the vent as shown in Figure 2-8 and tightening the fitting nut finger tight plus a small amount more with a wrench. Be sure the P-trap drain vent assembly is oriented with the vent portion vertical.

6. The P-trap must be filled with fluid to prevent sample loss. To accomplish this, raise the drain tubing attached to the P-trap drain vent assembly above the instrument and place 10 mL of liquid, such as isopropyl alcohol, into the tube. Make sure the fluid level in the tubing doesn’t exceed the level of the instrument case top. If the tubing is raised too fast, fluid may flow out the top of the vent tube causing a spill. Lift it high enough so the fluid enters the P-trap drain (Figure 2-8).
7. Route the end of the P-trap drain tube to a suitable waste fluid collection container. Be aware that as the tubing is lowered, several mL of isopropyl alcohol will run out of the tubing. This is normal and means that the P-trap is properly filled. Depending on your application, the P-trap fluid may need periodic replenishment to ensure maximum signal strength of the ELSD.

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

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

**Note**
If using outside of the hood, ensure that the exhaust port tubing is routed to an exhaust handling system that meets your safety and environmental requirements. In normal operation, the exhaust gas is dry and does not require a waste collection container.
2.7 Vapor Enclosure (Optional)

1. Secure the Vapor Enclosure with the three provided screws to the holes in the instrument (Figure 2-9). Two along the back of the fraction collection area and one on the side of the fraction collection area.

2. The back of the unit has a 2" O.D. port for attachment to an external exhaust (Figure 2-10). The external exhaust should meet the specifications of your environmental, health, and safety specifications.

Figure 2-9 Location of holes for the vapor enclosure

Figure 2-10 Location of the external exhaust port
2.8 Install Solid Load Cartridge Cap Ring Support and SLCC Storage Bracket (Optional)

1. Secure the solid load cartridge cap (SLCC) storage bracket to the left side of the instrument with the screws provided (Figure 2-11).

![Figure 2-11 Before (left) and after SLCC storage bracket is installed (right)]

2. Place the SLCC Ring Support in the hole on the injection port trolley (Figure 2-12).

![Figure 2-12 SLCC ring support]

3. Secure the support with the knobbled screw via the side hole (Figure 2-13).
2.9 Position the System

After completing the various connections, the system can be moved to its operating position. Turn the system so that the operator can access all of the front view features and controls (Figure 1-5). Use care not to damage the connections, tubing, and cables while moving the system.

**CAUTION**

Ensure that the CombiFlash NextGen system has at least 3 cm (1.25 inch) of air space behind it for ventilation. Position the solvent and waste containers as necessary.

**Note**

When using higher-density solvents such as dichloromethane (DCM), place the solvent container level with or above the CombiFlash NextGen unit. Placing solvent containers below the level of the unit can contribute to decreased flow due to the high vapor pressure of DCM. This problem becomes more pronounced as the ambient temperature increases.
2.10 Install Collection Tube Racks

Before beginning a run, you must load collection racks with tubes onto the system’s fraction collector tray.

Your system was shipped with two collection tube racks. The following tube rack sets are available:

- **60-5237-013** – Two racks for 13 x 100 mm test tubes (8 mL). Total tubes: 216.
- **60-5237-061** – Two racks for 16 x 100 mm test tubes (14 mL). Total tubes: 150.
- **60-5237-031** – Two racks for 16 x 125 mm test tubes (15.5 mL). Total tubes: 150.
- **60-5237-032** – Two racks for 16 x 150/160 mm test tubes (18 mL). Total tubes: 150.
- **60-5237-033** – Two racks for 18 x 150 mm test tubes (25 mL). Total tubes: 150.
- **60-5237-034** – Two racks for 18 x 180 mm test tubes (30 mL). Total tubes: 140.
- **60-5237-035** – Two racks for 25 x 150 mm vials (50 mL). Total vials: 60.
- **60-5237-040** – One rack for twelve 480 mL French square bottles.
- **60-5394-469** – Two racks for 20 mL (28 x 61 mm) scintillation vials. Total vials: 54.
- **60-5394-468** – Two racks for 40 mL (28 x 95 mm) scintillation vials. Total vials: 54.

To load the racks:

1. Insert test tubes, vials, or bottles into the rack (Figure 2-14). The system assumes all tube positions have tubes. If any tubes are missing, it is possible to have a fluid spill when the fraction collection system advances to that position.

⚠️ **CAUTION**

Risk of broken glass or equipment damage. Do not load test tubes longer than the length listed on the tube size label.
2. While holding the rack with the tube size label visible (Figure 2-15), insert the racks into the system. Slide the rack in until it stops. You can feel it drop into its seated position when it is properly loaded.

![Figure 2-15 Inserting the collection tube rack](image)

**CAUTION**

An incorrectly installed rack will cause the rack to be misaligned under the fraction collector arm. Misaligned racks might cause fractions to miss the tube opening or deposit in the wrong tube. Always ensure the tube size label is visible (that is, facing outward) and the rack is pushed in until it is seated.

When you turn the power switch to ON, the system will automatically detect the type of rack and configure program settings accordingly. Later, in Section 2.13.1, you can configure the maximum volume for each tube size if you want smaller fractions than the tube capacity.
2.11 Powering on the System

The system’s power switch is located on the lower right side panel, near the front of the instrument.

1. Switch the rear panel circuit breaker to ON.
2. Press the switch to turn the system on. The system will begin its startup routine, which includes self-diagnostics.

**Note**
The momentary power button on the right side of the instrument can be used to boot up the system or shut it down on a daily basis. The rear panel power switch is only used when you desire to completely remove power from all internal electronics.

3. The system is ready for operation when the PeakTrak® screen is displayed.

2.12 Configure the System

Before operating the system, it should be configured to best meet your needs. To configure the system, open the Configuration window by selecting TOOLS > CONFIGURATION from the PeakTrak menu.

The configuration window has 3 tabs: INSTRUMENT CONFIGURATION, NETWORK CONFIGURATION, USER PREFERENCES. Network Configuration settings are discussed later in Section 4. Adjust the selections on the INSTRUMENT CONFIGURATION or USER PREFERENCES tabs for the desired operation and then click OK to save the settings.

**Note**
Some configuration settings do not take effect until the system is restarted. See Section 2.13.4.

![Instrument configuration menu](image)
2.12.1 Solvents
When the system is installed, up to four solvents were connected to the back of the system. This section of the INSTRUMENT CONFIGURATION tab allows you to name these solvents for convenience. To do so:

1. Solvent names for drop down use are limited to a maximum of 16 including the prepopulated names, which can be removed. Names are stored in the order created.
2. From the Solvent 1 drop-down list box, select the solvent name connected to the 1 Solvent Inlet Port. Repeat for Solvents 2, 3, and 4.
3. If your desired name is not listed by the system:
   a. Click the ADD SOLVENT button.
   b. Enter the solvent name and click the OK button.
   c. Repeat steps 1 and 2 as needed.
4. Select the ENABLE SOLVENT LEVEL SENSING check box to enable this feature (recommended). Clear this box to disable this feature.

The solvent level sensing feature pauses the pump operation if the solvent level is below the configured level. In addition, if the same supply container has been used for a previous separation and has not been refilled, the system will estimate the amount of solvent remaining. It compares the volume required for a purification run with the estimated volume in the solvent container and alerts you when there is not enough before the separation begins.

5. If using the solvent level sensing feature, you can select the MINIMUM SOLVENT LEVEL (density dependent) as a range in centimeters.

Solvent level sensing works by sensing the fluid pressure at the inlet line level. This is specified as a range of variance in the density of most common chromatographic solvents. To understand this range, consider that dichloromethane is ~2x more dense than hexane. If the selected range is 2–4, the system triggers an alert when the dichloromethane level falls to about 2 cm above the weighted filter. At the same selected range, the system would trigger an alert when the hexane falls to about 4 cm above the weighted filter, since the fluid pressure of hexane is only half of dichloromethane of the same depth.

2.12.2 General Settings

**CombiFlash NextGen Name (optional)** – Use this option to name your system. The name will appear in operational displays and separation reports. This feature is useful when your laboratory has more than one CombiFlash system and you want to determine which system performed a given separation.

**Time Zone** – Select your time zone from the drop-down list. This allows the factory set time to be updated to correspond to your time zone. This also allows the system to adjust the system time for seasonal time changes (such as daylight savings time in
the United States). If the date of this time change is changed by
the local government, the system may not be correct based on the
changed effective date.

