Bio-Botanical Extraction

System Guide

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Revision D

General information

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Documentation conventions

Instructions in this documentation adhere to the following conventions.

>	Denotes the path for selecting menu commands, for moving from one menu to another, or for performing a task. **Example:** Click **File** > **Open**.
< >	Indicates that you must enter variable information, such as a path or filename. **Example:** Type open <filename>

Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice (GLP), and consult your organization's standard operating procedures as well as your local requirements for safety.

Safety hazard symbol notice

Documentation needs to be consulted in all cases where the symbol is used to find out the nature of the potential hazard and any actions which have to be taken.

High voltage hazard

Warning: To avoid electric shock, do not remove protective panels from system modules. The components within are not user-serviceable.

Power cord replacement hazard

Warning: To avoid electric shock, use SVT-type power cords in the United States and HAR-type (or better) cords in Europe. The power cords must be replaced only with ones of adequate rating. For information regarding which cord to use in other countries, contact your local Waters distributor.

CO_2 hazards to humans

Carbon dioxide exhibits three primary hazards for humans:

- Toxicity, at levels above 10,000 ppm (PEL 5000 ppm)
- Frostbite, from uncontrolled release of pressurized CO_2 to atmosphere, or contact with accumulated dry ice at a leak site
- Asphyxiation, caused by displacement of oxygen

For locations in which CO_2 is used and stored, the United States Occupational Safety and Health Administration (OSHA) has established the permissible exposure limit (PEL). Installing an ambient-air sensor and alarm capable of detecting CO_2 levels to 10,000 ppm and triggering an alarm at 5000 ppm (instead of, or in addition to, installing an oxygen monitor) complies with the PEL. When another asphyxiant, such as N_2, is used or stored in the same room as CO_2, Waters recommends installing an oxygen monitor with both audible and visual alarms in addition to the ambient-air sensor.

Before proceeding with any monitoring safety configuration, consult your environmental health and safety manager regarding applicable local, federal, and international safety regulations and requirements.

High temperature hazard

During system operation, the extraction vessels, cyclone separators, and tubing at the outlet of the heat exchanger can become heated to high temperatures. These components remain heated for several minutes after the heating devices are powered-off.

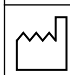
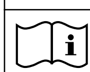
Warning: To avoid burn injuries, exercise care when handling the extraction vessels, separation cyclones, heat exchanger outlet tubing, or other components heated to high temperatures or cooled to low temperatures. Wait until the hot components have sufficiently cooled or the cold components have sufficiently warmed before you handle them.

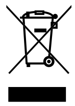
Operating the system

When operating the system, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

The following symbols can be present on the device, system, or packaging.

Symbol	Definition
	Manufacturer
	Date of manufacture
EC REP	Authorized representative of the European Community
	Confirms that a manufactured product complies with all applicable European Community directives
	Australia EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Consult instructions for use
	Alternating current

Symbol	Definition
	Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste. For compliance with the Waste Electrical and Electronic Equipment Directive (WEEE) 2012/19/EU, contact Waters Corporation for the correct disposal and recycling instructions.
SN	Serial number
REF	Part number, catalog number

Audience and purpose

This guide is intended for personnel who install, operate, and maintain the Bio-Botanical Extraction System. It provides much of the information required to set up, operate, and maintain the system's hardware and software components. For additional operational and maintenance details, consult the operator's guides for the individual modules that comprise the Bio-Botanical Extraction System.

Intended use of the Bio-Botanical Extraction System

Waters designed the Bio-Botanical Extraction System to function as a bulk scale, high-pressure separator for collecting extracted compounds to support a wide variety of applications, including quantitative recovery of botanicals and natural products, or for research. It is not intended for use in diagnostic or biologically hazardous applications.

ISM classification: ISM group 1 class A

This classification has been assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements.

Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment.

Class A products are suitable for use in all establishments other than residential locations and those directly connected to a low voltage power supply network supplying a building for domestic purposes.

There may be potential difficulties in ensuring electromagnetic compatibility in other environments due to conducted as well as radiated disturbances.

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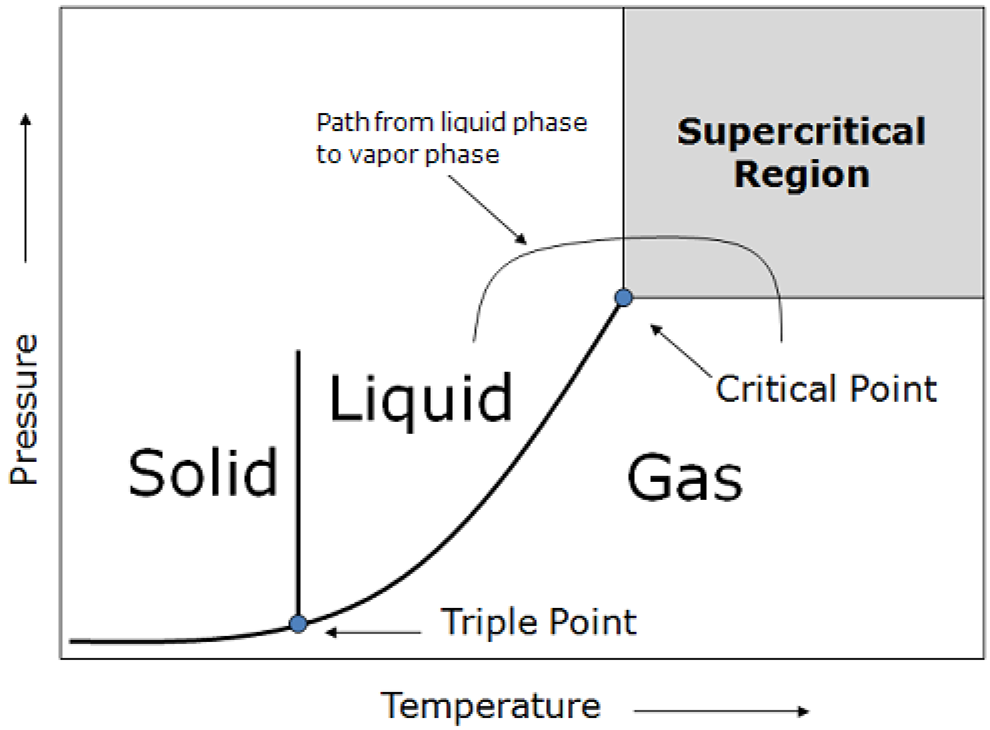
1 Getting started with the Bio-Botanical Extraction System

1.1 Extracting compounds using carbon dioxide

Historically, supercritical fluid extraction (SFE) refers to a technique for extracting compounds from solid or semi-solid substances using a supercritical fluid as the primary component of the extraction solvent. A supercritical fluid results when a substance is heated and pressurized above its critical temperature and pressure. For carbon dioxide (CO_2), this corresponds to 31 °C (88 °F) and 7398 kPa (74 bar, 1073 psi). Under this supercritical state, the solvent takes on advantageous properties that are in between those of liquids and gasses such as high diffusivity, low surface tension and viscosity, and tunable solvating power. Today, however, SFE is used most commonly to describe the use of CO_2 as the extracting solvent, regardless of its physical state. For example, a single extraction using CO_2 could start in the gas phase, transition to liquid, and then supercritical, and end up back in the gas phase at the end. In addition to CO_2, a co-solvent (usually methanol or food-grade ethanol) can serve as a modifier. CO_2 modifiers increase the polarity of the solvent and change the selectivity of the extraction. The Bio-Botanical Extraction System (BBES) takes advantage of the unique properties of CO_2 to extract compounds of interest from complex and convoluted matrices.

The BBES extracts and separates (fractionates) compounds by altering the solvent strength of the CO_2 solvent. As you increase temperature and pressure, CO_2 becomes a stronger solvent, capable of dissolving a wider range of compounds. SFE can still be compared to liquid extraction by comparing corresponding Log (P) data (the ratio of concentrations of a compound in two immiscible phases of solvents at equilibrium) and molecular weight. These principles render SFE predictable and scalable from analytical to process applications. With the BBES, the user can manipulate the pressure and temperature of the CO_2 solvent to selectively extract and collect the desired material, leaving a clean, concentrated extract with minimal processing steps in between.

Figure 1–1: Solvating strength of CO_2 is tunable by changing the pressure and temperature

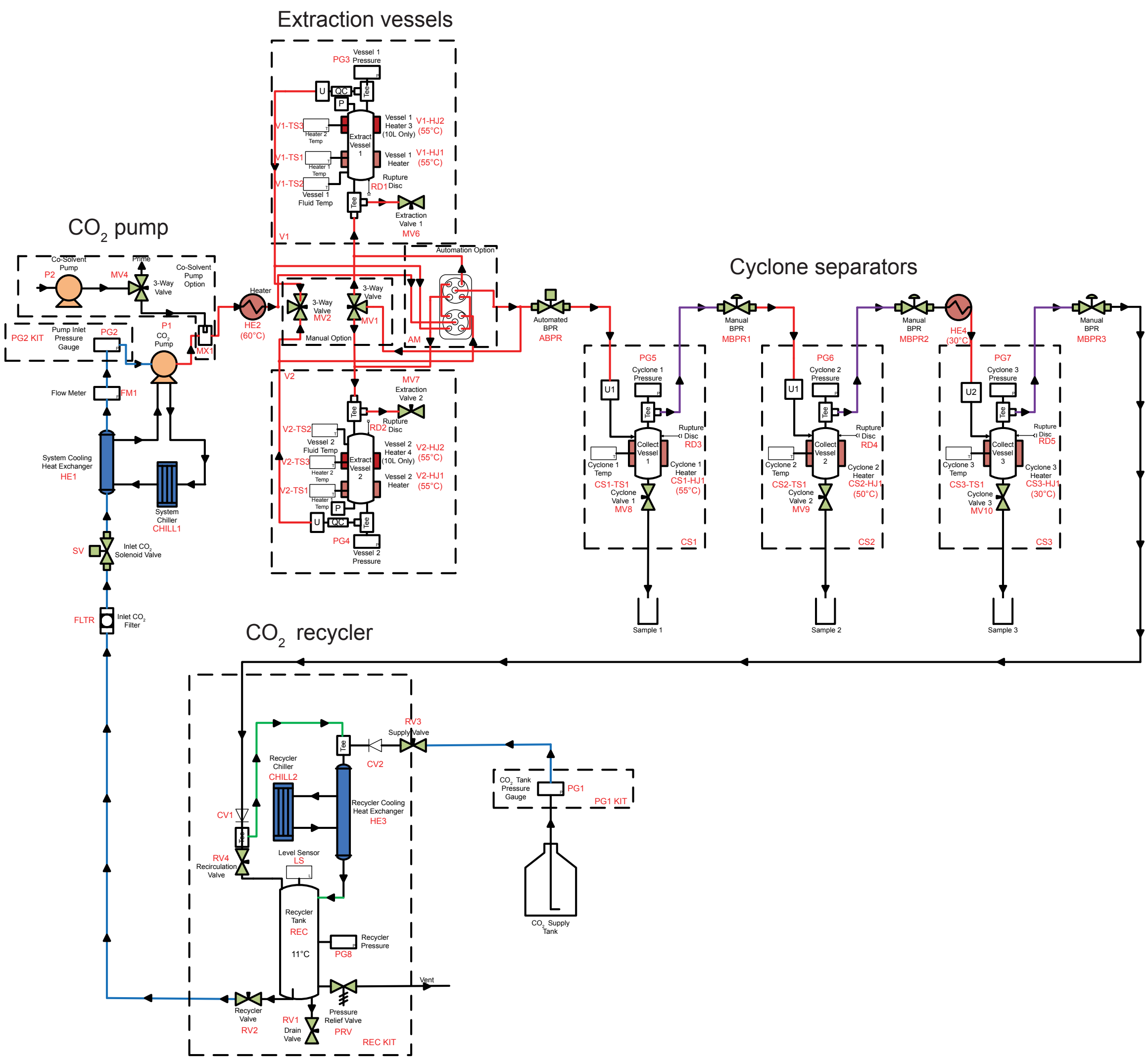


During extraction, raising the pressure of CO_2 in the system allows for the extraction of more polar compounds. Adding a co-solvent such as food-grade ethanol additionally increases this capability to extract even more polar compounds. During collection, decreasing the pressure of the CO_2 in the system causes extracted compounds to precipitate and fall out of solution in the collection vessels. The first compounds to precipitate tend to be the most polar compounds with the highest molecular weight. This is followed by the least polar, light, volatile compounds.

1.1.1 Bio-Botanical Extraction System flow

The Bio-Botanical Extraction System (BBES) functions as a high-pressure separator for extracting compounds from solid or semi-solid substances. By manipulating the pressure and temperature, CO_2 can selectively extract the desired material. On a system configured for purification and collection of extracted compounds, the sample is placed in an extraction vessel and pressurized with CO_2 and, depending on the application, a small percentage of co-solvent, to extract the compounds of interest. These dissolved compounds are then transferred from the extraction vessel to one or more cyclone separators by flowing CO_2, depending on the system's configuration. The automated back-pressure regulator (ABPR), located between the extraction vessel and cyclone separators, allows for controlled depressurization of the CO_2. After the ABPR, the system pressure is reduced, causing the CO_2 to lose its solvating power. When the manual back-pressure regulators (MBPR) are properly set, the extracted material precipitates out of the solution into the cyclone separators, which can be operated as a series of cascading pressure steps to isolate the collected compounds into fractions. The CO_2 is vented or diverted to a CO_2 recycler. The diagram below shows the key components comprising the system flow for a BBES configured with a co-solvent pump, two extraction vessels, and three cyclone separators.

Figure 1–2: Bio-Botanical Extraction System flow path



Note: The connection locations for V2 are representations only. The true inlet of the extraction vessel (from the CO_2 pump) is on the top, and the actual outlet (to the ABPR) is on the bottom. Refer to the V1 connections.

The following diagrams show the flow path in more detail.

Figure 1–3: CO_2 pump and extraction vessel flow path

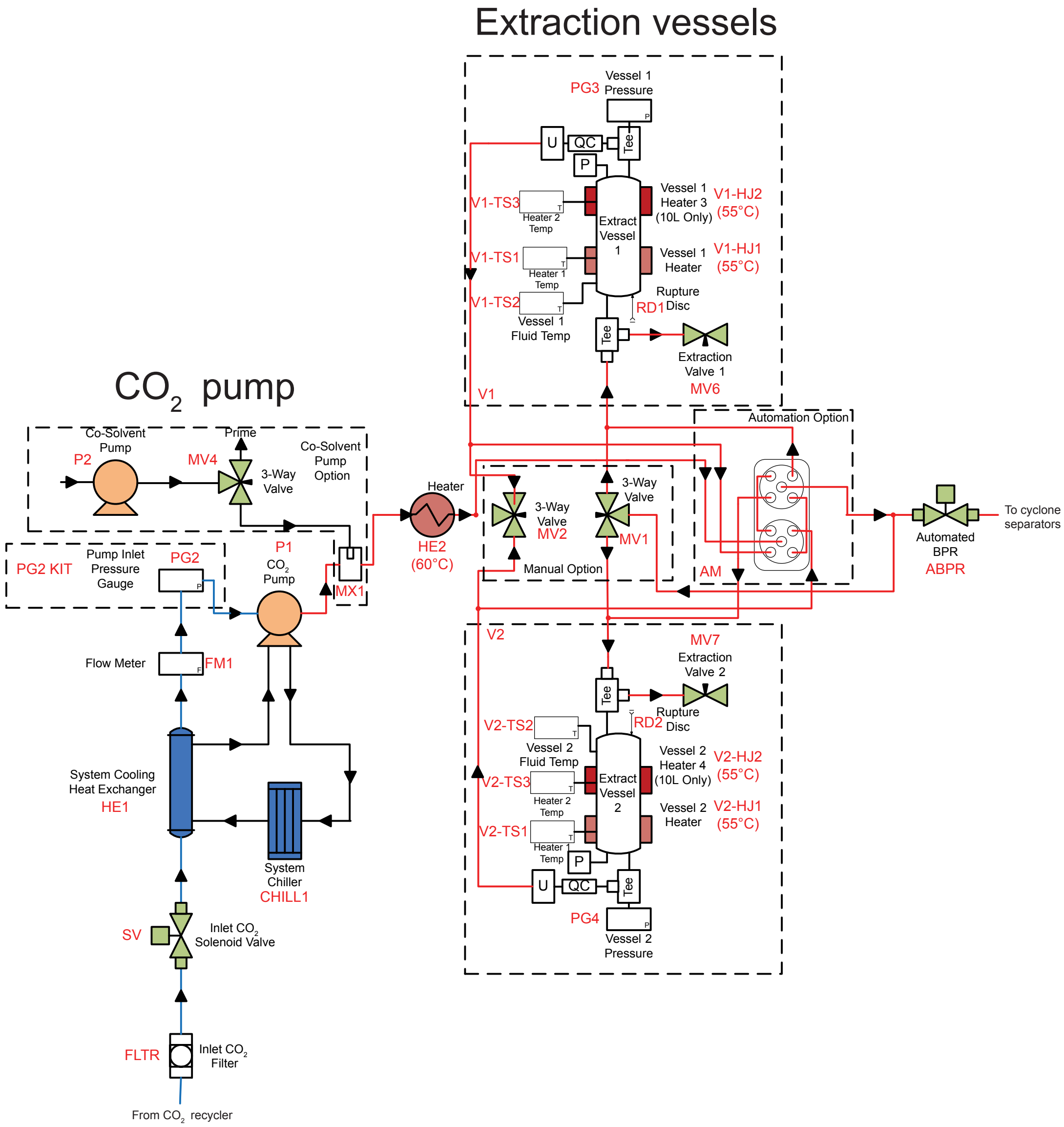


Figure 1–4: Cyclone separators flow path

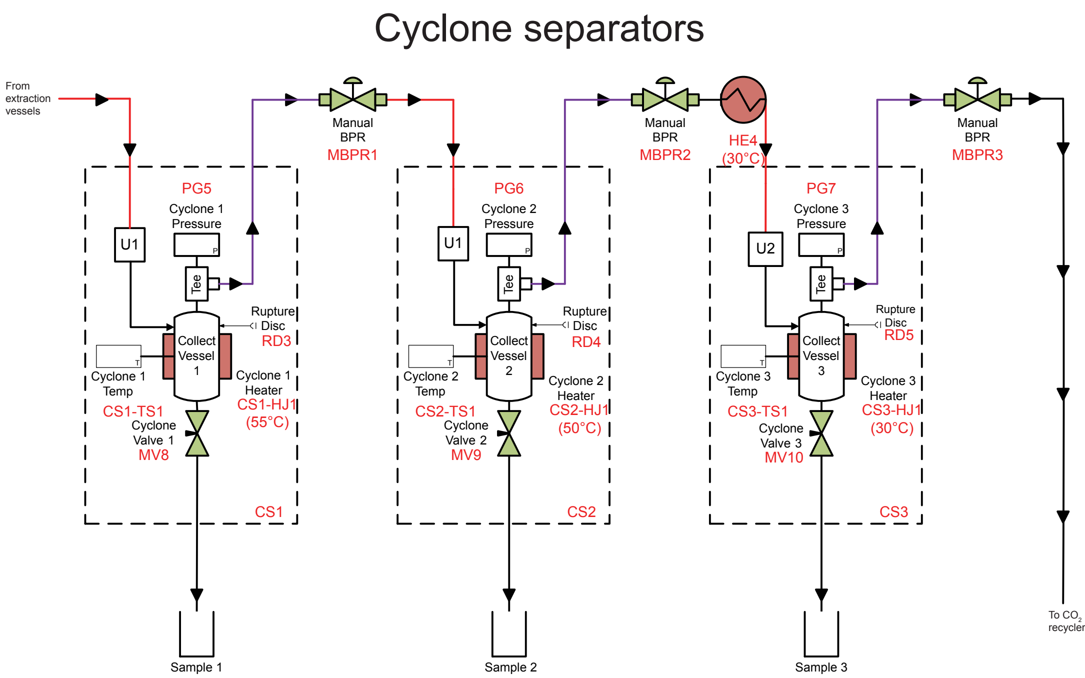
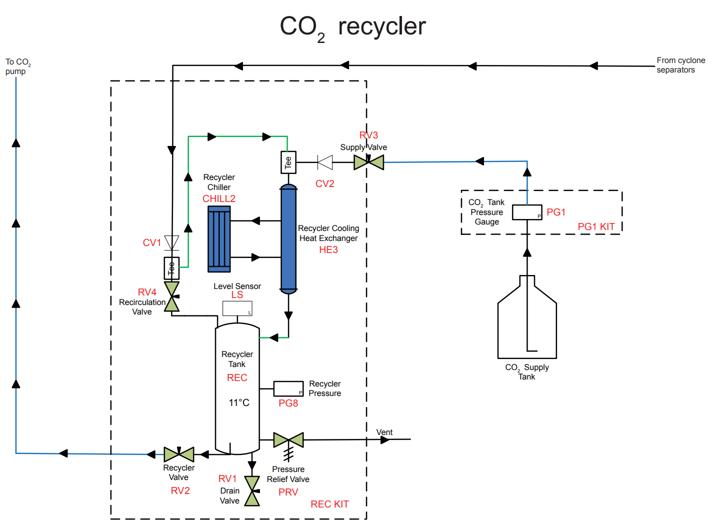


Figure 1–5: CO_2 recycler flow path

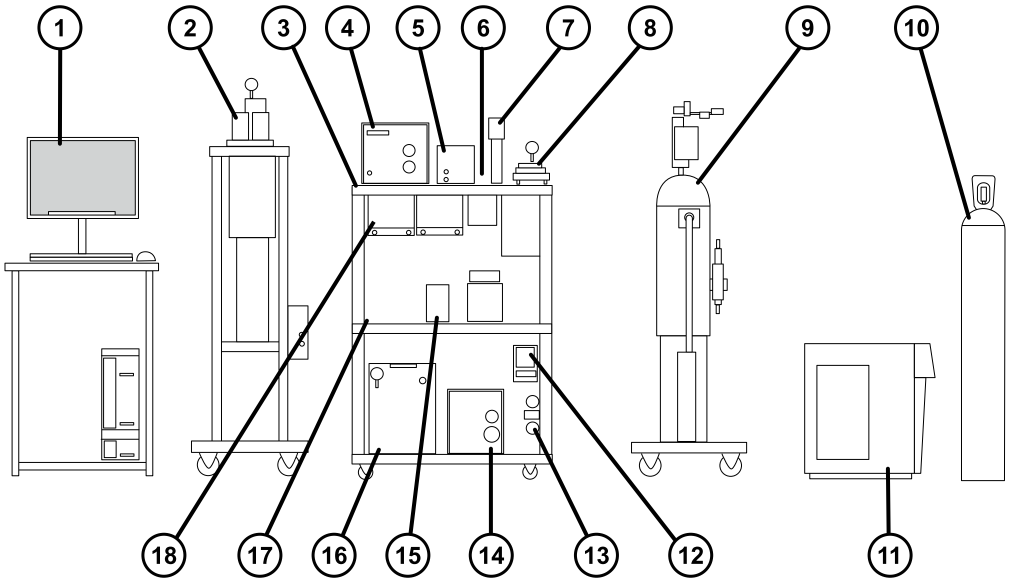


1.2 System components

The Waters SFE systems include skid-mounted, modular components. The specific components and options comprising a system depend on its configuration. Configurations vary to accommodate small-scale to large-scale extractions based on the size and number of extraction and collection vessels. The largest systems, Waters Bio-Botanical Extraction Systems (BBES), serve as bulk-scale, high-pressure separators for collecting extracted compounds from botanical samples. Below is an example of a BBES configured with a CO_2 recycler, one 5-L extraction vessel, and three cyclone separators.

See also: For information about proper component layout, refer to the *Bio-Botanical Extraction System Site Preparation Guide* (part number 715004529).

Figure 1–6: Example Bio-Botanical Extraction System components



1. ChromScope workstation – Runs ChromScope software, which enables fully automated instrument control and facilitates the process of developing SFE methods and purifying samples.

2. Extraction vessel – Stainless steel extraction vessel of 5-L volume (shown), equipped with a finger-tight cap, for simple opening and closing. The cap's seal is a polymide "C" cup fitted with a spring that is energized under pressure, forcing the seal's inner lip to contact the threaded cap. The seal's outer lip contacts the inner-vessel wall, forming a pressure seal. A cap at each end of the vessel, along with a seal, has a frit assembly, to provide even distribution of fluid during introduction. The vessel has a pressure gauge used for monitoring the internal pressure within the vessel's chamber. An electric heater-jacket encases the vessel and maintains the supercritical temperature of the extracting solvent within the sample matrix. A 10-L extraction vessel is also available, and a BBES can be configured with up to two extraction vessels, but both extraction vessels must be of the same volume. One is filled with bio-botanical material and extracted, while the other is emptied of extracted bio-botanical material and refilled with fresh bio-botanical material to prepare for the next extraction.

3 MV1 outlet valve – A 2-stem manual shutoff valve for systems equipped with manual switching valves and dual extraction vessels. During a run, the MV1 outlet valve is open from the extraction vessel to the ABPR, or closed, during periods of static soaking durations. The stem positions of the MV1 outlet valve and MV2 inlet valve control which extraction vessel is open or closed to the system flow. Additionally, after a run, with the system pumps shut off, the MV1 and MV2 valves allow siphoning of the remaining CO_2 flow from the pressurized vessel into the unpressurized vessel loaded with sample, to equalize the pressure between vessels and to reclaim CO_2 for the next run.

Tip: For systems configured with dual extraction vessels, to achieve the specified flow and pressure conditions uniformly within the sample matrix, perform runs using one vessel at a time. An exception to this case applies when the vessels are plumbed in series.

Note: Systems configured with a single vessel can be equipped with a single-stem MV1 valve that controls vessel outflow.

4 Automation module (optional) – Facilitates automatic switching between extraction vessels and allows unattended operation for two full extractions until collection (pulling) of the samples.

5 Automated back-pressure regulator – Uses an electronically controlled needle valve to pressurize the extraction vessel. The regulator is plumbed downstream from the extraction vessels, to maintain all components in the extraction flow path at the correct pressure. The outlet of the automated back-pressure regulator (ABPR) flows to the inlet of the first cyclone separator.

6 Inline collection heater – Heats the sample flowing from cyclone separator 2 (CS2) to cyclone separator 3 (CS3), which keeps the sample in a gas phase and ensures that there is no carryover to the CO_2 recycler.

7 Manual back-pressure regulator – Controls the working pressure in the cyclone separators, to prevent product loss during collection due to overly rapid expansion of CO_2. The system is configured with one manual back-pressure regulator (MBPR) per separator. On a system fitted with three cyclone separators, the manual back-pressure regulator of the first separator is set to the highest pressure. The second and third regulator valves are then set to create decreasing pressure steps that reduce the CS3 pressure to near atmospheric conditions. If using the optional CO_2 recycler, the CS3 pressure is set to approximately 5516 kPa (55 bar, 800 psi).

8 Cyclone separators – 2-L volume capacity fraction collectors that are plumbed in a serial flow. When the MBPRs are properly set, the cyclone separators collect, from the bottom of the vessels, compounds that precipitate from solution as the pressure across the three separators drops, and the CO_2 loses its solvating power. The flow path from the final separator exits to vent or an optional CO_2 recycler. The cyclone separators include an integrated splash guard and drain valves for collecting extracted compounds.

9 CO_2 recycler – Provides a continuous source of liquid CO_2 for the extraction system. During operation, the recycler reclaims the gaseous CO_2 that remains after the liquid or solid compounds collected from the extraction process are precipitated out in the cyclone separators. The recycler system consists of a storage vessel and a level sensor for carbon dioxide storage, a level-sensor module to display level, a condensing heat exchanger, a condensing cooling bath, and valves. The system also features a pressure relief valve that vents pressure in case of overpressurization or overfill conditions. Fill conditions in a system are dictated by operating conditions. Responding to the information appearing in the level-sensor module's display, the operator can switch the carbon dioxide supply to the system from the CO_2 source, or to the storage vessel in recycling mode. If the recycler is the automated model, the ChromScope software performs this function. For details and operating instructions, refer to the *CO_2 Recycler Overview and Maintenance Guide* that accompanies the system.

10 CO_2 tank – Supply cylinder for the CO_2 used in the system.

11 CO_2 recycler chiller – Condenses the CO_2 that flows to the CO_2 recycler. For product details and operating instructions, refer to the operator's guide that ships with the chiller unit.

12 Mass flow meter – Measures the liquefied carbon dioxide mass input to the high-pressure CO_2 pump. The feedback from the mass flow meter controls the pump and maintains an accurate and consistent CO_2 flow rate. For additional product details and operating instructions, refer to the mass flow meter's operator's guide.

13 System chiller – Provides a continuous supply of fluid at a constant temperature and volume. The unit consists of an air-cooled chiller unit, recirculating pump, coolant reservoir, and a microprocessor controller. Its primary function is to circulate temperature-controlled coolant through HE1 and the high-pressure CO_2 pump heads, and to cool and maintain the liquid state of the inlet CO_2, removing the heat of compression caused by pump operation. For product details and operating instructions, refer to the chiller unit's operator's guide.

14 P50 pump (optional) – Also referred to as the co-solvent pump. Used to pump co-solvent that acts as a modifier agent into the static mixing tee that combines the co-solvent with the CO_2 stream before it flows through the extracting solvent heat exchanger (HE2).

Note: When the P-50 pump is installed, the system also includes the static mixing tee and pump-priming valve (MV4).

15 Extracting solvent heat exchanger (HE2) – Heats the extracting solvent to a method-specified temperature before the flow enters the high-pressure extraction vessel.

16 P200 pump – Also referred to as the CO_2 pump. Pumps the liquefied carbon dioxide to the system. Its key features are as follows:

- Dual pump heads with cooling chambers for removing the heat of compression, to keep the CO_2 from converting to a gas as it is drawn into the pump
- A rupture-disc safety device, to prevent high-pressure damage to pump components
- A CO_2shutoff valve, which controls the flow of liquid CO_2 exiting the pump
- Control and monitoring of pump from the ChromScope workstation

17 MV2 inlet valve – A 2-stem, manual shutoff valve for dual extraction-vessel configurations. It serves to direct the extracting solvent flow to the designated extraction vessel for the run.

18 Temperature controller modules – Two 6-zone modules that control and monitor the temperatures of the mobile-phase heat exchanger and the electric heater-jackets that encase the extraction vessels and cyclone separators. The temperature set points and readings are controlled and monitored by the ChromScope software. Each controller can support as many as six independent control zones using standard J-type thermocouple inputs. Each zone requires its own thermocouple and control relay.

1.3 Safety practices and warnings

Observe Good Laboratory Practice when using the system. Always keep in mind these safety practices:

- Familiarize yourself with proper handling, storage, and disposal of all chemicals used with your SFE system. Refer to the Material Safety Data Sheet (MSDS) for each solvent you use, and know its chemical properties.
- Wear the appropriate personal protective equipment when working with any type of hazardous chemical.
- Wear eye protection while near the instrument to protect eyes from possible failure of valves or fittings.
- Wear hearing protection while venting the system to protect ears from loud noise owing to a high-pressure release of solvent.
- Ensure that the system is depressurized before loosening any fittings in the CO_2 flow path.
- Use care when loosening and opening the extraction and collection vessel caps. Loosening an in-line vessel's fitting with CO_2 in the system can cause a high-pressure release of sample and solvent.

Warning: To avoid explosion, do not use a highly flammable solvent as a cleaning agent for heated components. Leaks of flammable cleaning agents onto the heated vessel surfaces could cause flammable or explosive mixtures to form. The heated components can achieve temperatures as high as 90 °C. These temperature levels exceed the flash point of many flammable vapors. Avoid using any solvent for which the ignition temperature is in this range. Always inspect the system for leaks before operating it unattended.

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid burn injuries, exercise care when handling the extraction vessels, separation cyclones, heat exchanger outlet tubing, or other components heated to high temperatures or cooled to low temperatures. Wait until the hot components have sufficiently cooled or the cold components have sufficiently warmed before you handle them.