**Alert Sounds** – Used along with red lights in the fraction area
(on some systems) to alert the user to items such as collection
rack fill, solvent low, and serious errors. The alert sounds also
occur to alert the user to when the run is complete or equili-
bration is complete for liquid or solid (pause) loading techniques.

**Set Date/Time** – Click this button to set the system date and
time.

To prevent unauthorized time changes, this feature is password
protected. The system is shipped with the password set to
*combiflash*. Use the TOOLS > SET PASSWORD menu command to
change this password for greater security if desired.

**PurIon Delay** – Configuration for the connection tubing
included with the PurIon. If your installation has a PurIon Mass
Detector and no ELSD detector, this value is 11.0 mL. If the
system also has an ELSD detector included, this value is 4 mL.

**Automatic Column Purchase** – Enables a flash column
supply agreement that must be purchased separately from the
system. This allows us to supply columns as needed without need
for inventory management at the customer’s site.

### 2.13 Vapor Limit (if equipped)

The CombiFlash NextGen system has an internal vapor sensor
that detects vapors present inside the system. This sensor mon-
itors the system for premature pump seal failure or leaks of the
internal plumbing connections. Fluid vapors external to the
system may be detected when ambient air is drawn into the
system by the cooling fan. When the vapor level limit is exceeded,
the system will stop the pumps to minimize a hazardous con-
dition.

Currently, Teledyne ISCO ships all systems with this vapor limit
feature. The default setting of High sensitivity is recommended
because the response of the sensor to vapors from other solvents
may vary.

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

<table>
<thead>
<tr>
<th>Sensitivity Setting</th>
<th>Percentage (relative to the LEL of hexane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>45%</td>
</tr>
<tr>
<td>Medium</td>
<td>15%</td>
</tr>
<tr>
<td>High</td>
<td>5%</td>
</tr>
</tbody>
</table>
Medium and High sensitivity settings may result in false alarms (i.e., no 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.

Once 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 this case, 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, the user assumes any additional risk and has taken any additional steps to mitigate the increased risk of disabling or lowering the Vapor Limit sensitivity level.

### 2.13.1 Set Default Tube Volumes

Click this button to set the default volume of the collection tubes. There is some variation in the fraction size as a function of flow rate. Be sure you don’t overflow the containers by setting the default volume too close to the actual capacity.

The system will automatically advance to the next tube when this default volume is met in each tube. Keep in mind that this level can be set for each separation in the METHOD EDITOR. During the separation, other factors such as automatic tube advances for detected peaks will affect collected volume.
2.13.2 User Preferences

View the User Preferences tab to configure system operation for individual users. This is especially convenient if the system is shared by several individuals with different preferences (Figure 2-18).

![User Preferences Screen](image)

**Figure 2-18 User Preferences screen**

**Note**

New systems will only have one user account named “common.”

**Use ELSD by Default** – causes all runs after system boot up to use the ELSD detector unless disabled in the run requirement screen. After an initial separation, each subsequent separation uses the same ELSD selection unless changed during the Run Requirements screen.

**Language** – Select the user’s desired language from the drop-down list box.

**Automatically Print Report at End of Run** – If the system is on the network and a printer is configured, then this command allows a report to be printed automatically. If you want to automatically save the PDF to a network location, see Section 4 Network Configuration.

**Note**

This option requires the system to be configured for network operation and a connection to a printer on the network. Network settings are discussed in Section 4. Do not select this option for these initial installation steps.

**Default Run Units** – Run units are displayed along the X-axis of the chromatogram. Select Time (in minutes) or Column Volumes (CVs). A column volume unit is the time it takes to
pump enough solvent to exchange the volume held by the column. The duration in minutes will vary according to the column media and size, and the flow rate.

**Enable Run Length Extension** – When enabled, this option automatically extends the run if a peak is eluting at the end of the maximum %B gradient. This ensures that a late-eluting peak fully comes off the column and is collected.

An automatic run extension is a five-minute isocratic hold added to the end of the run’s maximum %B gradient profile. During the extension, the system continues to pump the maximum %B solvent mixture. Should the system still detect a peak after an extension, the system will add another, up to a maximum of three extensions.

Occasionally, compounds might come off the column once the %B returns to the minimum value at the end of a run. If the **RUN LENGTH EXTENSION** is enabled, it will automatically extend the run one time to clear the column and plumbing of any remaining material.

![Figure 2-19 Automatic Run Extension Examples](image)

Figure 2-19 illustrates the possible run extensions. The original method was programmed to rise to the maximum %B over seven minutes, hold for two minutes, and return to the minimum %B for a final minute. At nine minutes, a peak was eluting. The system extended the run, holding the %B at the maximum level for another five minutes. This also occurred at fourteen and nineteen minutes, resulting in the second and third extensions. At twenty-four minutes, the %B solvent strength returned to zero. Before the final minute elapsed, more compound was detected, causing the system to extend the run for a final five minutes at the minimum %B.

**Enable rapid equilibration** – Select this option to equilibrate the column at a higher flow rate.

**Note**

Due to the high pressure that is possible during rapid equilibration, this option may not be desirable for some column media or purification methods.
Enable Automatic Peak Hold – Inserts an isocratic hold for the duration of each detected peak.

2.13.3 User Management

User management allows creation of individual user accounts on the system. Password access can be added if desired. When configured, the user’s preferences are stored and their data is automatically saved to the user’s personal folder on the system.

To implement control over system usage, a system administrator can set varying levels of access for each user. The capability of each user is summarized in the chart below.

User Management allows each user of the system to have customized settings.

![User Management screen](image)

*Figure 2-20 User Management screen*
2.13.4 Restart the System

Some configuration settings do not take effect until the system is restarted. After completing the configuration, restart the system:

1. If the CONFIGURATION window is not closed already, click the OK button to close it.
2. Momentarily press the power switch on the side of the instrument and wait for the system to shut down.

**CAUTION**

Except during an emergency, never remove AC power immediately after placing the power switch in the OFF position. The power down sequence includes file maintenance that takes up to one minute to complete. Interrupting the file maintenance may corrupt files causing abnormal operation or a complete system failure.

3. After one minute or more, press the power switch.
4. The system is ready for operation when the PeakTrak screen is displayed.

2.14 Prime the Solvent Lines

Before the first use, the system should be primed. Ensure that the solvent containers are filled, then (NextGen 300 or 300+):

1. Select the TOOLS > AUTO PRIME menu command. Automatically primes each line with 50 mL of solvent in the order directed by the user, as shown in Figure 2-21. You can also rename your solvents by using this screen to switch solvents. These changes are also reflected in the configuration menu.
Figure 2-22 Auto Prime screen
2. There are 4 solvent selection lists labeled from first solvent through fourth solvent, describing the order in which solvents will be pumped. The solvent choices for each list are those defined by the CONFIGURATION window.

- If all solvents are of a similar polarity, it is recommended that the solvent be primed in order of decreasing solvent strength, with the weakest solvent primed last.
- If the 4 solvents are of mixed polarity (aqueous and nonpolar), ensure that sequential solvents are compatible, such as water, acetonitrile, ethyl acetate, hexane.
- If you are performing an auto prime after redefining some of the solvents, it may be beneficial to run 2 auto prime operations sequentially to get full flushing of the lines.

3. Click the START button to start priming the system.

**Note**

When priming the first time, inspect the solvent and waste connections to the system. If any leaks are observed, click the CANCEL button to stop the Auto Prime. Correct the leak by tightening the fitting an additional 1/4 turn, and then restart the Auto Prime from step 1.

To terminate an auto prime step in process and advance to the next Auto Prime step, you can click the TERMINATE button. The Auto Prime window closes when finished.

After Auto Priming, the system is ready for operation. If you are priming a new system for the first time, it may be beneficial to flush all of the fluid lines with the desired solvent to remove any isopropanol left from production testing.

**Note**

Use Auto Prime for quick solvent changes of similar polarity by pumping a fixed amount of solvent through the waste fluid path at 100 mL/min. For more advanced priming functions such as varying the flow rates, fluid paths, etc., or to purge the system with air before changing between normal and reversed phase solvent systems, use the TOOLS > MANUAL CONTROL option.