Warning: The system could potentially pose a rapid decompression hazard. Exposure to rapidly expanding fluids can cause injuries, including frostbite. To avoid this hazard, take the following precautions:

- Always depressurize the system before changing or working on the bulk CO_2 supply.
- Use only bulk CO_2 supply lines constructed of appropriate materials.
- To avoid a high-pressure release of sample and solvent, or damage to the vessel's cap, never use a wrench to force open a vessel cap. If the vessel's pressure-escape vent becomes plugged, the vessel can remain under pressure indefinitely. If you cannot easily remove the vessel's cap, allow the vessel to depressurize for at least 15 minutes, and then try again.
- To avoid the risk of a high-pressure release of solvent and extracted compounds, always use containers made of materials other than glass for collecting extracted compounds and waste.

Warning: To avoid injury when moving a system component, ensure that no objects are in the path of the cart wheels.

Warning: To avoid eye injury, always wear eye protection when working with components under pressure.

1.4 Typical system workflow

A typical workflow involves setting up the system and extracting and collecting compounds of interest. It comprises these tasks:

- Configuring settings for system modules, including their shutdown conditions
- Creating and loading extraction methods
- Preparing the sample and filling the extraction vessel
- Equilibrating the system
- Starting and monitoring the run
- Setting the manual back-pressure regulators to prepare for collection
- Collecting fractions
- Repeating the run with the second extraction vessel (if used)

You perform these tasks using ChromScope software and following the instructions in the online Help and system guide. Always observe Good Laboratory Practice (GLP) guidelines and your laboratory's standard operating procedures.

2 Component connections

2.1 Installation and operation requirements

You must plan well before moving the system to a different area, replacing its modules, or modifying your laboratory facility.

The topics in this section explain installation and operation requirements for the system. Refer to the "Specifications" section for additional system and component details.

Warning: To avoid spinal and muscular injury, do not attempt to lift a system module without assistance.

Warning: To avoid injury when moving a system component, ensure that no objects are in the path of the cart wheels.

2.2 Gas supply (liquid CO_2) requirements

The CO_2 must be industrial grade or better (depending on the application requirements) and supplied without the use of a regulator. CO_2 can be supplied from one of these sources:

- A pressurized cylinder equipped with a dip (eductor) tube, to pull liquid CO_2 from the bottom of a cylinder
- A BDS 500 gas delivery system
- An in-house supply of liquid CO_2 supplied at an ambient temperature of 18 to 24 °C and a minimum pressure of 5500 kPa (55 bar, 798 psi)

Depending on its configuration, a system can also include an optional CO_2 recycler system.

Warning: To avoid frostbite and other injuries associated with the sudden release of rapidly expanding fluids, observe these precautions:

- Depressurize the system before attempting to change or work with the bulk fluid supply.
- Exercise caution when opening fittings, to avoid being sprayed by rapidly expanding solvents.
- Fit supply lines made only of materials that can suitably withstand the highest pressure that the system can generate.

Table 2–1: Minimum requirements for CO_2 and its supply system

Item	Minimum requirement
Purity	Industrial grade
Supply cylinder	Dip-tube cylinder
Pressure	5500 kPa (55 bar, 798 psi)
Storage temperature	Ambient (above 15 °C)

2.3 Solvent requirements

Secondary solvents mixed with the primary solvent are called co-solvents and must be HPLC-grade or reagent-grade. Waters recommends using clean, high-purity, sub-micron-filtered solvents.

Many different organic solvents can be used in the system as long as they are miscible with supercritical CO_2 and compatible with the materials in the system's flow path. Those materials include types 316, 17-4, and 304 stainless steel, GFPM, Polyimide, Nitronic 60, Fluoropolymer, sapphire, ruby, and UHMWPE (optional).

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Notice: To avoid clogging the system, do not use water as a solvent in the mobile phase. At certain concentrations, water becomes immiscible with organic solvents and can separate and freeze.

Notice: To avoid damaging components in the system's flow path, do not use acidic solvents, particularly those containing any amount of hydrochloric acid.

Recommendation: When extracting compounds for edibles, Waters recommends using food-grade ethanol as the co-solvent.

2.4 Power requirements

Consider these power requirements when preparing your laboratory:

- All system modules require a dedicated, grounded power source, the receptacles of which must share a common ground and be accessible to all modules.

Warning: To avoid nonlethal electric shock caused by high current leakage, when installing or replacing the P-200 high-pressure pump, connect a secondary earth ground, or earth, to the rear of the pump chassis.

- The circulating bath requires a dedicated circuit.
- The individual modules, except for the circulating bath, relays, optional recycler system, heat exchanger between CS2 and CS3, and PC workstation, are connected to the system's attached power strip.
- Use a line conditioner or an uninterruptible power supply (UPS), for optimum long-term input-voltage stability.

For additional information about power requirements, refer to the *Bio-Botanical Extraction System Site Preparation Guide* (715004529).

2.5 Environmental requirements

2.5.1 General environmental requirements

Refer to these general environmental requirements when preparing the laboratory:

- Install the system in an environmentally controlled laboratory, in a draft-free location away from excessive amounts of dust.
- Do not locate the system directly beneath air-conditioning or heating ventilation.
- Do not locate the system in direct sunlight.
- The ambient relative humidity must be in the range of 20% to 80%, non-condensing.
- The system is intended for use in a well-ventilated area designed to accommodate the use of flammable solvents. The equipment must not be used in an environment capable of containing an explosive atmosphere.
- The ventilation system must provide an air exchange rate of four rooms per hour.

2.5.2 Temperature requirements

Refer to these temperature requirements and considerations when preparing your laboratory:

- The ambient temperature in the laboratory must be 18–24 °C.
- The optimum temperature range of the laboratory is 19–22 °C.
- Short-term thermal variations must not exceed 2 °C per 1.5 hours.

Important: Thermal variations in excess of 2 °C per 1.5 hours can adversely affect system performance.

Recommendation: To avoid significant thermal variations, do not locate the Bio-Botanical Extraction System in direct sunlight.

2.5.3 Vibration considerations

Do not locate the system close to heavy machines, such as compressors or generators, that can create excessive floor vibration.

2.5.4 Magnetic field considerations

Locate the system away from strong magnetic fields, such as those generated by NMR systems or magnetic-sector mass spectrometers.

2.5.5 Radio emission considerations

Locate the system in an environment where radio frequency (RF) emission from surrounding sources is minimal.

2.6 Safety considerations

Waters recommends the use of carbon dioxide monitors in laboratories in which the system is located. The monitors must be able to detect CO_2 levels to 10,000 ppm, and trigger an alarm at 5000 ppm.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

2.7 Venting and waste

CO_2 is toxic at levels above 10,000 ppm (PEL 5000 ppm). Additionally, it can displace air in a confined space, such as a laboratory. Ventilation for the system is therefore required. Plumb the exhaust tubing (not included with the system) to the laboratory's exhaust system, to properly vent gaseous CO_2 and the residue of solvent waste.

The system includes provisions for venting to a fume hood through a 6.4-mm O.D. compression fitting. Exhaust tubing connected to the fitting must be stainless steel and sized to allow unrestricted venting of the vapor. The system also includes provisions to vent the rupture discs to a hood or to the outside by 3/8-inch compression fittings attached to each rupture disc outlet. Tubing connected to each fitting should be of sufficient strength to withstand the pressures. Exhaust tubing is not included with the system.

Warning: To avoid explosion, do not use a highly flammable solvent as a cleaning agent for heated components. Leaks of flammable cleaning agents onto the heated vessel surfaces could cause flammable or explosive mixtures to form. The heated components can achieve temperatures as high as 90 °C. These temperature levels exceed the flash point of many flammable vapors. Avoid using any solvent for which the ignition temperature is in this range. Always inspect the system for leaks before operating it unattended.

Warning: To prevent death or injury in locations where CO_2 is used or stored install a CO_2 ambient-air sensor and alarm unit to comply with the permissible exposure limit for CO_2 prescribed by the US Occupational Safety and Health Administration. Install the CO_2 air sensor and alarm unit instead of, or in addition to, an oxygen monitor. The unit must be capable of detecting CO_2 levels to 10,000 ppm and issuing an alarm at 5000 ppm CO_2.

Warning: To avoid injuries caused by shattering glass fragments, do not use laboratory glassware to collect fractions or waste components. When opening drain valves, a high-pressure release of solvent or extracted compounds can knock a glass container out of your hand and cause it to shatter.

Notice: To avoid electrical damage to components from static charge build-up, ensure that the exhaust tubing is grounded.

2.8 Plumbing connections

A Waters technician equips and plumbs a system as part of its setup. All tubing connections between components are pre-fitted for their intended use. Extra fittings can be purchased in an accessories kit. If you need additional tubing or fittings, contact your Waters service representative for assistance.

Warning: To avoid injury from fittings that burst under high pressure, do not interchange ferrules and gland nuts between fitting types. Many fittings appear identical or similar. Use only the specified ferrule and gland nut for a fitting. If you are unsure about the nature of a particular type of fitting, contact your Waters service representative for assistance.

Warning: To avoid injuries from flailing tubing, ensure that tubing is secured at both ends before pressurizing the system.

Warning: To avoid injury from tubing that bursts under high pressure, use only tubing that can suitably withstand the highest pressure the system can generate. If you are unsure about the pressure rating of a particular type of tubing, contact your Waters service representative for assistance.

Notice: To avoid leaks caused by improper tightening of fittings, when making tubing connections or tightening their fittings, follow the fittings manufacturer's tightening instructions. Replace the tubing or fittings when proper tightening fails to stop the connection from leaking.

2.9 Electrical considerations

The system's operating voltage is configured at the factory to operate at on the standard line voltage for your location. If you move the system to an area where the line voltage differs from that of the original location, refer to the system's site preparation guide for the specific requirements for your location.

Before powering-on the system modules, ensure that all power cords are connected to appropriate sources of power. Insert the power cords only into socket outlets provided with a protective ground (earth) contact. Any interruption of the protective grounding conductor can result in a fault condition that can cause personal injury or death. All wiring constituting the ac supply must meet local electrical codes.

Warning: To avoid a fault condition that can result in personal injury or death from electric shock, do not disconnect or otherwise interrupt the continuity of an electrical socket's protective grounding conductor or the power cord's grounding conductor.

Warning: To avoid electric shock, observe these precautions:

- Use SVT-type power cords in the United States and HAR-type power cords, or better, in Europe. For requirements elsewhere, contact your local Waters distributor.
- Inspect the power cords for damage, and replace them if necessary.
- Power-off and unplug each module before performing any maintenance operation on it.
- Connect each module to a common ground.

Warning: To avoid electric shock, always disconnect the system from its source of electrical power before connecting or disconnecting external cables.

2.10 Connecting to the ac power source

Each module contains its own internal power supply. Connect the system power connections to an appropriate ac power source. For modules with On/Off switches, the switch is located on the rear panel of each module. To start the system operating, power-on each of its modules individually.

Requirement: You must wait at least 30 seconds after you power-on the last module before you start the ChromScope software. This waiting period is necessary for the modules to communicate with the software during start-up.

3 Getting started with ChromScope software

3.1 Starting ChromScope software

To start and log on to the ChromScope software, you must be a registered user, and you must provide your user name and password.

Exception: If the administrator disables the Secure System function, the ChromScope Login window is bypassed, allowing anyone, not only registered ChromScope users, to start and run the software.

Important: To enable communication between the software and system modules, ensure that the pumps and other hardware components are powered-on for at least 30 seconds before you start the software.

To start ChromScope software:

1. From the Windows desktop, double-click the ChromScope shortcut .

   Alternative: From the Windows **Start** menu, click **All Programs**, and click **ChromScope**.

2. Enter a valid user name and password.

   Requirement: Both are case-sensitive. If you do not know your user name and password, inform your system administrator.

   Requirement: When the software initially opens, you must provide these default settings:

   - Login: Administrator
   - Password: waters

3.2 Exiting from ChromScope software

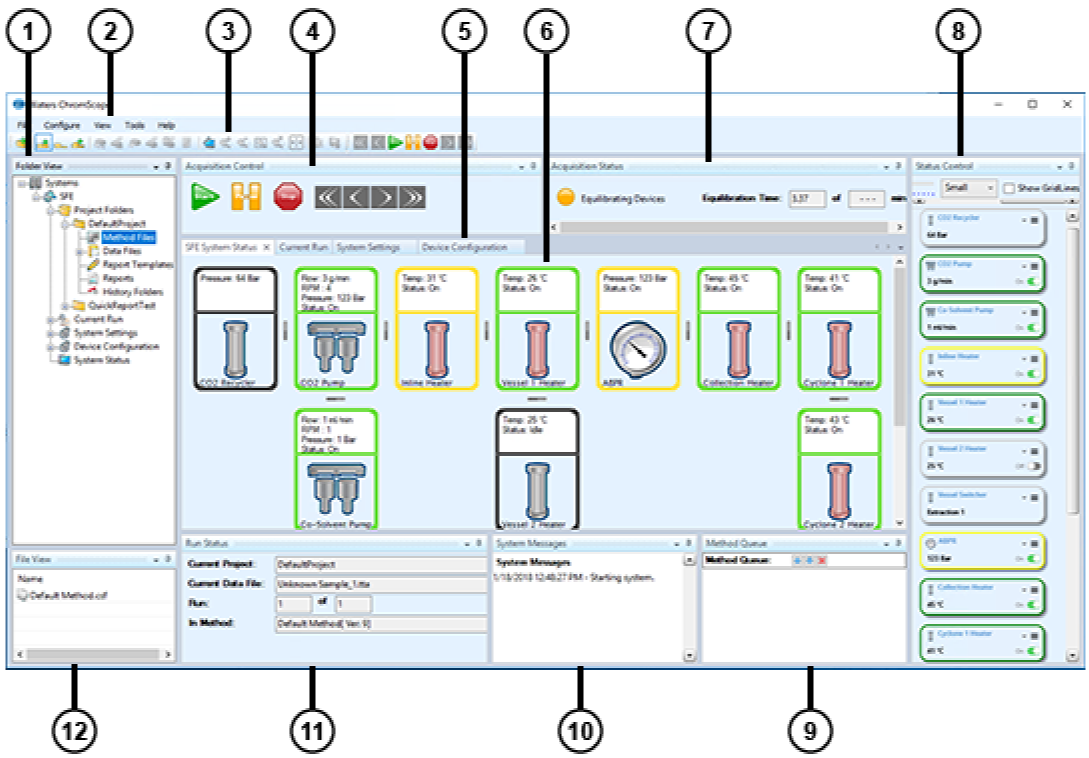
To exit from the ChromScope software:

1. From the **File** menu, click **Exit**.

3.3 ChromScope software interface

The default ChromScope main window shows projects, the system's status, and the configured modules. The window includes a system-status panel for monitoring system modules and a status-control panel from which you manage modules and manually control the application. From the Status Control panel's options menu, you can click **Configure** to change a module's device settings. You can customize the main window to display or hide individual panels, or to change their display format, according to user preferences.

Figure 3–1: ChromScope main window



1. Nodes in folder view provide controls and options for the files within it
2. Menu bar
3. Toolbar
4. Acquisition Control panel
5. Tabbed panels open in this area from the Folder View or File View panels to be viewed or edited.

6 System Status panel shows each module's status, at a glance

7 Acquisition Status panel

8 Status Control panel provides both running status and set-point boxes that you can use to monitor and manually control individual modules

9 Queue of pending methods

10 System Messages box lists system messages and shows the status of individual modules when you move the pointer over the module's representation in the System Status or Status Control panels

11 Run Status panel showing current run information

12 File-list view area displays the files listed in the selected project's subfolder node

The ChromScope software provides simple workflows for developing sample lists and running extractions. It also provides instrument control and automation and reporting. The software includes the key components and functions shown in the following table.

Table 3–1: ChromScope key components and functions

Component or function	Purpose
Account manager	Adds user accounts, as needed, so that all changes and generated data are digitally signed by the user performing the work.
Project manager	Groups data-log files, sample files, templates, and reports (PDF files) into logical categories under the project's name.
Navigation tree	Quickly accesses frequently chosen functions and software files that appear in the Folder View and File View panels.
Menu and toolbar	Provides logical access to most ChromScope functions.
Custom viewing and file opening options	Specify these user preferences: • Change the size of different display panels in the application window by dragging the split bar between different display panels (areas). • Change the ChromScope main window layout to enhance the visibility of desired elements, such as the current run status information. For details, see Customizing your ChromScope window layout. • Open data-log files in a **new** tab, **current** tab (replace), or **overlay**. • Open active plots in a separate window that offers an expanded view. You can also view all graphs of a file in a separate window.