**Manual Prime (NextGen models only)** – The pumping system in the base NextGen model requires the pumps to be manually primed. To do this, use the syringe port on the front of the instrument under the screen. From TOOLS select MANUAL PRIME and follow the directions on the screen. Pull the syringe to remove air from each of the solvent lines as directed by the on screen instructions.
2.15 System Verification

It is recommended that the system operation be verified. To do so, use the CombiFlash Universal verification kit, Teledyne ISCO P/N 60-523-4317:

- The instructions for Universal Verification Mix can be found in Instruction Sheet P/N 69-5233-870.
- For PurIon equipped systems the following test mixtures are also needed:
  - P/N 60-5234-315 Test Mixture for APCI probes, 5 mL.
  - P/N 60-5234-627 Test Mixture for ESI probes, 10 mL.
### 2.16 Installation Qualification Checklist

Table 2-1 may be completed to verify and document the installation procedures contained in Section 2 of this guide:

<table>
<thead>
<tr>
<th>Step</th>
<th>Section</th>
<th>Description</th>
<th>Installer Initials</th>
<th>Operator Initials</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1</td>
<td>Unpacking the unit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>Instrument location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.3</td>
<td>Connect power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.4</td>
<td>Connect solvent lines (if not pre-installed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.4</td>
<td>Connect waste lines (if not pre-installed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.5</td>
<td>External ELSD gas (optional)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2.6</td>
<td>Connect and route drain lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.7</td>
<td>Vapor Enclosure (optional)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.8</td>
<td>Install Solid Load Cartridge Cap Ring Support and SLCC Storage Bracket (optional)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.9</td>
<td>Position the system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2.10</td>
<td>Installation of the collection tube racks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.1 – 2.15</td>
<td>Install Purlon (optional)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2.11</td>
<td>Turn on the power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>2.12</td>
<td>Configure the system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2.14</td>
<td>Prime the solvent lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>2.15</td>
<td>System verification</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Certification of Section 2 Completion**

- **Installer Name (print):**
- **Installer Signature:**
- **Date:**

- **Operator Name (print):**
- **Operator Signature:**
- **Date:**

**Comments:**
CombiFlash NextGen Systems

Section 3 Operation

This section provides operating instructions for CombiFlash NextGen systems.

3.1 Flash Sample Preparation

Before starting a run, consider how the sample will be loaded onto the column media. There are three different loading methods: liquid injection, solid sample cartridges, and pre-loading the sample on the column. Sample loading is covered in the included CombiFlash NextGen Quick Start Guide.

3.1.1 Liquid Sample Injection

If the sample is soluble in the starting mobile phase or a solvent strength that doesn’t significantly impact the separation, it can be prepared as a solution and injected onto the column when prompted during the purification run.

3.1.2 Solid Samples

Some compounds are not soluble in solvents that are compatible with the chromatography. In addition, the compounds may have very limited solubility in any solvent, resulting in sample dissolution volumes that are impractical for good chromatography. The answer in this case is solid sample introduction.

Some samples of this type may be simply dissolved and placed into a pre-filled solid load cartridge and allowed to adsorb onto the media in the cartridge. You can then use the cartridge immediately or dry it before placing it on the system.

Other samples may need more care. Typically, you would dissolve the sample using a suitable solvent, then create a mixture of ~20% sample load to media (w/w). Then dry the mixture under conditions that will dry off the solvent without affecting the compounds of interest. These compounds remain bound to the media. Once dried, pour the media/sample mixture into an empty cartridge.
3.2 Loading a RediSep Column

⚠️ WARNING

The CombiFlash NextGen system has redundant safety devices to limit pressure to less than 300 psi (2068 kPa); 150 psi (1034 kPa) for systems without an automated injection valve. RediSep columns smaller than 100 g are CE certified using standard IEC61010-1 for use on the NextGen system. RediSep columns larger than 100 g meet Pressure Vessel Directive 97/23/EC.

⚠️ Note

For best results, always use RediSep columns. The system will not automatically detect other columns. The system also limits the maximum operating pressure to 100 psi when the column is not detected.

To load a RediSep column:

1. Select the proper RediSep column. The RediSep column selection guide that can assist you in selecting a stationary phase media and column size. In addition, the approximate sample loading capacity is printed on the RediSep column label.

2. Raise the column mount and insert the column into the top column mount. Note that the column fittings are keyed to ensure the correct flow direction.

3. Slowly lower the injection valve while aligning the bottom column fitting. The spring-loaded column mount will hold the column in place.

4. To seal the column fittings, give the column a slight twist (1/4 turn).

⚠️ Note

After loading a RediSep column, systems configured with RFID will use RFID technology to automatically detect the media type and column size (if available on the column). PeakTrak displays the detected column size on the MAIN and METHOD EDITOR windows. If the system does not detect the column, manually select the column media and size.

3.3 Starting a Separation

After completing the system installation steps, preparing the sample (See the CombiFlash NextGen Quick Start Guide for sample loading procedures), and inserting the column, you are ready to perform a run. The system will assume a default method based on the column size placed in the system. Default methods are optimized for the use of RediSep columns with run parameters typically used by chemists.
The default settings include:

- Pre-configured equilibration settings based upon column size and type.
- Set a flow rate and separation length suitable for the column loaded.
- Set to collect all fluid in the fraction collector rack to ensure peaks without UV absorbance are collected.
- Pre-programmed peak detection settings suitable for typical peak sizes based on the column size.
- Automatically advance to the next tube when a peak is detected and when a peak ends to maximize peak concentration.
- Advance to the next tube if a partially resolved peak has a shoulder or valley UV absorbing peaks based on slope detection or level threshold.

To start a separation from the PEAKTRAK MAIN window:

1. Enter a sample name if desired. If no sample name is specified, PeakTrak will enter the date and time as the sample name when you start the run.

2. Review the PEAKTRAK MAIN window settings. Some settings can be edited from the main menu. For example:
   - If the gradient conditions are not correct, the duration can be adjusted using the RUN LENGTH input field.
   - The gradient slope can be adjusted by grabbing an inflection point and dragging to the desired position.
   - Inflection points can be added or deleted if needed. Select the ADD POINT button and touch the screen where a point should be added, or DELETE POINT and touch the point to be removed.
   - For greater detail, the screen can be zoomed using a 2 finger pinch zoom, or panned using 2 fingers.
   - Other parameters may be edited such as peak detection parameters or numerical gradient point input using the METHOD EDITOR selection at the top of the screen.

3. Click the START button.

4. Select the sample LOADING TYPE from the list. This LOADING TYPE should support the sample preparation you chose. Possible sample loading types are:
   - Solid (requires the inject valve; optional on some systems) – Select this option if you have prepared the sample and placed it into a solid sample load cartridge before starting the separation. (Column plan subscribers must also specify the cartridge type and size).
     - The system will proceed automatically until the end of the programmed run length.
• **Solid (Pause)** – Select this option if you will place the sample into a solid sample load cartridge, but have not yet prepared the sample. (Column plan subscribers must also specify the cartridge type and size).

When you click the RUN button, the system will perform a column equilibration and then wait while you prepare the cartridge. After you have placed the cartridge on the system, click OK to continue with the run.

• **Liquid** – Select this option if you have prepared a liquid sample and plan to manually inject it into the injection port or directly onto the column after column equilibration.
  - If you inject using the Luer fitting on top of the column mount, inject the sample, then inject 3 mL of chase fluid to wash the sample completely onto the column. Make sure the chase solution is strong enough to prevent sample crashing, but not so strong as to adversely affect the chromatography.
  - **None (on column)** – Select this option if you have pre-loaded the sample on the column.
  - The system will skip column equilibration so that the sample will not be flushed from the column before the run.

5. Review or update the **START RACK and START TUBE**.

6. Review the **MINIMUM RUN REQUIREMENTS**:

   • The system will report the estimated solvent volumes, expected waste, collection tube usage, etc. You can use this information to verify that there will be enough solvent to complete the run, the waste volume does not exceed the collection container's capacity, and whether or not more tubes will be required during the run.

   • **End of Run Hold** – Select whether or not the run will undergo automatic valve wash and air purging without waiting for user input. If enabled, this allows the user to extend the run if needed to complete the separation before proceeding to the post run processes. This setting defaults to the selection used for the previous separation.

---

**Note**

The *CombiFlash* systems with solvent level sensing estimate the solvent volumes by monitoring the solvent level in the container and the known usage rate. The system continues to refine this estimate when in operation.
If equipped with solvent level sensing and the waste level sensing tube is inserted correctly into the waste container, the systems will automatically suspend operation before an overflow condition might exist. To prevent the run from being suspended before completing the run, ensure that the container will hold the expected waste volume.

7. If equipped with an ELSD, ensure that the ELSD option is selected if desired. The system defaults to ELSD detection parameters that are compatible with fluids typically used by the selected column. If performing a normal phase separation using a high boiling point solvent such as heptane or toluene, you may need to review the ELSD parameters in the METHOD EDITOR; otherwise the ELSD may be disabled. The system will automatically select or deselect use of the ELSD based on the previous run.

If you typically use normal phase solvents and the ELSD P-trap drain fluid hasn’t been replenished recently, you may need to ensure the P-trap is full for maximum ELSD signal strength. This can be done by lifting the drain line above the instrument and placing 10 mL of isopropyl alcohol in the drain line. Lift the line sufficiently that the fluid flows into the P-trap drain. Then place into a waste container so that the excess fluid can drain out of the trap. If you normally use higher boiling point solvents such as water, the P-trap will typically remain full and will not require replenishment.

If you are using a silica flash column and your solvent exceeds 7.5 pH, the ELSD should not be used. Basic conditions can dissolve silica. When the solvent with dissolved silica is evaporated in the ELSD, silica can build up over a period of time and eventually clog the ESLD, resulting in an expensive repair. Sample build up in the spray chamber is less of a concern since it is sent to the chamber in smaller quantities only during the peak and is more easily cleaned from the chamber during regular cleaning (see Section 5.6 ELSD Maintenance).

**Mass Spectrometer** – (PurIon systems only) click this button to enable mass spectrometer detection.

**PurIon Loading** – (PurIon systems only) sets the amount of sample sent to the mass spectrometer. Default is set to LOW to reduce saturation of the mass spectrometer detector. Select HIGH for lightly loaded columns or samples with weak detection on the mass spectrometer. For situations in-between, select MEDIUM.

**Detection Ions** – Enter up to 4 ions, or a range and up to 3 ions. The ions may be either positive or negative.
3.4 During the Separation

You may allow the system to proceed with the gradient while monitoring the progress on the PeakTrak main window. If desired, all method parameters may be modified during the separation without stopping the separation. Changes to the gradient may be made by dragging the solvent inflection points with your finger without stopping the pump.

As the run progresses, the absorbance trace(s) is displayed while the separation continues until the end of the run defined by the run length setting.

During the run, data is automatically saved to the internal data storage every few seconds. You can manually save a RUN FILE as text (in XML format) or PDF to a flash drive while stopped or any later time as long as the data file has not been deleted.

If a flash drive is in the USB port at the end of the separation, the PDF file is automatically saved on this drive in addition to the system’s internal hard drive.

To automatically save a text or PDF file to a network location after each run, configure the NETWORK FILE SAVE feature.

3.4.1 Run Control Buttons

The following information will correspond with Figure 3-1.

![Figure 3-1 MAIN SEPARATION screen](image)

**Start** – The START button starts the separation. During the separation, this becomes the ISOCRATIC HOLD button.

**Isocratic Hold** – The ISOCRATIC HOLD button holds the %B at the current value while the system continues to operate. Note that holding the run extends the separation length. While in the isocratic hold state, the button is renamed CONTINUE GRADIENT and you can resume the gradient by clicking the button, or stop the separation by clicking the STOP FLOW button. If you resume the separation, the system continues with the gradient curve at the %B when the system was placed in isocratic hold.
Stop Flow, Terminate, and Reset – The STOP FLOW button suspends the entire separation. Unlike the ISOCRATIC HOLD state, the pump, peak detection, and fraction collection will stop. While in the STOP FLOW condition, you can:

- resume the run by clicking the START button, or
- skip the rest of the separation by clicking the TERMINATE button which proceeds with the air purge (if the system is equipped with this feature), or
- end the run by clicking the RESET button, which clears the data from the screen and displays the now-truncated temporary method.

The advantage of TERMINATE vs. RESET is that TERMINATE advances to the air purge line flush, and detector wash while RESET performs none of these important maintenance functions.

If RESET is selected, it may be useful to manually flush the lines so that strong solvent remaining in the lines doesn’t affect subsequent separations.

Tube Advance – This button advances the fraction collector to the next tube position after the delay volume from the detector to the fraction collector has elapsed. This ensures the fractionation matches the detector signal at the time the button was pressed.

3.4.2 Bypassing the Solid Load Cartridge (Injection valve equipped systems only)

Occasionally, impurities precipitate in the solid load cartridge as compounds are purified. This may cause high back pressures resulting in reduced flow rates and long run times. Clicking this button changes the injection valve position, which removes the cartridge from the solvent path and relieves the back pressure. Any compound that hasn’t eluted from the solid load cartridge will remain in the cartridge and not be purified.

3.4.3 About Solvent Level Detection (systems equipped with this option only)

When enabled in the TOOLS > CONFIGURATION window, the system will estimate the solvent volume usage by measuring the solvent level in the container and the monitoring the usage rate. The system continues to refine this estimate of available solvent during operation.

At the beginning of a run, the system calculates the amount of solvent required to complete the run. The system will alert you if it detects that there is not enough solvent at the start of the run. Likewise, the system will alert you during the run if the estimated usage rate or the measured level indicates that there is insufficient solvent. Note that isocratic holds and automatic run extensions during a run will increase the required solvent volume.

The options available on the alert message will vary depending on when the condition occurs (before or during a run) and which method (measured level or estimated usage rate) indicated the condition.
To clear the alert message, the user must select one of the options below.

- **Continue** – Select this option after you have added more solvent to the container. After selecting this option the system measures the solvent level again. If the level is sufficient to complete the run, the run continues. If not sufficient, the system displays the alert message again.

- **Ignore** – This option is available when the estimated usage rates indicates that the solvent levels are insufficient. Select IGNORE to clear the message and continue the run. The system then ignores the estimated solvent usage rate for the remainder of the run, but continues to measure the solvent level. Should the level become insufficient, the system will display another alert message.

- **Disable** – Select this option to turn off solvent level measurements and estimated solvent usage rates for the remainder of the run.

- **Cancel** – This option appears before the run is started. Select this option to cancel the run and return to the main window which will display the current method.

### 3.5 Software Reference

Many features of the software function like a typical PC or tablet and therefore are not covered in detail. The remainder of this section covers areas that may be less common or unique to CombiFlash NextGen systems.

![NextGen Login screen](image)

**Figure 3-2  NextGen Login screen**

The CombiFlash NextGen system comes configured as an open access system with all work done in a single common area. If so configured, the screen above is not shown at startup. In addition, it can be configured for multiple, individual users who can be assigned individual passwords. In this case, the screen in Figure 3-2 is displayed after startup or individual logoff. In either case, the SELECT USER drop down allows the username to
be selected, followed by a login screen (if activated for the user). Once logged in, the MAIN SEPARATION screen is shown (Figure Figure 3-3).

3.5.1 Main Separation Screen

The MAIN SEPARATION screen (Figure 3-3) defaults to a 4 g silica method until an RFID tagged column is sensed, or the user selects a column via the COLUMN drop down list (for support of non-RFID tagged columns).

- If the displayed method is acceptable, the START button will start the separation.
- If a sample name is not entered, it defaults to the current date and time. Also, there is no need to save the method to perform a separation. The PeakTrak software treats methods as a temporary method discarded after each separation. If desired the user can save the method at any time for future reuse. All method parameters are stored with the data file and printed on the report so no information about the separation is lost.
- If the displayed method doesn’t meet your needs, it can be edited before use. For example if the suggested gradient isn’t optimal, the gradient can be adjusted by dragging the inflection points to the desired position. If more gradient inflection points are desired, select the INSERT POINT button followed by touching the point on the gradient to insert the point. The METHOD EDITOR button at the top left of the screen allows for further method adjustment.
- ZOOM and PAN can be performed by using a 2-finger pinch zoom (like many cell phones or tablets) or a 2-finger pan. A single-finger touch or drag is interpreted as a gradient edit, not a pan function.
• Fraction collection defaults to collecting ALL to ensure no peaks are lost due to lack of UV absorbing properties. Even when selected, the peak detector operates with default parameters based on the column size (unless disabled by the user). This will cause the system to advance to the next tube whenever a peak starts or ends to ensure cleanly cut fractions with maximum concentration. The tube changes and peaks will be marked and the fraction collector will move after the delay volume has passed.

- **PEAKS** collects fractions only when a peak is detected and sends the non compound containing fluid to waste.

- **NONE** is used conjunction with INITIAL WASTE AND TIME windows (Figure 3-7) to minimize fractions or when the user wants to manually select when to collect while the remainder is sent to waste. This is commonly used when a large single peak is desired and is selected by the user.