Table 3–1: ChromScope key components and functions (continued)

Component or function	Purpose
Method editor	Specify and save the parameter settings of temperature, flow, pressure, and timing (static and dynamic). Later, you can edit or modify the methods, as needed, load the methods into a queue, and then run them to perform extractions or purify sample.
System Status, Current Run, and Status Control panels	Monitor performance, configure settings, perform diagnostic tests, and maintain the system and its modules. In the Current Run panel, you view real-time plots for temperature, flow, and pressure activities.
Reporting	Prints custom or quick reports to a PDF file or an installed printer.
Batch process	Prints reports of groups of data-log files in batch mode.
Exporting	Exports raw data from the data-log files to MS Excel and CSV formats.
Copy and Save	Copy plot images to the clipboard or save plots to a file.

3.4 Customizing your ChromScope window layout

You can customize the ChromScope main window layout according to your preferences to display or hide individual panels or to change their format. For example, you can reposition or hide individual panels within the ChromScope main window, as needed, to maximize the space available for display of the plots or chromatograms from the current run.

From an individual panel's options menu, you can change its layout by selecting any of the following options:

- **Float** – Allows you to move and resize the panel in various locations of the ChromScope main window.
- **Dock** – Opens the panel according to its last layout.
- **Dock as Document** – Opens the panel in the tabbed area.

  Tip: When a panel is docked as a document in the tabbed area, you can right-click on it to select additional display characteristics, including having it open in a new horizontal or vertical tab group.
- **Auto Hide** – Closes the panel from view and allows you to quickly redisplay it by clicking on the panel's name, which appears along the margin of the ChromScope main window.
- **Close (X)** – Closes the panel or tab from view. To redisplay the panel or tab, from the **View** menu, click **Show**, and then click the name of the panel or tab that you want to redisplay.

  Alternative: To redisplay a closed panel or tab, from the navigation tree in the Folder View panel, click the panel's node or the tab's folder, respectively.

Tip: You cannot close any panels or tabs that you cannot open from the Folder View panel.

Important: To reset the panels to their default layouts, from the **View** menu, click **Load Views** > **Default Views** > **DefaultUI**.

Additional customization features:

- Enlarge or shrink the subpanels that make up the Status Control panel to maximize the space available for display of the subpanels in a particular layout or view. You can also reposition them within the panel individually, or as a group, according to your preferences.
- Edit and save customizations to the user-interface layout as individual views, which you can later select and load, as needed, to change the user-interface layout.
- Lock or unlock the main window to prevent or allow customizations to the user-interface layout.
- Lock or unlock the Status Control panel to prevent or allow customizations to the Status Control panel's user-interface layout.

3.4.1 Repositioning a panel or tabbed component within the ChromScope main window

You can customize the ChromScope window layout, or view, according to your preferences by moving any of the individual panels or tabbed components that make up the ChromScope main window to a new "docked" location. When you do so, the new layout persists until it is changed again or is overridden by loading a different view.

Exception: Except for reports and report templates, components that open from the File View panel, including methods, sample lists (for SFC and LC systems), data-log files, and history records, are intended to open as tabbed components within the "editor panel", which in the ChromScope default layout view, is in the center area of the main window. Although you can reposition any of the opened files or tabbed components out of the editor panel to another location in the ChromScope main window, after closing these files, any files that you subsequently open, do so in the editor panel, instead of the new location. Accordingly, use care when repositioning or resizing the editor panel so that it can fully display these files for viewing or editing.

Requirement: The lock/unlock control for the ChromScope main window must be set to unlock.

To reposition a panel or tabbed component, do as follows:

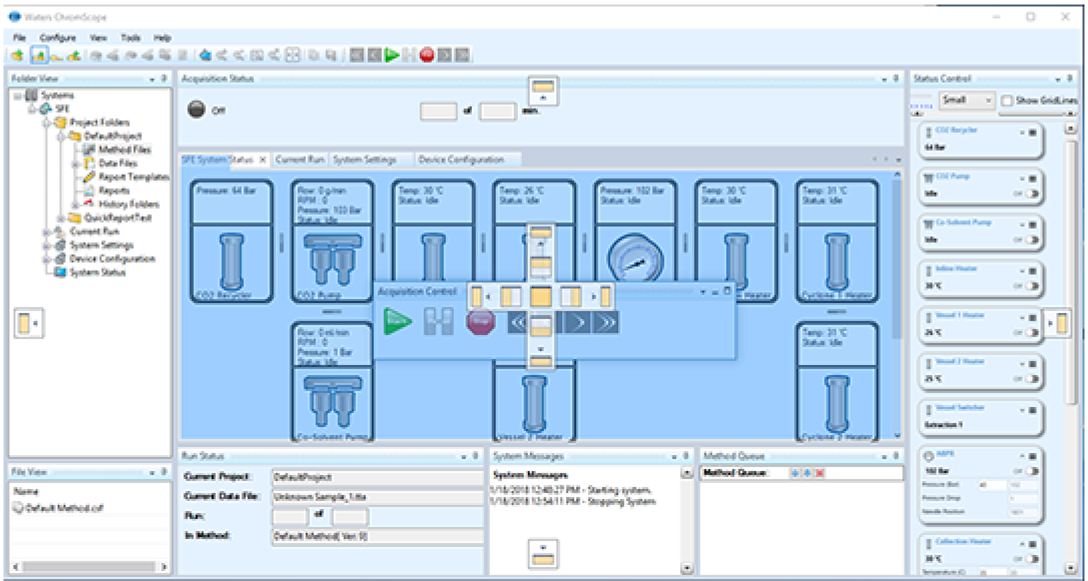
1. From the individual panel's or tabbed component's options menu, select **Float**.

   Result: The panel or component s temporarily resized so that you can easily move it over a repositioning guide that indicates its new location.

2. Click within the panel's title bar and drag it slightly to reveal the repositioning guides on the ChromScope main window.

Alternative: In place of steps 1 and 2, click on the panel's title bar or component's tab and drag it slightly to reveal the repositioning guides on the ChromScope main window.

Figure 3–2: Acquisition Control panel set to Float, showing the repositioning guides on the ChromScope main window



3. Continue dragging the panel or tabbed component onto the repositioning guide that indicates its new location, and then release it.

   Note: The repositioning guide becomes active when you drag the panel or component onto it.

4. Resize the repositioned panel or component, as needed.

   Tip: To prevent unintended changes to the newly customized layout, lock the ChromScope main window layout by clicking **View** > **Lock Main UI Control**.

3.4.2 Resizing and repositioning the subpanels within the Status Control panel

You can enlarge or shrink the subpanels that make up the Status Control panel to maximize the space available for display of the subpanels in a particular layout or view. You can also reposition them within the panel individually, or as a group.

Requirement: You must set the lock/unlock controls for the Status Control panel and the ChromScope main window to unlock.

To resize the Status Control subpanels, do as follows:

1. Open the Status Control panel and, if necessary, reposition it within the ChromScope main window.

See also: Repositioning a panel within the ChromScope main window.

2. At the top of the Status Control panel, use either of these techniques, according to your preference, to resize the subpanels:
   - Click the box next to the **Resize Bar** and select **Small**, **Medium**, or **Large**.
   - In the **Resize Bar**, click and drag the slide control to shrink or enlarge the subpanels.

   Result: Each of the subpanels adopts the new size characteristic, which persists until they are resized again.

3. Reposition the subpanels within the Status Control panel using these techniques:
   - To align the subpanels along a vertical or horizontal grid, right-click in the Status Control panel and select **Align Vertical** or **Align Horizontal**.
   - To reposition the subpanels individually, click and drag each subpanel to its new location.

   Tip: To display a grid for repositioning the individual panels, select the **Show Grid Lines** check box.

   Result: The subpanels retain their new positioning, which persists until they are repositioned again.

   Tip: To prevent unintended changes to the newly customized Status Control panel, at the top of the Status Control panel, click **Lock**  to lock the panel.

3.4.3 Saving a view

You can save the current layout of the ChromScope main window as a new view, or as an update to a previously saved view.

To save the current ChromScope user-interface layout, do as follows:

1. From the **View** menu, click **Save View**.
2. In the Save View dialog box, type the *name* of the new view, or select an existing view that you want to update with the current main window layout characteristics.
3. Click **OK** to save the view.

3.4.4 Loading a view

You can load a previously saved view to change the layout of the ChromScope main window.

To load a view, do as follows:

1. From the **View** menu, click **Load Views**.
2. In the Load Views dialog box, select an existing view that you want to load.

3. Click **OK** to load the view.

4. Click **Yes** to accept the new ChromScope layout.

3.4.5 Locking the ChromScope window or Status Control panel layout

You can prevent users from unintentionally modifying the ChromScope main window or Status Control panel layouts by locking the controls for these layouts. Doing so prevents customization of the window or the panel until you unlock the layout controls.

3.4.5.1 Locking the ChromScope main window layout

To lock the ChromScope main window layout, do as follows:

1. From the **View** menu, click **Lock Main UI Control**.

   Result: Changes to the ChromScope main window layout (including the Status Control panel) are prevented until you unlock the control by clicking **View** > **Unlock Main UI Control**.

3.4.5.2 Locking the Status Control panel layout

Requirement: To lock or unlock the Status Control panel layout, the ChromScope main window layout control must be unlocked.

To lock the Status Control panel layout, do as follows:

1. From the **View** menu, click **Lock Status Control**.

   Result: Changes to the ChromScope Status Control panel layout are prevented until you unlock the control by clicking **View** > **Unlock Status Control**. You can still make other changes to the ChromScope main window layout.

   Alternative: At the top of the Status Control panel, click the **Lock/Unlock** or icon to lock or unlock the Status Control panel layout.

3.5 Project management

In the ChromScope Folder View panel, the Project Folders node provides access to all defined projects and their associated files. Within the node, a sub-node structure for each project stores methods, data-log files, report templates, reports, and history records.

You can create a new project or choose to work with existing projects. When you create a new project in the system, a project folder with the project name appears under the Project Folders root node. Inside the project folder, ChromScope software creates a subfolder structure to organize each file type that exists in the project. Click any of these sub-nodes to display the files of that type in the file-list view, or right-click a sub-node for more options.

The table "ChromScope project subfolder structure" describes each of the project's subfolders and the files they contain.

Table 3–2: ChromScope project subfolder structure

Subfolder	Description
Method Files	Contains the methods used to define run conditions.
Data Files	Contains the data-log files that store data, plots, and associated method information from previous runs. It can have a subfolder for organizing the data-log files.
Report Templates	Contains the Quick Report template and any custom report templates.
Reports	Contains the reports saved as PDF files.
History Folders	Contains the history records for each session that ChromScope automatically logs. These records, organized by date, provide a running log of system activities.

These are the options for setting up a new project:

- Define a subfolder-naming convention that organizes all of a project's data-log files by date, sample name, or user name.
- Import files from another project.

4 Administration

4.1 User management

As a ChromScope system administrator, you can use the Account Management function to accomplish these tasks:

- Control access to the ChromScope software functions by establishing and maintaining user accounts.
- Enforce limits on what ChromScope components a user can see and control by assigning the user specific component rights.
- Choose whether the ChromScope software runs in a secured or open environment.

If you elect to run the software in a secured environment, the system security and audit trail functionality provide these features:

- You prevent users from operating the system until they successfully log in using a registered user account.
- After a user logs into the system, the user-name entered becomes attached to all data-log files generated by the system during that session. In addition, reports generated from data recorded during the session include user name information for both the user who generated the data file and the user who created the report.

Rule: Users who are not also system administrators, can use the Account Management Current User Information window only to view their personal user account information and change their passwords.

Restriction: Only an administrative user can set the Secure System preference and create, modify, or delete a user account.

4.1.1 Specifying security preferences

1. From the **Configure** menu, click **Account Management** > **Secure System or User Manager**.
2. Specify whether the software will operate in a secured or unsecured environment by selecting or clearing the **Secure System** check box.

   Important: When you clear the check box, the log-in screen is hidden, and audit trail functionality for generated logs or reports is disabled.

4.1.2 Creating a user account

1. From the **Configure** menu, click **Account Management** > **User Manager**.
2. In the User List area of the User Manager window, right-click, and then click **Add User**.
3. If you want to assign the new user administrator privileges, select the **Administrator** check box.
4. Enter the new user's authentication information, and then click **OK**.

4.1.3 Configuring a user account

1. From the **Configure** menu, click **Account Management** > **User Manager**.
2. In the User Manager window, select the user from the user list, and click **View Components**.
3. In the User Information window, click the **Component Type** list to add, modify, or remove component rights for the user.
4. To change the user's login password, click **Change Password**.

4.1.4 Removing a user account

1. From the **Configure** menu, click **Account Management** > **User Manager**.
2. In the User Manager window, right-click the user name in the user list, and then click **Delete User**.

5 System configuration and methods

5.1 Viewing or modifying the system configuration

From the System Configuration panel, you can view all of the system's configured devices and, when necessary, update them to mirror the addition or removal of optional devices you installed on, or removed from, the system.

For example, with a Prep SFE system, you could add the vessel switcher with CO_2 recycler or change the number of collection vessels the system uses for the product you are extracting.

To view or modify system devices:

1. From the **Configure** menu, click **System Configuration**.
2. In the upper box, view the present configuration, which lists serial numbers and firmware versions for devices with these features.
3. To update the devices in the System Configuration panel to mirror the addition, or removal, of optional devices you installed on, or removed from, the system, click **Add** or **Remove**, as needed, to change the optional devices that appear in the **Attached Optional Devices** box.
4. If you modified your system configuration:
    a. Click **Apply** to save your changes.
    b. Close and then reopen the ChromScope software to validate the update process.
5. If you made no changes to your system configuration, click **Close**.

5.2 System and module settings

In the System Settings and Device Configuration panels, you define your preferences for shutting down the system in response to certain operating conditions and for configuring the individual module's alert thresholds and usage settings. You can also view a module's real-time device status for maintenance or diagnostic purposes.

5.2.1 Specifying the system shut-down and equilibration conditions

1. In the Folder View panel, click the **System Settings** node to open the System Settings panel.
2. Click the **Start/Shutdown Options** subpanel to display the system start-up and shut-down settings.
3. In the Start/Shutdown subpanel, configure the system referring to the following tables.

Table 5–1: System shut-down options

Setting or control	Purpose
Stop system when a device returns a warning	Initiates a system shutdown when a module issues a warning. Clear the check box to allow the system to continue operating when a module issues a warning.
Stop system when a device becomes unequilibrated	Initiates a system shutdown when events, like an empty solvent bottle or CO_2 supply tank, prevent the system from maintaining a specified set point. For example, if this option is enabled and the CO_2 flow becomes restricted, the system fails to maintain the specified pressure set point and initiates a system shutdown. Clear the check box to allow the system to continue operating whenever it fails to maintain a user-specified set point.
Stop system if the vessel pressure drop exceeds *x* bar	Initiates a system shutdown whenever the system detects a pressure drop exceeding the level specified by this option. Before specifying a value for pressure drop, consider the consistency of the sample material you are extracting and the run's flow rate. For example, separations of viscous sample material or those that run at very low flow rates tend to exhibit more pressure fluctuation than those unaffected by such conditions. This is a required entry, and the maximum pressure drop you can specify is 60,000 kPa (600 bar, 8702 psi). **Recommendation:** Waters recommends a "stop system" vessel pressure drop level of 15,000–20,000 kPa (150–200 bar, 2176–2901 psi).