• The MS button displays the current mass spectrum on PurIon systems, and the current UV button displays the UV (or UV-Vis) spectrum.

### 3.5.2 Method Editor Screen

The **METHOD EDITOR** screen (Figure 3-4) allows control of less commonly used parameters that aren’t available on the main screen.
3.5.3 Edit Gradient Options

The **EDIT GRADIENT** option is on the **METHOD EDITOR** screen. This option:

- Allows you to more precisely define a gradient than possible with a finger drag, by displaying the gradient in a tabular format (Figure 3-5).
- Allows solvents to be changed with each segment of the separation.
- Set a constant percentage of a third solvent as modifier in this screen (in the lower right corner of the screen; Figure 3-5).
- The table can be collapsed using the **EDIT GRADIENT** button again.

![Figure 3-5 Edit Gradient screen](image)

Other options on this screen include:

**Equilibration Volume** – Sets the amount of starting gradient solvent to equilibrate the column. The volume can be adjusted depending upon solvent used or column chosen (size and media). The equilibration solvent mixture is the same as that defined at the start of the run.

**Air Purge** – Sets the amount of time air is passed through the column after completion of the separation to remove free solvent from the column. (Only available on systems that have level sensing capability.)

**Tube Volume** – Sets the capacity for collected fluids. This volume can be the default maximum volume (Max option) for the tube size as defined in the **CONFIGURATION** window, or a method-specific volume less than the default maximum capacity.
This is useful for collecting fractions smaller than the maximum tube volume when the peak can’t be detected and must be located after the run via other detection techniques. Note that the actual fraction size may be less if a newly detected peak causes a tube change, or if you click the NEXT TUBE button.

Fraction volume may also be less than the max volume if the system is configured with an ELSD or MS.

### 3.5.4 Peak Detection

**UV or UV-Vis** – Selecting the λ button allows you to specify the wavelength for detection

- Up to two different wavelengths can be monitored or used to trigger fraction collection. This can be set up in the details menu (described below).

**Details** – Allows customizing of the detection methods and detection parameters.

- **Signal Gain** is a mathematical scaling of the collected signal for display.

- **Slope Based** – Allows peaks to be cut based on the slope of the signal.
  - Select the average peak width for your chromatography. Peak widths are measured at the baseline. The slope detector will detect peak widths ranging from about 0.2 to 2 times the peak width setting for a moderately sized peak.
  - For example, if you entered a peak width of 1 minute, the range would be 12 seconds to 2 minutes.
  - For best operation, the peak width should be set to just over the average peak width being separated. For instance, if the average peak width is 45 seconds, you should enter a peak width of 1 minute. For most flash chromatography, 1 minute is a good starting point for a peak width.

- **Threshold** – Identifies a peak if the absorbance exceeds the selected value.
  - It will continue to identify a peak until the absorbance drops 0.01 AU below the programmed threshold to minimize false peaks due to a noisy signal.
  - If both slope based and threshold are selected, these signals are logically “ORed” together; if either is active, a peak is collected.

- **Monitor** – Displays the detector signal for reference, but won’t trigger fraction collection based on the signal.

- **Baseline Correction** – Allows compensation of absorbance due to solvent UV absorbance.
• Selecting this option enables a pre-run gradient to provide a ‘blank’ run that can be used to adjust for absorbance changes due to changing concentration of UV-absorbing solvents throughout the run. This requires ~1 minute.

• Baseline correction may impact peak intensity. Additionally, this setting may effect peak shape as the software distinguishes between sample and solvent.

• Selecting baseline correction for any wavelength will apply the correction to all wavelengths and may impact peak height even in areas where the solvent absorbance is low.

Figure 3-6 DETECTION OPTIONS screen

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

• This algorithm compares the current total spectrum of absorbance and compares it to the solvent spectrum. The more that the spectrum deviates from the solvent spectrum, the higher the signal. It is useful to limit the range of wavelengths used in this algorithm to better differentiate the compound spectrum from the solvent background spectrum.

ELS Detector – Enable this option to use the optional evaporative light scattering (ELS) detector.

When enabled, click the DETAILS button to configure additional settings:

• Sensitivity; Low, Normal, or High sets the preamp gain in the detector.

• Most flash work should be done at normal sensitivity to keep large peaks on scale. High sensitivity is recommended when using higher boiling point solvents such as heptane, or reverse phase solvents such as water, methanol, or acetonitrile.
**Signal Gain** – You may modify the gain to increase the detector signal. This is a mathematical multiplication of the signal to increase the size of peaks on the display.

**Slope Based and Threshold** – Refer to these features described.

**Spray Chamber Temperature** – The default settings are 30 °C (normal phase default methods) and 15 °C (reverse phase).

- This setting may be adjusted from 10 to 60 °C so that detection can be optimized for the solvent system in use. However, the lower limit is 5 °C below the ambient temperature.

**Drift Tube Temperature** – The default settings are 60 °C (normal phase default methods) and 60 °C (reverse phase).

- This setting may be adjusted from 30 to 90 °C, but is limited to a range of 5 °C below and 60 °C above the ambient temperature.

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

- **Threshold** – Signal level used to detect a peak. This is based on the baseline noise. The baseline noise is measured during the first column volume; this is multiplied by the value entered in the THRESHOLD control to generate a trigger value. A peak is collected if the signal is greater than this trigger value.
  - For the PurIon mass detector, only threshold peak detection is allowed. In this case the threshold is set as a factor of the measured noise signal. The noise for the first few seconds of the chromatogram is used to determine average noise level. Any mass signal that exceeds this level by the selected factor will be collected as a peak.

- **Monitor** – Clicking on this option prevents fractionation based on the mass spectrometer signal.

- **Terminate On Target** – Stops the run after all mass spectrometer detection ions have been detected.

- **Detection Ions** – Used to set ions for detection or to be monitored. Up to 4 single ions may be chosen, or a range of ions and up to 3 single ions may be chosen. Detected ions may be a mixture of positive and negative ions.

**Purity Measure** – When using two wavelength detection options, a ratio of the two wavelengths can be displayed which can provide an indication of compound purity.

- For example, if a pure compound is eluting, the absorbance is linearly related to the concentration of the compound in the solvent regardless of wavelength. If the compound absorbs differently at different wavelengths, the absorbance at each wavelength may be different, but is still linearly related to the concentration. If the concentration doubles, the absorbance at each
wavelength doubles (at least within the limitations for Beer's Law). 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, this constant value is displayed as a horizontal line.

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

- The SHOW SPECTRAL PURITY selection measures purity by using a comparison of UV-spectra measured at differing time; it is not limited to 2 wavelengths. The algorithm used is the “similarity index.”
  - The SPECTRAL PURITY DETECTION button allows fractionation based on spectral purity. If the spectra within the peak are similar, it is considered 1 peak. If the composition of the peak changes during the peak, the spectra will not be similar and the purity index will decrease from the default value of “1” indicating a change in purity from the early portion of the peak. This allows the system to advance to the next fraction as the purity within a detected peak changes.

**Initial Waste & Time Windows** – The number of fractions collected can be minimized by setting PEAK COLLECTION to NONE on the MAIN screen and selecting only portions of the separation for fraction collection.

- Initial waste can be used to send fluid to waste before the first peak of interest. Time windows can be used to identify areas of interest that are collected within the separation. For example, if you know your peak of interest will elute between 3 and 4 minutes, you can create a time window to collect all during this period, or to collect peaks only during this period.
3.5.5 Tools

**Time to CV** – Allows the user to change the units used for the horizontal axis during the separation from the configured units.

**Column Information** – Displays column usage history. This is useful for reusable columns such as C18. It can display the age of the column, number of separations, and fluids left in column in the previous use.

**Gradient Optimizer** – Allows a gradient prediction based on TLC values and is targeted at improving the results for difficult separations.
Manual Control – Capability is also included to allow the user to wash individual flow paths.

- This is useful for troubleshooting or cleaning flow paths of potential obstructions or changing from normal to reverse phase solvent systems.
- If changing solvent systems from reverse to normal phase or vice versa, the entire flow path should be flushed with an intermediary solvent that is miscible with both reverse and normal phase solvents such as isopropanol, ethanol, or acetone.
- Able to set the flow rate, solvent mixture ratio, and volume pumped in this menu. Also able to choose to collect into a certain tube or rack in order to flush fraction collection arm tubing.
- Contains a lamp energy window to indicate if the flow cell windows are dirty. An indicator graphically shows the current level. Any value in the green portion of the graph is acceptable.
- Offers the ability to manually air purge the cartridge or column manually (if the system is configured with solvent level sensing capability).