Table 5–1: System shut-down options (continued)

Setting or control	Purpose
Depressurize system to end pressure *x* bar over a duration of *x* min	Depressurizes the system, according to specified conditions, after the last run ends and the sample list queue clears. Before specifying the pressure according to which the system depressurizes, consider the effects of temperature change on the sample material and specify appropriate values for end-pressure and time duration. Alternatively, clear the check box if you do not want to set the conditions for depressurizing the system after a run ends. **Important:** • During depressurization, the system pressure does not always achieve the specified end-pressure setting because the ABPR cannot reduce the system pressure below the level established by the MBPR1. In this situation, specifying an end-pressure below that set by the MBPR1 has no effect. • Generally, the shorter the duration you specify for depressurization, the more quickly you allow the liquid CO_2 to convert to gas, which increases the temperature drop to which the sample is exposed. Accordingly, due to the increased volume of CO_2, allow more time to depressurize larger extraction vessels. **Tip:** If you use this option to set the end pressure, you can also depressurize the system at any time to these conditions by clicking **Depressurize System** from the **Tools** menu.
Leave devices running at the end of a run	Select this check box to enable modules to continue running at sample list conditions at the end of a run. Alternatively, clear this check box to allow modules to go to a Standby state ("Off") at the end of a run. **Recommendation:** Waters recommends selecting this check box to keep the inline collection heaters running until you power them down.
Leave extraction vessel heaters running at the end of a run	Select this check box to maintain the temperature in the vessel heaters at the specified set points in the run.

Table 5–2: System equilibration options

Setting or control	Purpose
Pressure Equilibration Band (+/-)	For all system modules with system pressure set points, specifies the amount of variation, in bar, that the actual pressure reading can deviate from its specified set point before the module attains equilibration and maintains its ready state (indicated by green in the ChromScope software).
Temperature Equilibration Band (+/-)	For all system modules with temperature set points, specifies the amount of variation, in degrees Celsius, that the actual temperature reading can deviate from its specified set point before the module attains equilibration and maintains its ready state (indicated by green in the ChromScope software).
System Equilibration Timeout	For all system modules, specifies the maximum time duration, in minutes, that is allowed for a module to equilibrate to its set points and attain its ready state before the software triggers an alarm and initiates a system shutdown. **Note:** When specifying the System Equilibration Timeout, consider the size vessels you are heating. Larger vessels generally require more time to equilibrate than smaller ones.

4. Click **Apply** to save your system shut-down and equilibration settings.

5.2.2 Specifying settings or viewing device status for the CO_2 recycler

For systems that include an automated vessel switcher and CO_2 recycler, you can specify device settings and view device status for the CO_2 recycler in the Vessel Switcher configuration subpanel under the Device Configuration node. For details, see "Specifying settings or viewing device status for the vessel switcher".

5.2.3 Specifying settings or viewing device status for the CO_2 pump

In the Device Configuration panel, you can specify device settings, such as the high-pressure alarm or view the pump status.

To specify settings and view device status for the CO_2 pump:

1. In the Folder View panel, click the **Device Configuration** node to open the Device Configuration panel.
2. Click **CO2 Pump** to open the CO2 Pump configuration subpanel.

Alternative: In place of steps 1 and 2, in the Status Control CO2 Pump subpanel's options menu, click **Configure** to open the CO2 Pump configuration subpanel.

3. In the CO2 Pump configuration subpanel, view the device or pump status and configure device settings referring to the tables "CO_2 pump: device status" and "CO_2 pump: device settings".

Table 5–3: CO_2 pump: device status

Status item	Indicates
Device Status	The operating mode of the pump. During normal operation, and during the period prescribed by the Dynamic Duration sample list setting, the device status indicates that the pump is operating in the flow-control mode. When the pump is running during the period prescribed by the Static Duration sample list setting, the device status indicates that the pump is operating in the pressure control mode. **See also:** "Mode" under Device Settings below.
Flow	The flow rate of the pump in g/min.
Pressure	The pump's current pressure in bar.
RPM	The operating rate of the pump. **Tips:** • A high pump RPM can mean that the CO_2 supply in the CO_2 recycler is below the minimum required for operation. Check and fill the recycler with fresh CO_2. • If the pump RPM is high and you have confirmed that the CO_2 recycler still contains CO_2, check the mass flow sensor density. If the density is below -.88, add more CO_2 to the system from the recycler source. • When a pump's check valve fails, the pump's outflow decreases, causing it to run faster in order to maintain the specified flow rate. If the flow rate is significantly lower than the pump RPM, one or more check valves may need to be replaced. Other possible problems include restriction on the inlet, leaking piston seals, and a low supply of CO_2. • If the cooling heat exchanger or chiller are not performing properly, the pump will require a higher RPM to deliver the specified mass flow. If you suspect a low CO_2 supply is causing this, based on the pumping rate, check the mass flow sensor temperature and density. If the mass flow sensor temperature is normal (below 12 °C) and its density is low, the CO_2 supply may be low. If the mass flow sensor temperature is high (above 12 °C), the chiller or cooling heat exchanger may require service.

Table 5–4: CO_2 pump: device settings

Setting or control	Purpose
Mode 	If you are not running a sample list and are manually controlling the CO_2 pump from the Status Control panel, you can select either **Flow Mode** or **Pressure Mode**. **Important:** The flow mode is the pump's normal mode, used when pumping CO_2 through the system at a designated flow rate. The pressure mode is an alternative mode that enables the pump to maintain the user-specified pressure, instead of a specified flow rate. Under manual control of the system, you typically select, via the CO2 Pump configuration panel, the pressure mode only during the Static Duration period, when the extraction vessel's outlet valve is closed and the pump maintains a set pressure within the vessel. Under sample list control of the system, the ChromScope software automatically makes the selection from the flow mode to the pressure mode, and vice versa.
Maximum Set Flow	Specifies the flow-rate threshold above which the software's flow-rate control function issues an alarm and shuts down the pump.
Maximum Set Pressure	Specifies a limit for the maximum-pressure set point specifiable for the pump.
High Pressure Alarm	Specifies the pressure threshold above which the software's pump-pressure control function issues an alarm and shuts down the pump. **Recommendation:** Set the alarm pressure threshold at 5000 kPa (50 bar, 725 psi) above the maximum extraction pressure. **Notice:** To avoid the risk of a high-pressure burst of the pump's rupture disc, set the alarm pressure threshold below 40,000 kPa (400 bar, 5802 psi).
Flow Control Settings	For use by Waters service technicians only.
Pressure Control Settings	For use by Waters service technicians only.
Apply	Saves all new device settings for the CO_2 pump.

5.2.4 Specifying settings or viewing device status for the co-solvent pump

In the Device Configuration panel, you can configure co-solvent threshold and usage settings and view the pump's device status.

To specify settings or view device status for the co-solvent pump:

1. In the Folder View panel, click the **Device Configuration** node to open the Device Configuration panel.
2. Click **Co-Solvent Pump** to display the pump's device status and level settings.

   Alternative: In place of steps 1 and 2, in the Status Control Co-Solvent Level subpanel's options menu, click **Configure** to open the Co-Solvent Pump configuration subpanel.
3. In the Co-solvent Pump configuration subpanel, view the pump status and specify settings as follows.

Table 5–5: P-50 co-solvent pump: device status

Status item	Indicates
Operating mode device status	The operating mode of the pump. During normal operation, and during the period prescribed by the Dynamic Duration sample list setting, the device status indicates that the pump is operating in the flow-control mode. When the pump is running during the period prescribed by the Static Duration sample list setting, the device status indicates that the pump is operating in the pressure-control mode. **See also:** "Mode", under Device Settings, below.
Flow	The flow rate of the pump, in mL/min.
Pressure	The pump's current pressure, in bar.
RPM	The operating rate of the pump. **Tip:** When a pump's check valve fails, the pump's outflow decreases, causing it to run faster, to maintain the specified flow rate. A typical pump's flow rate (mL/min) is slightly under or equal to the pump RPM, depending on the output pressure. If the flow rate varies significantly from the pump RPM, you may need to replace one or more check valves. Other possible problems include leaking piston seals and a low supply of co-solvent.

Table 5–6: P-50 co-solvent pump: device settings

Setting or control	Purpose
Mode 	If you are not running a sample list and are manually controlling the co-solvent pump from the Status Control panel, you can select either the **Flow Mode** or **Pressure Mode**. **Important:** The flow mode is the pump's normal mode, used when pumping co-solvent through the system at a designated flow rate. The pressure mode is an alternative mode that enables the pump to maintain the user-specified pressure, instead of a specified flow rate. When you manually control the system, you typically select the pressure mode, via the Co-Solvent Pump configuration panel, only during the Static Duration period, when the extraction vessel's outlet valve is closed and the pump maintains a set pressure within the vessel. When the sample list controls the system, the ChromScope software automatically makes the selection from the flow mode to the pressure mode, and vice versa.
Maximum Set Flow	Specifies the flow-rate threshold above which the software's flow-rate control function issues an alarm and shuts down the pump.
Maximum Set Pressure	Specifies a limit for the maximum-pressure set point specifiable for the pump.
High Pressure Alarm	Specifies the front-end pressure threshold above which the software's pump-pressure control function issues an alarm and shuts down the pump.
Flow Control Settings	For use by Waters service technicians only.
Pressure Control Settings	For use by Waters service technicians only.
Apply	Saves all new device settings for the co-solvent pump.

5.2.5 Specifying settings or viewing device status for the heater controller modules

Depending on a system's configuration, it can include as many as two 6-zone heater controller (CN6) modules. When two modules are provided, you configure and view device status for the second heater-controller module on the Heater Controller 2 configuration subpanel. Each panel presents settings and device status for zones 1 through 6 that correspond to input/output-paired connections on the module's rear panel. Typical assignments for these zones include the electrical vessel heaters and in-line heat exchanger. Zones that are not assigned a heating role have the **Enabled check box** cleared.

Tip: Unassigned zones may have additional temperature-related equipment connected and be activated for diagnostic purposes.

To specify settings or view device status for the heater controller:

1. In the Folder View panel, click the **Device Configuration** node to open the Device Configuration panel.
2. Click **Heater Controller** or **Heater Controller 2** to open the configuration subpanel.

   Alternative: In place of steps 1 and 2, in any of the Status Control Heaters' subpanel's options menu, click **Configure** to open the Heater controller or Heater Controller 2 configuration subpanel.
3. In the Heater Controller configuration subpanel, identify the zones used to control and monitor the heat exchanger or electric heaters for the system.
4. For each assigned zone, verify that it is enabled, view its current temperature, and verify its maximum temperature limit and alarm set point referring to this table:

Table 5–7: Example heater controller settings - Zone 1

Setting or control	Purpose
Zone Name	Specifies the name given to the zone (for example, Zone 1). Zone 1 could also be identified by a name that indicates the controller and the heater device it controls or monitors (for example, "Vessel Heater 1"). **See also:** Role **Rationale:** Zone 1 of heater controller 1 controls the temperature of the electrical vessel heater for extraction vessel 1. Also, zones can be configured for monitoring only (associated with a connection having no output control cable), such as the zones used for monitoring the temperature of the fluid inside the extraction vessels.
Max. Temp.	Specifies the upper limit for specifying the temperature set point for the zone.
Alarm Set Point	Specifies the temperature threshold above which the software's overtemperature control function issues an alarm and shuts down the heater.
Cycle Time	For use by Waters service technicians only.
Dead Band	For use by Waters service technicians only.
Proportional	For use by Waters service technicians only.
Integral	For use by Waters service technicians only.

Table 5–7: Example heater controller settings - Zone 1 (continued)

Setting or control	Purpose
Derivative	For use by Waters service technicians only.
Upper Integral B	For use by Waters service technicians only.
Lower Integral B	For use by Waters service technicians only.
TC Type	Identifies the type of connector used to control and monitor the temperature of the heater device. For Waters heater devices, specify the TC type as `J`, to enable control and monitoring. Alternatively, specify `None`, to indicate that no connector is used.
Role	Assigns this zone to a particular device. **Important:** Usually, an administrator uses this setting for troubleshooting or adding a new heating device to the system. If you change the role setting, you must also reconfigure the thermocouple and control cabling between the heater controller module and the heater device identified in the changed role. **Warning:** To avoid electric shock, always disconnect the system from the AC power supply before connecting or disconnecting external cables.

5. Click **Apply** to save the heater controller settings.

5.2.6 Specifying settings or viewing device status for the ABPR

In the Device Configuration panel, you can specify settings or view device status for the automated back-pressure regulator (ABPR).

To specify settings or view device status for the ABPR:

1. In the Folder View panel, click the **Device Configuration** node to open the Device Configuration panel.
2. Click **ABPR** to open the ABPR configuration subpanel.

   Alternative: In place of steps 1 and 2, in the Status Control ABPR subpanel's options menu, click **Configure** to open the ABPR configuration subpanel.
3. In the ABPR configuration subpanel, view the status of the automated back-pressure regulator and configure its control and needle position settings referring to the tables below.

Table 5–8: ABPR status

Status item	Indicates
Valve temperature	The temperature of the pressure-regulator valve.
External heater temperature	The temperature reading of the optional external heater, when connected. (If an external heater is not connected, the temperature reading is 0 °C.)
Valve needle position (units)	The extent of the valve's open position. The software can automatically open the valve to a maximum needle count of 3000. To fully open the valve and depressurize the system, you can manually set a needle count of 6000. A needle count of 0 fully closes the valve.

Table 5–9: ABPR threshold and usage settings

Setting or control	Purpose
Valve heater set point	For use by Waters service technicians only.
External heater set point	For use by Waters service technicians only.
External temperature alarm	For use by Waters service technicians only.
High-pressure alarm	For use by Waters service technicians only.
Attenuation Factor	For use by Waters service technicians only.
Proportional Constant	For use by Waters service technicians only.
Integral Constant	For use by Waters service technicians only.
Output Max	For use by Waters service technicians only.
Derivative Constant	For use by Waters service technicians only.

Table 5–9: ABPR threshold and usage settings (continued)

Setting or control	Purpose
Desired needle count value/Set Needle Count	Opens the pressure valve to depressurize the system, or closes the valve if it is open. The software can automatically open the valve to a maximum needle count of 3000. To fully open the valve and depressurize the system, you can manually set a needle count of 6000. A needle count of 0 fully closes the valve. The ABPR can be either in the "on" or "off" position. "On" indicates that the ABPR is controlling the set pressure, and "off" indicates that the ABPR is not automatically controlling the set pressure. Change the needle position when the ABPR is not automatically controlling the back pressure. **Rationale:** The system uses this value to calculate the position of the needle valve. **Rule:** The ABPR must be idle before you can use this control to open or close the needle valve.
Apply	Saves all new control settings and needle count value changes.

5.2.7 Specifying settings or viewing device status for the vessel switcher

For systems equipped with the automation module, the Vessel Switcher configuration subpanel provides four additional heater zones for controlling or monitoring auxiliary heating devices. It also includes controls for specifying the duration of the extraction vessels' equilibration, and if the system includes a CO_2 recycler, the extended vessel fill time and max pressure of the automated CO_2 recycler.

Notes:

- While using the automated vessel switcher, when a method is extracting from one vessel and about to switch to the next vessel, ensure that the cap is on the next vessel. Do not open the cap of a vessel while the system is extracting from that vessel or about to switch to that vessel.
- Lower the pressure on a system equipped with an automation module to 10,000 kPa (100 bar, 1450 psi), so that when equilibration occurs, the pressure is not exceptionally high and the frit on the extraction vessel cap is not damaged.

To specify settings or to view device status for the vessel switcher:

1. In the Folder View panel, click the **Device Configuration** node to open the Device Configuration panel.
2. Click **Vessel Switcher** to open the Vessel Switcher configuration subpanel.

Alternative: In place of steps 1 and 2, in the Status Control Vessel Switcher subpanel's options menu, click **Configure** to open the Vessel Switcher configuration subpanel.

3. Identify the zones used to control and monitor any auxiliary heating devices configured for the system.

4. For each assigned zone, verify that it is enabled, view its current temperature, and verify its maximum temperature and alarm set point referring to this table:

Table 5–10: Example Vessel Switcher heater controller settings - Zone 1

Setting or control	Purpose
Enabled	Select the **Enabled** check box to use the zone's settings to control and monitor the specified heating device, or clear the check box to disable the zone.
Zone Name	Specifies the name given to the zone (for example, *Zone 1*). Zone 1 could also identify a name that indicates the controller and the heater device it controls or monitors. **See also:** Role
Max Temperature	Specifies the maximum temperature set point a user can specify for the zone.
Max Temperature Alarm	Specifies the temperature threshold above which the software's overtemperature control function issues an alarm and shuts down the heater.
Proportional Constant	For use by Waters service technicians only.
Integral Constant	For use by Waters service technicians only.
Derivative Constant	For use by Waters service technicians only.
Role	Assigns the zone to a particular device. **Important:** Usually, an administrator uses this setting for troubleshooting or adding a new heating device to the system. If you change the role setting, you must also reconfigure the thermocouple and control cabling between the heater controller module and the heater device identified in the changed role. **Warning:** To avoid electric shock, always disconnect the system from the ac power supply before connecting or disconnecting external cables.