3.5.6 Results Screen

At the end of a separation, the final chromatogram report is shown. At this point, the user can: print the report; customize the displayed report by adding multiple UV absorbance or mass spectra to the report; format the display; reuse the final (potentially modified) method for a subsequent separation; scale up the
method for a separation on a different column size; or add time to
the current separation to continue if the desired compound
hasn't eluted yet.

If SCALE METHOD is selected, simply chose the size of column for
scaling from the displayed drop down list (Figure 3-10). The
method will be scaled for immediate use, or can be saved for later
use (Figure 3-11).

![Figure 3-10 Scale Method screen](image)

3.5.7 PurIon Operation

The PurIon functions like another detector in the system. In
addition, operation of the Mass Detector has been integrated into
the PeakTrak control software. To use the PurIon, turn the
PurIon power ON (if not already on). When turned on, the PurIon

![Figure 3-11 Files screen](image)
requires 30 minutes to achieve proper vacuum levels. A
countdown window will monitor progress. Separations can be
completed without the PurIon during this period.

**Operate/Standby/Shutdown:** When ready to use the PurIon,
select the MS drop down and select OPERATE. This will turn on
the gas flow, heaters, and detector voltage. When not in use,
select STANDBY to turn off the detector voltage and heaters. The
standby mode will prolong the life of the detector when not in
use. The SHUTDOWN command is used to power down the turbo
pump in the PurIon in preparation for repair or repositioning of
the system. Moving the system without shutting down can cause
major damage to the PurIon. Turbo speed is monitored during
the shutdown process.

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

- To use the METHOD DEVELOPMENT screen, dissolve a
  minute amount of crude sample in a minimal amount of
  suitable solvent. Dilute this by placing 1 drop in
  ~ 20 mL of solvent. Place the front panel inject valve
  into the load injection and inject >20 µL using a
  22 gauge blunt-tipped needle. Within a few seconds the
  mass spectra will be displayed.

- If your ion of interest isn’t visible, it could be due to the
  formation of adducts or fragmentation. If you select the
  ION FINDER and enter your desired mass, it will compare
  the 10 most intense ions with a list of common adducts
  or fragments to determine if your desired mass was
  detected as one of the peaks. If so, you can select the
  peak for detection in your separation.

- The displayed spectrum will continue to collect mass
  information until the window is closed or the display
  cleared operation of the injection valve will clear the
  current display.

- If your compound isn’t detected, you can adjust the
  ionization settings by selecting from the drop down list
  (Figure 3-12). The factory selections include:
  - ROBUST for compounds that do not easily ionize.
  - TYPICAL works well for most compounds.
  - FRAGILE for those compounds that are delicate or
easily fragment.
When one of the ion settings is selected, you have the option of using the suggested setting, or editing the parameters to optimize the ionization settings. The following are setting examples:

- **Factory Settings** – Restore the ROBUST, TYPICAL, and FRAGILE ion settings to the factory default values.

- **Probe** – The probe nebulizes and ionizes the sample. There is a choice of ESI (electrospray interface) or APCI (atmospheric pressure chemical ionization) probes. The software will change the labels on the control to reflect the probe installed in the mass spectrometer.

- **Gas Temp** – The temperature of the nebulization gas for the probe. Lower temperatures are used for more delicate, heat-labile compounds. The temperature is set to quickly evaporate the carrier solvent. (Note that PurIon S and PurIon L systems will display a single temperature for both positive and negative ionization.)

- **Voltage (Current)** – This displays a voltage setting (ESI probes) or current value (APCI probes). Lower values are used for more delicate compounds.
  - The capillary is heated to complete the evaporation of solvent. It also carries a voltage; lower voltages are used for more delicate compounds.

- **Source Voltage** – The source voltage settings have the greatest effect on fragmentation. Higher values induce more fragmentation but also reduce adduct formation.

- **Offset** – OFFSET is the voltage applied to all masses. Large values tend to increase fragmentation but reduce adduct formation.
• **Span** – SPAN voltage defines an increased voltage applied as the mass increases. As with the offset, larger values increase fragmentation.

• **Close** – Closes the window.

---

**Figure 3-13 Ionization Settings**

Once the Mass Spectrum is displayed and represents the information you want to see, it can be printed or saved for future reference. To view more Mass labels, use the FORMAT GRAPH button.

The fluid pressure indicator is used during troubleshooting if there is a blockage in the fluid interface. The TIC indicator is a relative measure of background Total Ion Count and therefore a measure of PurIon cleanliness. It will never read completely 0; rather, it indicates the progress of any cleaning activity performed.
CombiFlash NextGen Systems

Section 4 Network Configuration

4.1 Network Configurations

The system has a factory-assigned IP address — 192.168.1.51. In many cases, this address must be reconfigured for use on a corporate network. To reconfigure the IP address:

1. From the touch screen panel, select the TOOLS > CONFIGURATION menu command.
2. Select the NETWORK CONFIGURATION tab.
3. Select Static IP as the NETWORK TYPE.

Note
The CombiFlash NextGen system also provides limited support for DHCP connections. If DHCP is desired, select DHCP as the network type and skip steps 4 through 7. The system will display instructions required to complete a DHCP connection.

4. Enter the IP Address, Netmask, and Gateway information provided by your network administrator.

Note
Should your network administrator request the MAC address, it can be found in the HELP > ABOUT PEAKTRAK window.

5. Click the OK button to save the settings.
6. Confirm that your network administrator has completed any necessary network changes to support the system.
7. Locate the CAT5 connection cable (P/N 480-6545-01) in the accessory package. Insert one end into the Ethernet port on the back panel. Connect the other end of the cable to your network access port.

You should be able to connect to the CombiFlash NextGen system from a PC on the network (Section 4.1.1) and configure other network features such as network printing (Section 4.1.2) and network file saving (Section 4.1.3). Networks with Wi-Fi access points may also support remote control with mobile digital devices.
4.1.1 Network PC Access

A successfully networked CombiFlash NextGen system can be accessed by a PC that meets the recommendations listed in Table 4-1.

Table 4-1 Personal Computer Recommendations

<table>
<thead>
<tr>
<th>Operating Systems:</th>
<th>Microsoft Windows 7, Windows 8, Windows 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardware:</td>
<td>The computer hardware must meet the minimum required specifications of the selected operating system.</td>
</tr>
<tr>
<td>Network Connection:</td>
<td>Ethernet, 100 Mbit/s or faster</td>
</tr>
<tr>
<td>Display:</td>
<td>1280 x 800 pixels, minimum</td>
</tr>
<tr>
<td>Internet Browser:</td>
<td>Recent versions of Microsoft Edge, Google Chrome, and Mozilla Firefox</td>
</tr>
</tbody>
</table>

a. This table shows supported PC configurations. Other configurations or AJAX-compatible browsers may be possible but are unsupported by Teledyne ISCO.

To access the system, simply open an Internet Browser and enter the IP address as “http://...”, where ‘...’ is replaced by the selected address. The browser window will load PeakTrak after you enter the address.

4.1.2 Network Printing

The CombiFlash NextGen system can print to a network printer and supports both JetDirect and line printer (LPR) queues.

Consult with your network administrator to determine the IP address of selected printer. If the printer uses an LPR print queue, you must also find out the queue name. If the printer uses a JetDirect print queue, also ask for the port number.

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

1. Select the TOOLS > CONFIGURATION menu command.
2. Select the NETWORK CONFIGURATION tab.
3. Follow the on-screen instructions for entering the address and queue information.
4. Click OK to save the settings. The CombiFlash NextGen system will send a test page to the printer.

After successfully printing a test page, the network printer will be available for printing using the FILE > PRINT menu command or through the Automatically Print Report at END OF RUN OPTION on the TOOLS > CONFIGURATION USER PREFERENCES tab.

4.1.3 Network File Save Configuration

To save all run histories automatically as a text or PDF file, configure the NETWORK FILE SAVE option. This allows the CombiFlash NextGen system to access the corporate network and save the file in a selected Network Share folder. Consult with your network administrator to configure these fields.

4.2 Direct Connection

A direct connection supports communication between the CombiFlash NextGen system and a single PC that meets the recommendations in Table 4-1.
CombiFlash NextGen Systems

Section 5 Maintenance

5.1 System Standby and Shut Down

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

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

⚠️ WARNING

As long as the AC mains power cord is connected, power is inside the unit. The mains power cord is the disconnect device. Position the CombiFlash NextGen system so that the power cord can be unplugged, or use a power strip where the plug can quickly be removed from the outlet in the event of an emergency.

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

⚠️ CAUTION

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

5.2 Preventive Maintenance

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

As Needed – Perform these tasks as conditions require:

- Cleaning (Section 5.3).
- Quick flow cell cleaning when recommended by a system alert message (Section 5.5).
- Wipe cone on Purlon system with wipe soaked with methanol or water to remove visible residue near cone inlet. (Purlon systems only).
Every Run – Perform these tasks for each run:
• Inspect Solid Load Cartridge Cap (Section 5.4.1).
• Allow the separation run to finish with a high percentage of solvent B to flush residual compounds from the column, internal tubing, and flow cell (Section 5.5).
• Allow Cone wash to run to completion (PurIon systems only) to wash residual compounds from the fluid interface, probe, and to clean the cone area.
• Allow Valve Wash sequence to run to completion to wash residual compounds from the injection valve and ELSD (if installed) flow path.

Monthly – Perform these tasks at least monthly, more frequently if conditions warrant:
• Tubing Inspection (Section 5.4.2)
• Collection rack and tray cleaning (Section 5.3.1).
• Monthly flow cell cleaning (Section 5.5.3).

Annually – Perform these tasks at least annually, more frequently if conditions warrant:
• Change roughing pump oil (PurIon systems only).

5.3 Cleaning

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

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

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

5.3.1 Collection Rack and Tray Cleaning

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

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

**WARNING**
Risk of fire or equipment damage. Faulty or worn seals, 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.

5.4.1 Solid Load Cartridge Cap Inspection
Before using the solid load cartridge cap ensure that the seal inside the cartridge cap is in good condition and not showing any signs of wear or deterioration.

Monitor the solid load cartridge (and RediSep column) for leaks while in use. If a solvent leak exists, stop the separation and correct the leak by either cleaning the sealing surfaces or replacing the seal on the solid load cartridge.

5.4.2 Tubing Inspection
Perform a tubing inspection monthly:

1. Visually inspect the solvent, waste, and drain tubing. The tubing must be free of any damage, kinks, or deterioration. Fittings should show no signs of leaks.

2. Test the collection tray drain and top shelf drain by connecting a vacuum or air supply source to the outlet end of the drain tubes. Then, verify the presence of such vacuum or air supply source on the drain hole (Figures 5-1 and 5-2).
Correct any deficiencies before returning the instrument to operation.

**Figure 5-1 Fraction collection tray drain hole**
5.5 Flow Cell Cleaning

5.5.1 Post Separation

As a preventive measure, all default column methods finish the separation run with a high percentage of solvent B (Figure 5-3). This brief time (one to six column volumes) of strong solvent flushes residual compounds from the column, flow cell, and internal tubing.

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 5-4).
Typically, chemists STOP and then TERMINATE the run after the last compound elutes. This action skips the post-separation flush. If any of the above conditions appear, consider allowing some of the runs to continue through the flush, or run a high percentage of %B solvent through the system for a few minutes at the end of each day.

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

---

**CAUTION**

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

---

### 5.5.2 Quick Cleaning when Recommended

When the lamp energy is lower than normal, the system will recommend flow cell cleaning (Figure 5-4) before starting a separation run.

![Flow cell cleaning message window](image)

**Figure 5-4 Flow cell cleaning message window**

When the system displays this message you can:

- **Cancel Run** (recommended) – Click the CANCEL RUN button so you can perform a quick cleaning described in the following steps.
- **Continue Collect All** – Click this button to ignore the message. Because the peak detection operation might be impaired, the system automatically collects all fluids to avoid diverting compounds of interest to waste.
- **Help** – Click this button to display the flow cell cleaning on-line help topic.

To perform a quick cleaning:

1. After clicking the CANCEL RUN button, select the TOOLS > MANUAL CONTROL menu option.
2. From the MANUAL CONTROL window, note the Raw Lamp Energy level at 254 nm. The Raw Lamp Energy gauge has two ranges: red and green. (Figure 5-5),
5-6 EAR99 Technology Technology Subject to Restrictions Contained on the Cover Page

CombiFlash NextGen Systems
Section 5  Maintenance

Figure 5-5  Raw lamp energy gauge

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

- **Green** – Lamp energy is sufficient to detect peaks within typical system limits.

3. Remove the column and insert a bypass tube between the upper and lower column mounts. A bypass tube (P/N 209-0165-46) can be found in the accessory kit shipped with your system.

4. Set the Flow Rate to 40 mL/min (Figure 5-6).

![Manual Control Settings - Quick Cleaning](image)

Figure 5-6  Manual Control Settings - Quick Cleaning

5. Select **THROUGH COLUMN** for the VALVE POSITION option.

6. Select **NEXT** for the Pump into Tube # option. By pumping into a collection tube, the diverter valve is also cleaned of any residue during this operation.

7. Click the **PRIME B** button to pump 100% Solvent B through the bypass tube and into the collection tubes.

**Note**

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

8. Monitor the Raw Lamp Energy gauge. As the system pumps solvent, the raw lamp energy should gradually
improve. Pump solvent for two to five minutes or until the indicator reaches the far-right of the green range. If after five minutes the indicator is still in the red range, repeat the cleaning steps using an alternative solvent. Or, complete the steps in the Monthly Flow Cell Cleaning procedure (section 5.5.3).

**Note**

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

### 5.5.3 Monthly Flow Cell Cleaning

Perform this procedure as part of your scheduled preventive maintenance, or when Quick Cleaning As Required (section 5.5.2) does not improve the lamp energy.

1. Remove the column and insert a bypass tube between the upper and lower column mounts. A bypass tube (P/N 209-0165-46) can be found in the accessory kit shipped with your system.
2. From the menu, select TOOLS > MANUAL CONTROL. This opens the MANUAL CONTROL window.
3. Set the Flow Rate to 40 mL/min.
4. Select THROUGH COLUMN FOR THE VALVE POSITION option.
5. Select NEXT for the Pump into Tube # option.
6. Place the B1 Solvent inlet line into a reservoir of methanol, acetone, or a strong solvent that readily dissolves residual sample material.
7. Click the PRIME B button to pump 100% Solvent B through the bypass tube and into the collection tubes.
8. After three minutes, click the STOP button. Allow the system to stand for at least six hours. Overnight is recommended.
9. Return the B1 solvent line to the original solvent container.
10. Perform the Quick Cleaning As Required (Section 5.5.2 and monitor the Raw Lamp Energy. (Figure 5-5)

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

### 5.6 ELSD Maintenance

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

#### 5.6.1 Cleaning the ELSD Detector

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

![Detection Options screens](image)

**Figure 5-7 Detection Options screens**

5. Lift the P-trap drain tubing up to approximately case top level. Using a wash bottle, syringe, or suitable measuring device, slowly fill the drain line with up to 40 mL of acetone. Lift the end of the tubing as needed to transfer most of the liquid into the spray chamber. Make sure the fluid level in the tubing doesn’t exceed the level of the instrument case top. If the tubing is raised too fast, fluid may flow out the top of the vent tube causing a spill. Hold the tubing up for at least 1 minute after the fluid has been transferred to the spray chamber.
6. Place the open end of the P-trap drain line in a 100 mL (or greater) beaker.

7. Allow the acetone to drain out of the unit by dropping the end of the P-trap drain line, while it is in the 100 mL beaker, below the P-trap drain line level.

8. Fill the P-trap pump drain line with up to 40 mL of isopropyl alcohol using the same procedure as step 5.

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

10. Allow the isopropyl alcohol to drain out of the unit by dropping the end of the P-trap drain line, while it is in the 100 mL beaker, below the P-trap drain line level.

11. Use the DETAILS menu to set the spray chamber to 60 °C.
12. Fill the P-trap with up to 40 mL of isopropyl alcohol as before and drape the P-trap drain line of the top of the unit and let it set for at least 20 minutes.

Figure 5-10 P-trap pump drain line draped over the unit

13. Allow the isopropyl alcohol to drain out of the unit by dropping the end of the P-trap drain line, while it is in the 100 mL beaker, below the P-trap drain line level.

14. To ensure that there is no fluid remaining in the drift tube, set the drift tube to 90 °C. Go to the MANUAL CONTROL window to turn the gas on and let run 10 minutes. Turn off the gas.

15. Press FILE and then NEW to reset the method temperatures. Use the instrument normally.

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

5.7 PurIon Maintenance

5.7.1 ESI and APCI Removal from PurIon S and PurIon L

Refer to Figure 5-11.