Table 5–10: Example Vessel Switcher heater controller settings - Zone 1 (continued)

Setting or control	Purpose
Set Max Pressure (bar)	If the system is configured with a CO_2 recycler, specify the pressure set point at which the CO_2 recycler starts venting. **Note:** High CO_2 recycler pressure increases the risk of sample carryover to the recycler. Setting the pressure to 6000 kPa (60 bar, 870 psi) releases pressure on the recycler if the room temperature gets too hot, reducing the risk of sample carryover.
Current Pressure (bar)	Displays the current pressure of the CO_2 recycler.

5. For Set Vessel Equilibration Time, specify the duration in minutes for the pressure to equalize between extraction vessels during a run when the equilibration setting is specified as the method's Extraction Vessel parameter.

   Tips:

   - For larger-volume vessels, specify a duration of three minutes to allow sufficient time for the CO_2 to siphon into the alternative, unpressurized extraction vessel.
   - When the automation module is set to equilibration mode in the method, both vessels are disconnected from the system and connected in a series in order to empty one vessel into another.

6. If the system is configured with a CO_2 recycler for Set Extended Fill Time, optionally specify the duration in minutes for the automated recycler to continue filling after the second-level switch is triggered. The filling stops when this duration expires.
7. Click **Apply** to save the vessel-switcher settings.

5.3 Methods for controlling system functions

In the method, you specify and save the settings that control the rate and composition of the extracting solvent, system pressure, vessel temperatures, extraction durations, and other important run conditions. Later, you can edit the method, as needed, load it into a queue, and run it to perform extractions, maintenance, or troubleshooting tasks.

Observe these guidelines when creating and managing your project's methods:

- You create, view, and edit methods using the method editor.
- You can create and save methods for controlling system functions used for maintenance or troubleshooting. For example, you can create methods for cleaning the system and for powering it down.

- While observing a run, from the Status Control panel, you can override the settings for the method currently in progress. You can also save the new settings by renaming the method and clicking **Save**.
- Use the method settings in concert with those specified in the System Settings panel to protect system components from unsafe conditions, such as excessive pressure drop or vessel overtemperature.

Tips for working with methods:

- You can hide and later redisplay optional columns in the method table as needed for your application. For Prep SFE systems, these optional columns include:
  - Sample Name
  - Extraction Vessel 2 Temperature
  - Co-Solvent Flow
  - Dynamic Duration 2
  - Static Duration
  - Collection Vessel Temperature 2
  - Cycles To Execute
  - Report Template and Report Print Options

  Note: If you hide a column that contains settings, and then add the method to the queue, a message appears stating that these settings are ignored during the run. Later, if you edit the method and redisplay the column, the settings for the column reappear and are available when the method is next run.
- When creating multiple step extractions, you can use the first row in the method to automatically fill multiple rows. You can also use a particular cell to "fill down" the column in multiple rows.

When following the instructions in this section, always observe Good Laboratory Practice (GLP) guidelines and your laboratory's standard operating procedures.

5.3.1 Viewing or editing a method

You view or edit a method using the method editor.

To view or edit a method:

1. In the Folder View panel for the project, click the **Method Files** node to display the currently stored methods in the File View panel.
2. From the list of available methods, click the method file to open it in the method editor.
3. If you are modifying the method, make any necessary changes, and click **Save**.

Result: The method file is saved to a new version, and the previous version of the method is archived.

5.3.2 Creating a method for running extractions

You can create and save as many methods as necessary for controlling the conditions used to operate single-step or multi-step extraction runs. Once saved, the methods are stored in your `Project` folder under the Method Files node.

To create a new method and configure its parameters:

1. In the Folder View panel for the project, right-click the **Method Files** node and select **Add new method**.

   Alternative: From the **File** menu, click **Create new project files** > ***project name*** > **Create Method File**.

2. On the **New Method** tab, type a *name* for the method.
3. Specify the name of the data-log file created when the extraction step runs. You can specify a data-file name or select one from a list of available naming options.

   Result: After each step in the running method, a data-log file is created in the `Data Files` subfolder.

4. Specify the required settings for the first row or **Step 1** of the method, referring to the table below.
5. Click the next row and specify the required settings for the next step of the method.
6. Repeat step 5, as necessary, for any additional, required steps.

   Tip: When creating multiple step extractions, you can use an existing row or cell in the method to automatically fill additional rows or cells with the same settings. To create duplicate rows, right-click on the first column in the row that you want duplicated and use the Copy Row(s) or Insert Row(s) functions to fill the new rows with the previously specified settings. You can also right-click in a particular cell and use the **Set Fill Value** function to "fill down" the column for a specified number of rows.

7. After you specify the settings for each step or row that you require, click **Save** to save your method.

   Tip: You can view the total amount of time, in minutes, for the running method in the **Total Duration** box.

Table 5–11: Method settings

Setting	Purpose
Co-Solvent Flow[1]	The flow rate for co-solvent flow, in mL/min **Range:** For the P-50 pump: 1 to 50 mL/min is allowed, 5 to 50 mL/min is recommended for proper pump function. For no flow, type 0.00.
CO_2 Flow	The flow rate for CO_2 flow, in g/min **Ranges:** For the P-200 pump: 3 to 200 g/min is allowed, 20 to 200 g/min is recommended for proper pump function. For no flow, type 0.00.
Extraction Vessel	For systems equipped with manual extraction-vessel switching valves, provides these selections: • Extraction 1 – Specifies manually setting the MV2 and MV1 valves to extraction vessel 1 for this method step. • Extraction 2 – Specifies manually setting the MV2 and MV1 valves to extraction vessel 2 for this method step. For systems equipped with automation, provides these selections: • Extraction 1 – Sets the valves to extraction vessel 1. • Extraction 2 – Sets the valves to extraction vessel 2. • Equilibration – Equalizes the pressures between extraction vessel 1 and extraction vessel 2. Typically, you use this setting as a transitional step to reclaim CO_2 and increase throughput by directing the system to siphon the CO_2 from the pressurized vessel into the unpressurized alternative vessel before it switches to the alternative vessel in the next step. **Note:** You specify the duration for equilibration on the Vessel Switcher configuration subpanel located under the Device Configuration node. • Series 1 to 2 – Connects the outflow of extraction vessel 1 to the inflow of extraction vessel 2. • Series 2 to 1 – Connects the outflow of extraction vessel 2 to the inflow of extraction vessel 1. **Note:** Series configurations are limited to configurations with 5-L extraction vessels equipped with the series configuration kit.

Table 5–11: Method settings (continued)

Setting	Purpose
Extraction Vessel 1 Temperature and Extraction Vessel 2 Temperature[1]	The temperature set points for the first and second extraction vessels. **Note:** If the system configuration does not include a second extraction vessel, the temperature setting for extraction vessel 2 is inactive. **Range:** Ambient to 90 °C: a setting of `0` affords no temperature control.
Cyclone Vessel 1 Temperature, Cyclone Vessel 2 Temperature[1], and Cyclone Vessel 3 Temperature[1]	The temperature set points for the first, second, and third cyclone separators (CS1, CS2, and CS3). **Note:** If the system configuration does not include a second or third cyclone separator, the temperature settings for CS2 and CS3 are inactive. **Range:** Ambient to 90 °C: a setting of `0` affords no temperature control. **Tip:** Cyclone Vessel 2 Temperature and Cyclone Vessel 3 Temperature are optional columns you can hide by right-clicking the header and selecting **Hide**. To redisplay the column, right-click in any column's field and select **Show** and the title of the hidden column.
Inline Heater Temperature	The temperature set point for the heat exchanger, which heats the extracting solvent to the temperature specified in the sample list. The extracting solvent comprises the combined CO_2 and co-solvent flows. **Important:** This setting is critical for maintaining the temperature of the extracting solvent. It is typically set approximately 5 to 10 °C warmer than the desired extraction temperature.
Inline Collection Heater Temperature	The temperature set point for the heat exchanger, which heats the sample flowing from cyclone separator 2 (CS2) to cyclone separator 3 (CS3), to keep the sample in a gas phase and ensure that there is no carryover to the CO_2 recycler.

Table 5–11: Method settings (continued)

Setting	Purpose
Extraction Pressure	The system-pressure set point. **Range:** 0 to 40,000 kPa (0 to 400 bar, 0 to 5802 psi): a setting of 0 affords no pressure control beyond the inlet CO_2 pressure. **Tips:** • The Extraction Pressure setting is critical for maintaining the system pressure throughout the extraction flow path. • Pressure is the primary factor affecting extractions and is a useful technique for fine-tuning them. It changes the solvating power of the supercritical CO_2. • Use care when specifying depressurization settings. Reduce pressure in a controlled manner. If the depressurization step is too steep, temperature-sensitive sample can be cold-stressed and valuable analyte lost. • In the Start/Shutdown subpanel under System Settings, specify the maximum allowed pressure drop to control the amount of pressure an extraction vessel can sustain before the system shuts down automatically.
Dynamic Duration 1	The duration, in minutes, for exposing the sample to the flow and pressure conditions defined for this extraction run. Specify this value in concert with values assigned the Static Duration and Cycles to Execute parameters.
Static Duration[1]	The duration, in minutes, for restricting the flow from the vessel, exposing the sample to the pressure condition defined only for this extraction step. Specify this value in concert with values assigned the Dynamic Duration and Cycles to Execute parameters. **Recommendation:** Static duration is not recommended when performing bio-botanical extractions. **Note:** When the static duration is in effect, the reported device status for the CO_2 pump is `Running in pressure control`. When the static duration expires, the pump status reverts to `Running in flow control`. **Important:** For systems equipped with manual extraction-valve switching, during the run, at the start of the static duration period, ChromScope prompts you to close the MV1 outlet valve stem for the extraction vessel undergoing static duration. Likewise, when the static-duration timer expires, it prompts you to reopen the valve.

Table 5–11: Method settings (continued)

Setting	Purpose
Dynamic Duration 2[1]	The second duration, in minutes, for exposing the sample to the flow and pressure conditions defined for this extraction step. Specify this value in concert with the Static Duration and Cycles to Execute parameters.
Cycles to Execute[1]	The number of cycles for dynamic and static durations for this extraction step.
Sample Name[1]	Specifies a name to record for the sample.
Report Template and Report Template Print Options[1]	Provides information for reporting.
Export Data File To[1]	Provides information for exporting the data-log file.
User Notes[1]	Provides your comments for the method.
[1] This is an optional column you can hide by right-clicking the header and selecting **Hide**. To redisplay the column, right-click in any table row and select **Show** and the title of the hidden column.	

5.3.3 Exporting a method

You can export a method from one project to another.

To export a method:

1. In the Folder View panel for the source project, right-click the **Method Files** node and select **Export To**.
2. Select the destination project from the list.

   Result: The method appears in the new project location.

6 Extraction runs and fraction collection

6.1 System startup and extraction workflow

To start the system, perform an extraction run, and collect fractions, use these procedures in conjunction with Good Laboratory Practice (GLP) guidelines and your laboratory's standard operating procedures:

- Start the chillers for the CO_2 pump and recycler, and ensure that the cooling flows are circulating.
- Verify that the CO_2 and co-solvent supply sources are ready.
- Apply power to the system components, including the heaters, chillers, pumps, and the automated back-pressure regulator.
- Prime the co-solvent pump.
- Start ChromScope software and log in.
- Prepare the extraction vessels and sample.
- Load the extraction vessels with sample.
- Load an extraction sample list for the run and start the method.
- Set the manual back-pressure regulators to the method conditions.
- Monitor the system and module status for the extraction run.
- Collect fractions, starting with the cyclone separator having the highest pressure, and then proceed sequentially with the next highest-pressure separator.

6.2 Starting the chillers for the CO_2 pump and recycler

The chillers, and their associated cooling baths for the optional CO_2 pump and recycler, are required to cool and liquefy the CO_2 before it enters the recycling vessel, and also during pumping.

Requirement: Before operating the chillers, inspect the level of coolant in the cooling bath, to ensure that it is adequate. Refer to the chiller's operation manual for an explanation about how to set up the chiller.

Important: For best performance, and in order to maintain the chiller manufacturer's factory warranty, use the coolant fluid recommended by the chiller manufacturer.

Warning: To avoid serious injury or death, do not ingest ethylene glycol, which is toxic even in small amounts. Instead of ethylene glycol, use propylene glycol/water 1:1 as the coolant in cooling baths. For specific warnings, consult the operator's manual for the chiller or the Material Safety and Data sheet for the coolant.

Notice: To avoid damaging the chiller, do not use automotive antifreeze as the cooling agent.

To start the chillers and verify their operation:

1. Power-on each of the chillers and set their operating temperatures as follows:
    - Pump chiller: 3 °C
    - Recycler chiller: 11 °C
2. Verify that the coolant is flowing through each of the chiller's associated cooling baths.
3. Inspect the cooling-tubing connections for leaks.
4. Monitor the chillers via their front-panel display to ensure that they reach the temperature set point and stabilize for at least 30 minutes before starting a run.

6.3 Verifying that the CO_2 supply is ready

Before you start the system for running an extraction, verify that the CO_2 supply is sufficient for system equilibration and run-time operation.

Recommendation: To ensure that the system is performing properly, maintain a log of the inlet CO_2 temperature and pressure, along with the pump RPMs for a given flow rate.

Tips:

- A standard CO_2 tank filled to capacity contains approximately 33 lbs (15 kg) of usable CO_2.
- A system with dual, 5-L extraction vessels, without a CO_2 recycler, uses approximately one full CO_2 tank every 90 minutes of operation.
- A system with 5-L extraction vessels, with a CO_2 recycler, uses approximately one full CO_2 tank every 630 minutes of operation.

Warning: To avoid serious injury, including asphyxiation, that can result from disconnecting a pressurized CO_2 line, before disconnecting the line from the CO_2 source, stop the flow of CO_2 from the system, and shut the valve at the CO_2 supply.

To verify that the CO_2 supply is ready:

1. If you are using CO_2 tanks without a CO_2 recycler, ensure that at least two additional full tanks are available for use, to ensure sufficient capacity for system equilibration and run-time operation.
2. If you are using the optional CO_2 recycler, ensure that the level sensor is powered-on, and that the recycler pressure and temperature readings are normal.
3. If you are using a manual recycler, and if the recycler CO_2 level is below the low-level indicator, open the CO_2 supply valve to add CO_2 to the level of the low-level indicator.

   Important: For manual recycler systems, do not fill the recycler level above the low-level indicator.

   See also: The *CO_2 Recycler Overview and Maintenance Guide*.

6.4 Starting system modules and components

Ensure that the heater controllers, pumps, ABPR, and all other modules and components are powered-on before you start the ChromScope software.

To start system modules and components:

1. Power-on the individual units by actuating the power switch on the rear panel of each.
2. Power-on the PC workstation.

6.4.1 Configuring the in-line collection heater

Version 2.0 and later Bio-Botanical Extraction Systems are shipped with the in-line collection heater enabled. For earlier versions, configure the heater system as described below.

To configure the in-line collection heater:

1. In the Folder View panel, click **Device Configuration** > **Heater Controller 2**.
2. Click the **Zone 5** tab (or the Zone configured for the Inline Heater role) .
3. Select the **Enabled** check box.
4. Click **Apply**.

6.4.2 Configuring the automated back-pressure regulator

The automated back-pressure regulator (ABPR) is a needle valve that creates the pressure for supercritical CO_2.

To configure the automated back-pressure regulator:

1. In the Folder View panel, click **Device Configuration** > **ABPR**.
2. Set the Control Settings Valve Heater Set Point to `45 °C`.
3. Click **Apply**.

   Notes:

   - The current needle position displays whenever the ABPR is pressurizing the extraction vessel or releasing material to the collection vessels.
   - When the needle count is 0, the ABPR is closed and pressurizing the extraction vessel.
   - When the needle count is between 300 and 1000, the ABPR is releasing material to the cyclone separators.
   - The software can automatically open the valve to a maximum needle count of 3000.
   - To fully open the valve and depressurize the system, you can manually set a needle count of 6000.