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.
### 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 $\frac{1}{4}$-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 µm syringe filter. To unplug the capillary, complete the following:

1. Connect a 1 mL syringe, using adapters as needed, to the ESI or APCI capillary and push fluid through to remove plug. If this procedure does not resolve the issue, an HPLC pump can be connected to the inlet fitting.

2. If this procedure fails to unplug the capillary, refer the instructions for the rebuild kit provided with your PurIon system.

3. Reinstall the probe following the appropriate procedures for your system.

### 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 $\frac{1}{4}$-28 PTFE tube fitting labeled as “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 troubleshoot an overpressure error, complete the following:

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

### 5.7.6 Check Valve Cleaning

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

1. Remove the inlet and outlet lines from the check valve holders (Figure 5-12), and then remove the check valve holders. Pliers may be needed to remove the holders.

![Figure 5-12 Check valve holder](image)

2. Remove check valves from pump head and place in a beaker of methanol. Sonicate check valves for 15 to 20 minutes.

3. Reinstall check valves into pump head, making sure that the ends of the check valves (with multiple holes) are facing up towards the outlet of the pump.

4. Reinstall check valve holders and tighten finger tight. Then using pliers tighten an additional ¼ turn.
5. Check the flow rate delivery. If the flow rate delivery is still incorrect, replace the check valves. The correct flow rate depends on the back pressure of the carrier solvent. The flow will be 0.5 mL/minute until the fluid interface reaches operating pressure, then reduces the flow rate to 0.2 mL/minute under typical operating conditions for the fluid interface. The operating mode can be determined by listening to the pump operate. While running at 50 mL/min, the pump motor speed is continuous. Once operating pressure is reached, the pump has a rapid refill stroke approximately that occurs ~8 seconds.

5.7.7 Replacing Check Valves

1. Install new check valve cartridges (P/N: 250-0001-17) into pump head housing making sure the ends of the cartridges, with three small holes, are facing upwards towards the outlet (Figure 5-13).

![Figure 5-13 Correct orientation of cartridge](image)

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

5.7.8 PurIon Cone Cleaning

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

5.7.9 Capillary Inlet Cone Removal

1. Set the PurIon to standby.
2. Wait ~15 minutes for the cone to cool.
3. Remove the ion source assembly, section 5.7.6.
4. Wearing gloves (typical lab gloves are usually sufficient), place your fingers on the top surface of the cone and turn counterclockwise to unscrew the capillary inlet cone. Many times this is sufficient to remove the capillary. If you are unable to unscrew the part manually, use an adjustable wrench on the flats of the cone to unscrew. The flats are not large; they may be difficult to see while keeping a wrench seated on them.
5. Remove the O rings from the capillary inlet cone.

5.7.10 Capillary Inlet Cone Cleaning

1. Remove the O-ring under the capillary base, then sonicate the capillary in a methonal:water (50:50) mixture for 30 minutes (Figure 5-14).

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, and methanol, then dry the capillary using nitrogen air.

5.7.11 Capillary Inlet Cone Installation

1. Ensure that the O-ring seals are in position.

2. Place the capillary inlet cone into the opening and press down until the threads are able to engage. There may be slight restriction in a downward motion as the part is almost completely inserted. This restriction is caused by 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.

5.7.12 PurIon Troubleshooting

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

If the line cord is connected properly, check circuit breaker on the system's rear panel to ensure it is switched to the ON position.
### Table 5-1 Common PurIon Error Codes and Resolutions

<table>
<thead>
<tr>
<th>Error Description</th>
<th>Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purlon temperatures are stabilizing. [r] seconds remain. (where [r] is a number)</td>
<td>The Purlon has several areas with heaters. The software has a set timer to allow temperatures to come up to operating conditions. After that time, a separate error is thrown if the heaters are not within an acceptable band. The default time is 300 seconds after entering the operate condition. During the standby condition most heaters are set to OFF except the inlet capillary with is set to 50 °C during standby.</td>
</tr>
<tr>
<td>The Purlon vacuum level is too low to operate. Please verify that the roughing pump is on and operating correctly. Pirani Pressure: [s1] mbar. (where s1 is the vacuum reading).</td>
<td>The Pirani pressure must be below 5.5E-3 mbar before the Purlon turbo pump will operate. If trying to place the Purlon in operate without turning on the roughing pump, this message will appear. This error generally occurs if the user forgets to turn the roughing pump back on after cleaning the capillary or changing the pump oil.</td>
</tr>
<tr>
<td>The Purlon has been shut down. It will be unavailable for use until the NextGen unit has been rebooted. Please ensure the Purlon and fluid interface are both turned on before rebooting the NextGen unit.</td>
<td>This message is displayed after the Purlon has been successfully shut down through the SHUTDOWN command. It serves as a reminder that a NextGen reboot will be necessary for the Purlon to be enabled again by the NextGen system.</td>
</tr>
<tr>
<td>The splitter valve seals have exceeded their recommended life. The Purlon will continue to operate, but there is an increased possibility of leakage at the splitter valve and loss of Purlon detection during a separation.</td>
<td>The splitter valve supplier has stated that typical valve life exceeds 1,000,000 actuations. After that time, there is no method of determining when it will leak. The valve can be rebuilt using the valve rebuild kit (P/N:60-5234-629) or continue to use and monitor for leakage. Leakage should not drip out the bottom of the fluid interface front cover.</td>
</tr>
<tr>
<td>No ion source is detected on the Purlon. Please ensure that the ion source is properly installed and connected. If the source is still not detected, contact a qualified service technician. Error 309</td>
<td>The Purlon has reported that the ion source high voltage cable is not properly plugged into its socket. This is the cable with the round connector and is to the right and behind the ion source. This generally occurs upon changing or cleaning probes.</td>
</tr>
<tr>
<td>A plug has occurred in the Purlon fluid lines. The separation can be continued without Purlon detection or continued if the plug is corrected. Error 310</td>
<td>This error message is displayed if a plug is detected during a separation.</td>
</tr>
<tr>
<td>The Purlon inlet cone may be plugged which could prevent detection. Continued operation will not cause damage. Please contact a qualified service technician to clean the cone; Pirani Pressure: [s1] mbar. Error 315</td>
<td>During normal operation, the Pirani pressure should be &gt;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 Capillary Inlet Cone Cleaning (5.7.10).</td>
</tr>
<tr>
<td>A plug has occurred in the Purlon fluid lines. The plug must be corrected to allow continued operation. Error 316.</td>
<td>This error occurs if the tubing is plugged while the method development screen is in use. See cleaning the probe capillary (5.7.3).</td>
</tr>
<tr>
<td>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.</td>
<td>On Purlon systems (not Purlon S or Purlon L), the probe isn’t seated properly. Loosen the thumbscrew on the front of the ion source housing, push down on the probe, then tighten the thumbscrew.</td>
</tr>
<tr>
<td>The fluid interface pressure is too low. Error 325</td>
<td>This could be due to lack of carrier fluid, loss of pump prime, or leakage.</td>
</tr>
</tbody>
</table>
### Table 5-2 General PurIon Troubleshooting

<table>
<thead>
<tr>
<th>Error/Symptom</th>
<th>Solution</th>
</tr>
</thead>
</table>
| No signal on any mass, or very weak signal         | • Verify nitrogen pressure. Nitrogen is required to nebulize the sample.  
• Check carrier solvent level, refill carrier solvent and re-prime the carrier solvent pump. Carrier solvent is needed to bring sample to the mass spectrometer.  
• Check that the priming valve is tight. A loose priming valve prevents sample from getting to the mass spectrometer.  
• Verify the splitting valve is not leaking. This causes loss of carrier solvent preventing sample from getting to the mass spectrometer.  
• Check for leaks around other fittings on the solvent interface. |
| Delayed signal during run.                         | • Verify nitrogen pressure. Sample may drip through the cone and still be detected later.  
• Check priming valve tightness. A slight leak will delay sample.  
• Verify the splitting valve is not leaking.  
• Check for leaks around other fittings on the solvent interface. |
| Sample masses observed but weak, or masses other   | • Check polarity to see if the sample ionizes under the opposite polarity.  
• Try another ionization settings.  
• Compound fragments – use fragment mass or try another ionization setting.  
• Compound forms adducts.  
• Compound fails to ionize using the selected probe. Some compounds ionize better using APCI rather than ESI, for example. |
| than the analyte observed.                         |                                                                                                                                              |