6.4.3 Configuring the extraction vessel switcher

Note: Vessel switcher configuration is only required for systems with an automation module, with or without the automated CO_2 recycler.

To configure the extraction vessel switcher:

1. In the Folder View panel, click **Device Configuration** > **Vessel Switcher**.
2. Ensure that the Set Vessel Equilibration Time is 3 minutes.
3. For systems with the automated CO_2 recycler, change the **Set Max Pressure** from 7000 kPa (70 bar, 1015 psi) to 6000 kPa (60 bar, 870 psi).

   Note: High CO_2 recycler pressure increases the risk of sample carryover to the recycler. Setting the pressure to 6000 kPa (60 bar, 870 psi) releases pressure on the recycler if the room temperature gets too hot, reducing the risk of sample carryover.
4. Click **Apply**.

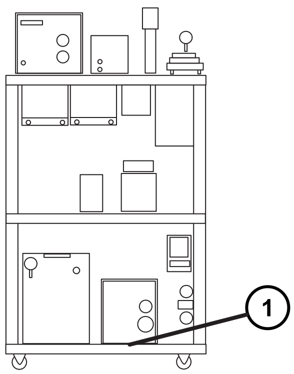
6.5 Priming the high-pressure, co-solvent pump

To properly operate a system equipped with the optional co-solvent pump, you must prime the pump during initial system start-up, and whenever you change solvents or replace a solvent reservoir.

To prime the pump:

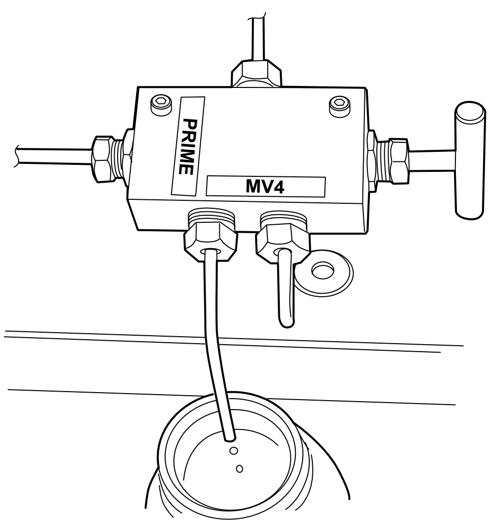
1. In the software, stop the operation of the co-solvent pump.
2. Place the solvent reservoir from which you are drawing solvent at the same level as the pump or higher.
3. Ensure that the weighted solvent-filter inlet line is fully submerged in the solvent reservoir.
4. Place a waste receptacle beneath the priming-valve outlet to collect the solvent.

Figure 6–1: Location of MV4 valve on module cart



1 Location of MV4 valve

Figure 6–2: Priming the co-solvent pump using the MV4 valve



5. Close the MV4 system valve (located on the right-hand side of the valve in the figure above), which directs flow to the system.
6. Slowly open the MV4 Prime valve, which directs flow to the priming outlet.

   Tip: Open the priming valve slowly. Any pressure remaining in the system escapes through the valve.

7. Start solvent flowing, observe the flow until a steady stream exits the outlet of the priming valve, and then stop the pump operation.

   Notice: To avoid the risk of a high-pressure burst of the co-solvent pump's rupture disc, always leave one side open on the MV4 valve. Operating the pump with both sides of the MV4 valve closed will cause the rupture disc to immediately burst.

8. To complete the priming operation, close the MV4 Prime valve, which closes the flow path to the priming valve, and open the MV4 system valve, which opens the flow path to the system.

6.6 Starting the ChromScope software and setting initial conditions

After all of the system modules and components are powered-on, you can start ChromScope, and log on to set initial conditions and monitor components.

Requirement: You must wait at least 30 seconds after powering-on all of the system modules and components before you start the ChromScope software. Doing so is necessary for the components to communicate properly with the software during startup.

6.7 Preparing for a botanical extraction in a system with manual valves

Setting up for a botanical extraction in a system with manual valves involves these steps:

1. Verifying the room conditions.
2. Verifying the CO_2 tank conditions.
3. Verifying the CO_2 recycler level and settings.
4. Preparing the extraction vessel.
5. Preparing the sample.
6. Loading the sample.
7. Setting the extraction valves in the proper position.
8. Verifying that the cyclone separator valves are closed.

6.7.1 Verifying the system operating environment

To ensure that the system functions properly, you must first verify the environmental conditions of the room where the system is operating.

To verify the system operating environment:

1. Ensure that the room temperature is between 18 and 24 °C.

   Important: Thermal variations in excess of 2 °C per 1.5 hours can adversely affect system performance.

2. Inspect the CO_2 monitor and ensure that it displays <1000 ppm.

   Notes:

   - The typical concentration of CO_2 in occupied indoor spaces is 350 to 1000 ppm.
   - CO_2 concentrations in occupied indoor spaces in the range of 1000 to 2000 ppm will cause drowsiness and elicit complaints of poor air quality.

   Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

3. Ensure that the ventilation system is functioning.
4. Ensure that each system module is powered on.

6.7.2 Verifying the CO_2 tank conditions

To verify the CO_2 tank conditions:

1. Ensure that the CO_2 tank pressure is greater than 5861 kPa (59 bar, 850 psi).
2. Ensure that the CO_2 tank is equipped with a dip tube.
3. Ensure that the CO_2 tank is equilibrated at room temperature for approximately 8 hours.
4. If the CO_2 tank was stored outdoors in cool weather, wrap a gas cylinder heater to raise the tank pressure to greater than 5861 kPa (59 bar, 850 psi).

   Tip: Tanks stored in sunlight will have high pressure and may not deliver adequate liquid CO_2 to the CO_2 recycler.

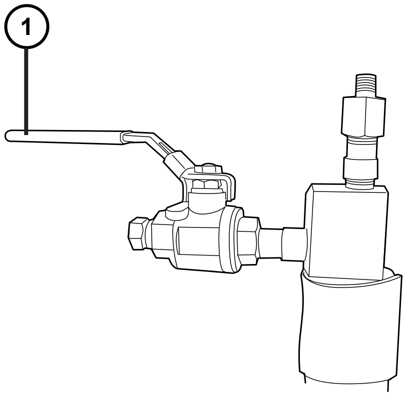
6.7.3 Verifying the manual CO_2 recycler level and settings

To verify the manual CO_2 recycler level and settings:

1. Partially open the manual CO_2 supply valve to refill CO_2 to the recycler.

   Tip: To prevent freezing, do not completely open the CO_2 supply valve.

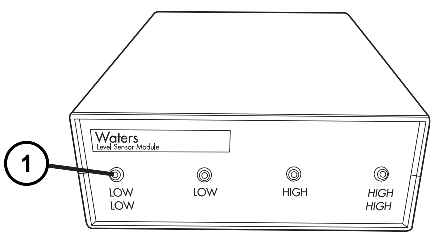
Figure 6–3: Manual CO_2 supply valve



1 Manual CO_2 supply valve handle

2. Check the level-sensor module. If the "Low-Low" LED is illuminated, add CO_2 to the recycler.

Figure 6–4: Level-sensor module

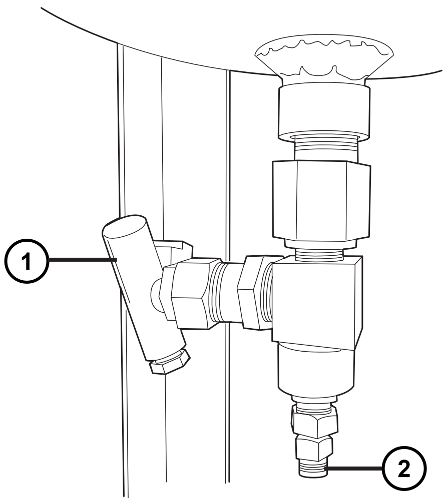


1 "Low-Low" LED

3. Ensure that the "Low" LED on the level-sensor module is illuminated.

Tip: If the "High" or "High-High" LED on the level-sensor module is illuminated, use the recycler's manual drain valve to vent CO_2 until the "Low" LED is illuminated.

Figure 6–5: CO_2 recycler manual drain valve

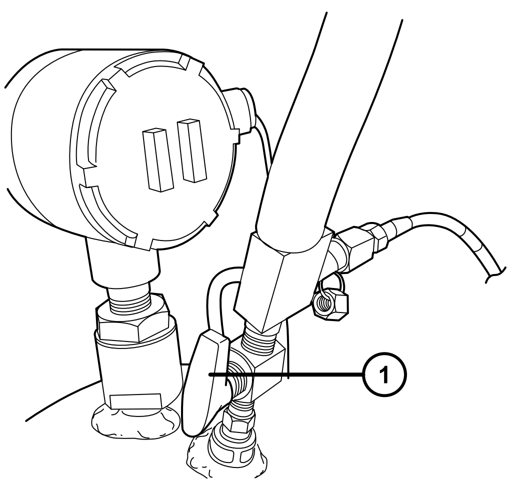


1 Drain valve handle

2 Drain port

4. Set the CO_2 recycler recirculation valve to the operate (horizontal) position.

Figure 6–6: Manual CO_2 recycler recirculation valve handle



1 Recirculation valve handle (vertical position shown)

5. Verify that the pressure gauge on the CO_2 recycler displays 4826 to 4999 kPa (48 to 50 bar, 700 to 725 psi). If the pressure is not in this range, do one of the following:

- If the pressure is less than 4826 kPa (48 bar, 700 psi), increase the CO_2 recycler chiller temperature.
- If the pressure is greater than 4999 kPa (50 bar, 725 psi), decrease the CO_2 recycler chiller temperature.

Tip: Adjust the CO_2 recycler chiller temperature in 0.5 °C increments, waiting 15 minutes between adjustments.

6.7.4 Preparing the extraction vessel

Required tools and materials

- Food-grade ethanol
- Shop vacuum
- Steel-toed shoes
- Ultrasonic bath

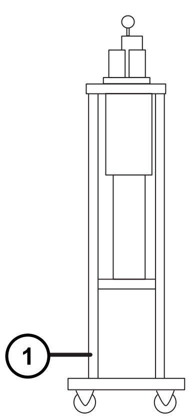
To prepare the extraction vessel:

1. Ensure that the extraction vessel being loaded has cooled to a safe handling temperature.
2. If the vessel is connected to the system, ensure that the pumps and ABPR are switched off, and that the vessel is switched out of the system flow path.
3. Depressurize the extraction vessel to at least the pressure of cyclone separator 1 (CS1).
4. Gradually open the MV6 exhaust valve to drain the CO_2 from the extraction vessel.

   Tip: The MV6 exhaust valve is located on the left, backside of the extraction vessel.

   Important: For safety, this valve must be exhausted to an outside vent.

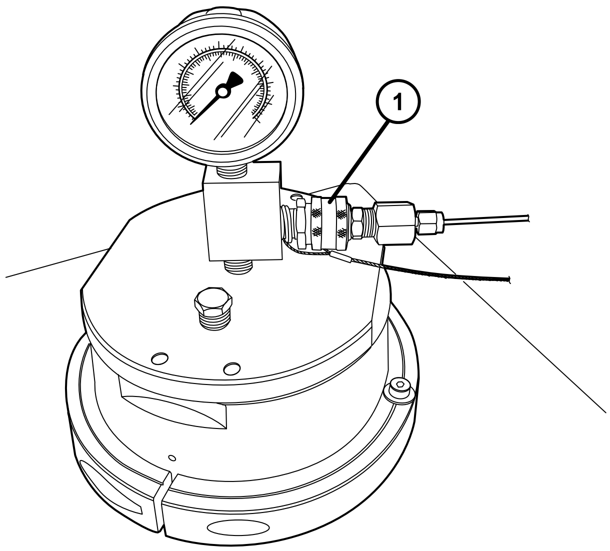
   Figure 6–7: Location of MV6 valve on back side of extraction vessel



1 Location of MV6 valve

5. After draining the CO_2 from the extraction vessel, close the MV6 valve.
6. Confirm that the extraction vessel's pressure gauge displays 0 kPa, (0 bar, 0 psi) to ensure that all CO_2 has been drained from it.
7. Use the quick-disconnect fitting to disconnect the MV1–V1 tubing from the extraction vessel's cap.

Figure 6–8: Quick-disconnect fitting on extraction vessel



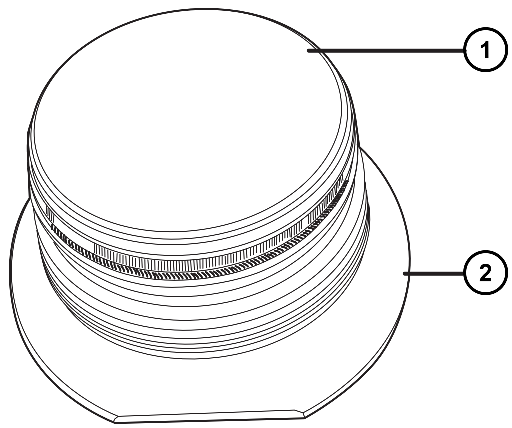
1 Quick-disconnect fitting

Warning: To avoid a high-pressure release of sample and solvent, or damage to the vessel's cap, never attempt to force open a vessel cap using a wrench. In the event that the vessel's pressure-escape vent becomes plugged, the vessel can remain under pressure indefinitely. If you cannot easily open the vessel's cap, allow the vessel to depressurize for at least fifteen minutes, and then try again to remove its cap.

Warning: To avoid hand or foot injury caused by accidently dropping a vessel's cap, wear steel-toed shoes and use care when handling the cap. Depending on its size, the cap can weigh as much as 5 kg.

8. Loosen and remove the extraction vessel's cap by turning it counterclockwise.
9. Use the shop vacuum to remove any dry waste from the extraction vessel.
10. If you observe any sludge under the cap, do the following:
    a. Remove the frit from the cap by turning it clockwise.

Figure 6–9: Extraction vessel cap with frit



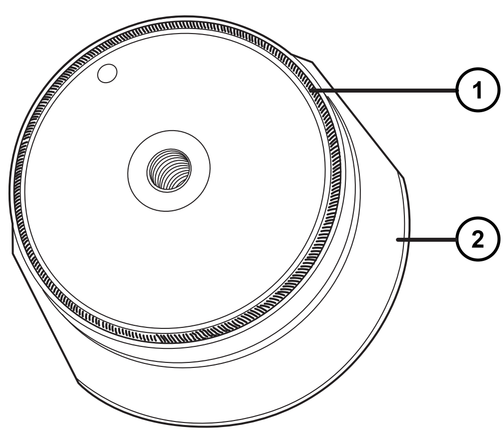
1. Frit
2. Extraction vessel cap

b. Inspect the frit and clean it with 95% food-grade ethanol, if needed.

c. If the frit is damaged, replace it.

d. Inspect the spring in the seal and sonicate it with 95% food-grade ethanol, if clogged.

Figure 6–10: Extraction vessel cap with frit removed



1. Spring in seal
2. Extraction vessel cap

e. If the spring is damaged, replace it.

f. Reinstall the frit on the cap.

11. Repeat the steps above to prepare a second extraction vessel.

6.7.5 Preparing the sample for extraction

Required tools and materials

- Suitable container for sample material
- Sample material to be extracted

To prepare the sample for extraction:

1. Grind the sample to at least a coffee ground consistency.
2. Dry the sample to a moisture content of 5% to 8% wt/wt.
3. Place the sample material in the container and mix well.

6.7.6 Loading the sample into the extraction vessel

Warning: To avoid burn injuries, exercise care when handling the extraction vessels, separation cyclones, heat exchanger outlet tubing, or other components heated to high temperatures or cooled to low temperatures. Wait until the hot components have sufficiently cooled or the cold components have sufficiently warmed before you handle them.

Required tools and materials

- Funnel
- Glass marbles
- Plastic or wooden tamping device
- PTFE spray
- Sample material to be extracted
- Soft bristle brush
- Steel-toed shoes

To load the sample into the extraction vessel:

1. Use a funnel to begin loading the extraction vessel with the sample material.

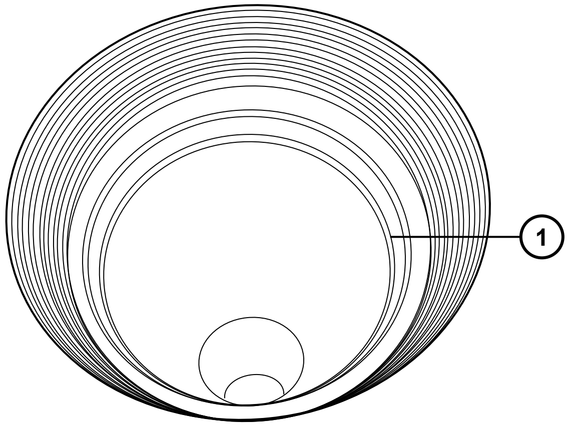
   Tip: Load about 1/2 pound (220 grams) of sample material at a time into the extraction vessel.

2. Use a plastic or wooden tamping device to pack the material firmly into the vessel.

   Tip: Pack the material uniformly to prevent channels from forming in the extractor.

   When the vessel is full, the packed sample material must reach the level of the fill line inscribed on the vessel's interior wall.

Figure 6–11: Extraction vessel fill line



1 Vessel's fill line

Requirement: To achieve proper extraction conditions, the extraction vessels must be filled to capacity. If you lack sufficient sample material to fill the vessel, you can add an inert filler, such as glass marbles, to bring the combined volume of sample and filler to the level of the vessel's fill line.

3. Use a soft bristle brush to clean any sample material from the vessel threads.
4. Place the extraction vessel's cap on the extraction chamber without screwing in the threads.
5. Lightly lubricate the extraction vessel's cap threads using a PTFE spray lubricant, taking care not to contact the sample material with the lubricant.

   Requirement: Be sure to spray all of the cap threads.

   Warning: To avoid hand or foot injury caused by accidently dropping a vessel's cap, wear steel-toed shoes and use care when handling the cap. Depending on its size, the cap can weigh as much as 5 kg.

6. Hand-tighten the cap by rotating it clockwise onto the vessel.
7. Use the quick-disconnect fitting to reconnect the MV1–V1 tubing to the cap.

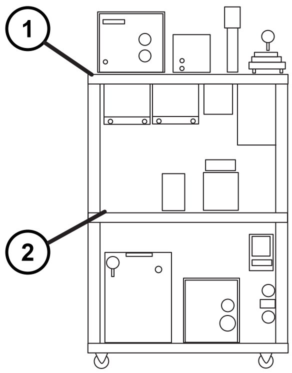
6.7.7 Setting the extraction valves in the correct position

To set the extraction valves in the correct position:

1. Completely open the MV2-V1 valve, and then close the MV2-V2 valve.

   Tip: The MV2-V1 and MV2-V2 valves are located on the middle shelf.

Figure 6–12: Location of MV1 and MV2 valves on module cart



1 Location of MV1-V1 and MV1-V2 valves

2 Location of MV2-V1 and MV2-V2 valves

2. Completely open the MV1-V1 valve, and then close the MV1-V2 valve.

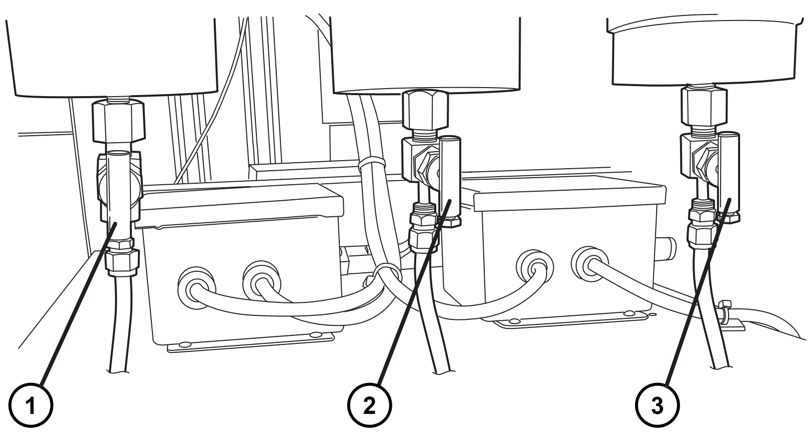
   Tip: The MV1-V1 and MV1-V2 valves are located on the top shelf.

6.7.8 Verifying that the cyclone separator drain valves are closed

To verify that the cyclone separator drain valves are closed:

1. Locate the cyclone separator drain valves at the bottom of each cyclone separator vessel.

   Figure 6–13: Cyclone separator drain valves



1 CS1 drain valve

2 CS2 drain valve

3 CS3 drain valve

2. Ensure that the cyclone separator drain valve handles (MV8, MV9, and MV10) are closed.

6.8 Preparing for a botanical extraction in a system with an automation module

Setting up for a botanical extraction in a system with an automation module involves these steps:

1. Verifying the room conditions.
2. Verifying the CO_2 tank conditions.
3. Verifying the CO_2 recycler level and settings.
4. Preparing the extraction vessel.
5. Preparing the sample.
6. Loading the sample.
7. Verifying that the cyclone separator valves are closed.

6.8.1 Verifying the system operating environment

To ensure that the system functions properly, you must first verify the environmental conditions of the room where the system is operating.

To verify the system operating environment:

1. Ensure that the room temperature is between 18 and 24 °C.

   Important: Thermal variations in excess of 2 °C per 1.5 hours can adversely affect system performance.

2. Inspect the CO_2 monitor and ensure that it displays <1000 ppm.

   Notes:

   - The typical concentration of CO_2 in occupied indoor spaces is 350 to 1000 ppm.
   - CO_2 concentrations in occupied indoor spaces in the range of 1000 to 2000 ppm will cause drowsiness and elicit complaints of poor air quality.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

3. Ensure that the ventilation system is functioning.
4. Ensure that each system module is powered on.

6.8.2 Verifying the CO_2 tank conditions

To verify the CO_2 tank conditions:

1. Ensure that the CO_2 tank pressure is greater than 5861 kPa (59 bar, 850 psi).
2. Ensure that the CO_2 tank is equipped with a dip tube.
3. Ensure that the CO_2 tank is equilibrated at room temperature for approximately 8 hours.
4. If the CO_2 tank was stored outdoors in cool weather, wrap a gas cylinder heater to raise the tank pressure to greater than 5861 kPa (59 bar, 850 psi).

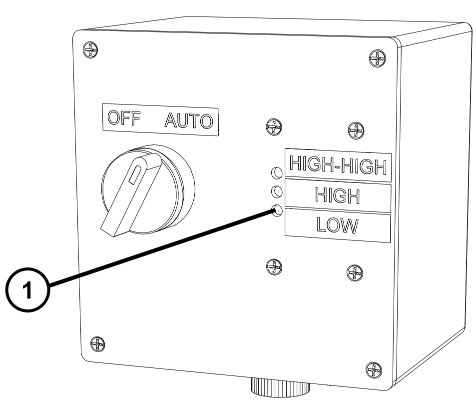
   Tip: Tanks stored in sunlight will have high pressure and may not deliver adequate liquid CO_2 to the CO_2 recycler.

6.8.3 Verifying the automated CO_2 recycler level and settings

To verify the automated CO_2 recycler level and settings:

1. Ensure that the CO_2 recycler indicator displays "Low".

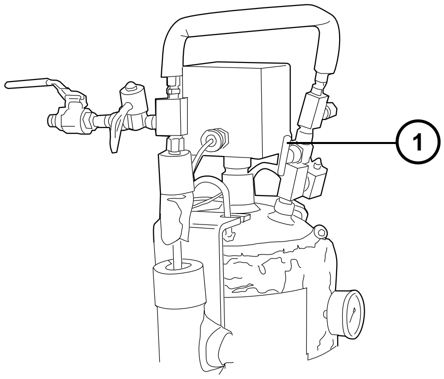
Figure 6–14: Automated CO_2 recycler indicator



1 "Low" LED

2. Set the CO_2 recycler recirculation valve to the operate (horizontal) position.

Figure 6–15: Automated CO_2 recycler recirculation valve handle



(1) Recirculation valve handle (vertical position shown)

3. Verify that the pressure gauge on the CO_2 recycler displays 4826 to 4999 kPa (48 to 50 bar, 700 to 725 psi). If the pressure is not in this range, do one of the following:
   - If the pressure is less than 4826 kPa (48 bar, 700 psi), increase the CO_2 recycler chiller temperature.
   - If the pressure is greater than 4999 kPa (50 bar, 725 psi), decrease the CO_2 recycler chiller temperature.

   Tip: Adjust the CO_2 recycler chiller temperature in 0.5 °C increments, waiting 15 minutes between adjustments.

6.8.4 Preparing the extraction vessel

Required tools and materials

- Food-grade ethanol
- Shop vacuum
- Steel-toed shoes
- Ultrasonic bath

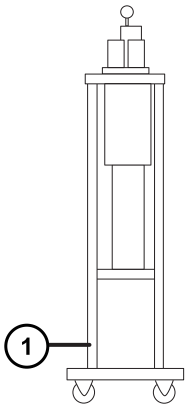
To prepare the extraction vessel:

1. Ensure that the extraction vessel being loaded has cooled to a safe handling temperature.
2. If the vessel is connected to the system, ensure that the pumps and ABPR are switched off, and that the vessel is switched out of the system flow path.
3. Depressurize the extraction vessel to at least the pressure of cyclone separator 1 (CS1).
4. Gradually open the MV6 exhaust valve to drain the CO_2 from the extraction vessel.

Tip: The MV6 exhaust valve is located on the left, backside of the extraction vessel.

Important: For safety, this valve must be exhausted to an outside vent.

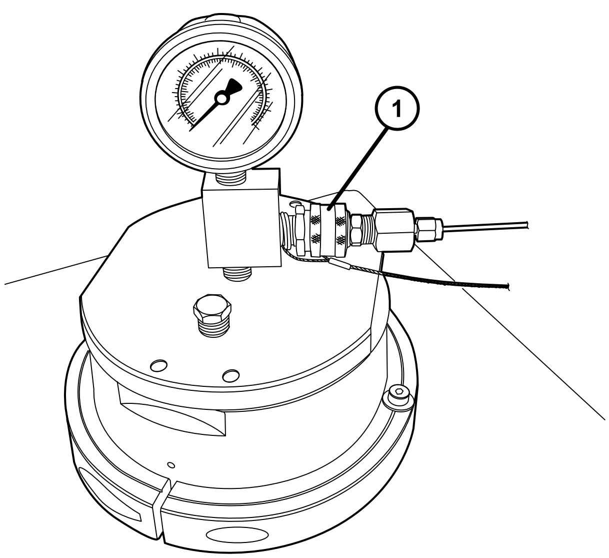
Figure 6–16: Location of MV6 valve on back side of extraction vessel



1 Location of MV6 valve

5. After draining the CO_2 from the extraction vessel, close the MV6 valve.
6. Confirm that the extraction vessel's pressure gauge displays 0 kPa, (0 bar, 0 psi) to ensure that all CO_2 has been drained from it.
7. Use the quick-disconnect fitting to disconnect the MV1–V1 tubing from the extraction vessel's cap.

Figure 6–17: Quick-disconnect fitting on extraction vessel



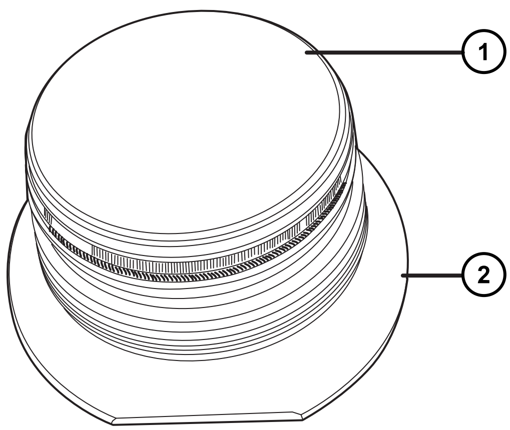
1 Quick-disconnect fitting

Warning: To avoid a high-pressure release of sample and solvent, or damage to the vessel's cap, never attempt to force open a vessel cap using a wrench. In the event that the vessel's pressure-escape vent becomes plugged, the vessel can remain under pressure indefinitely. If you cannot easily open the vessel's cap, allow the vessel to depressurize for at least fifteen minutes, and then try again to remove its cap.

Warning: To avoid hand or foot injury caused by accidently dropping a vessel's cap, wear steel-toed shoes and use care when handling the cap. Depending on its size, the cap can weigh as much as 5 kg.

8. Loosen and remove the extraction vessel's cap by turning it counterclockwise.
9. Use the shop vacuum to remove any dry waste from the extraction vessel.
10. If you observe any sludge under the cap, do the following:
    a. Remove the frit from the cap by turning it clockwise.

    Figure 6–18: Extraction vessel cap with frit

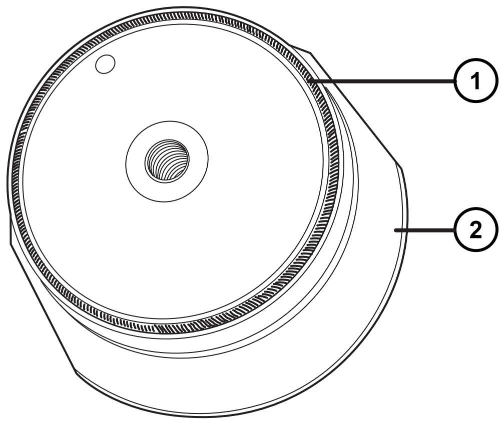
    

    (1) Frit

    (2) Extraction vessel cap

    b. Inspect the frit and clean it with 95% food-grade ethanol, if needed.
    c. If the frit is damaged, replace it.
    d. Inspect the spring in the seal and sonicate it with 95% food-grade ethanol, if clogged.

Figure 6–19: Extraction vessel cap with frit removed



1 Spring in seal

2 Extraction vessel cap

e. If the spring is damaged, replace it.

f. Reinstall the frit on the cap.

11. Repeat the steps above to prepare a second extraction vessel.

6.8.5 Preparing the sample for extraction

Required tools and materials

- Suitable container for sample material
- Sample material to be extracted

To prepare the sample for extraction:

1. Grind the sample to at least a coffee ground consistency.
2. Dry the sample to a moisture content of 5% to 8% wt/wt.
3. Place the sample material in the container and mix well.

6.8.6 Loading the sample into the extraction vessel

Warning: To avoid burn injuries, exercise care when handling the extraction vessels, separation cyclones, heat exchanger outlet tubing, or other components heated to high temperatures or cooled to low temperatures. Wait until the hot components have sufficiently cooled or the cold components have sufficiently warmed before you handle them.

Required tools and materials

- Funnel
- Glass marbles
- Plastic or wooden tamping device
- PTFE spray
- Sample material to be extracted
- Soft bristle brush
- Steel-toed shoes

To load the sample into the extraction vessel:

1. Use a funnel to begin loading the extraction vessel with the sample material.

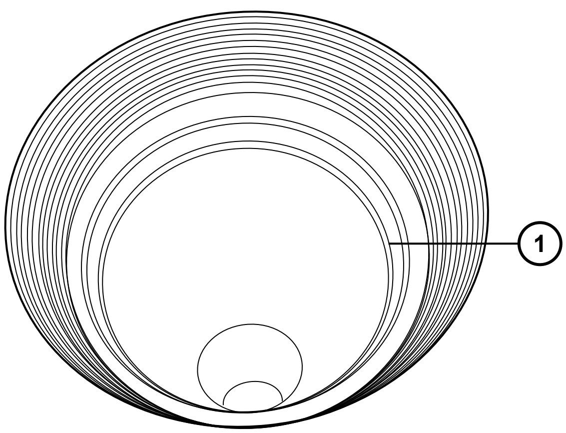
   Tip: Load about 1/2 pound (220 grams) of sample material at a time into the extraction vessel.

2. Use a plastic or wooden tamping device to pack the material firmly into the vessel.

   Tip: Pack the material uniformly to prevent channels from forming in the extractor.

   When the vessel is full, the packed sample material must reach the level of the fill line inscribed on the vessel's interior wall.

   Figure 6–20: Extraction vessel fill line

   1 Vessel's fill line

   Requirement: To achieve proper extraction conditions, the extraction vessels must be filled to capacity. If you lack sufficient sample material to fill the vessel, you can add an inert filler, such as glass marbles, to bring the combined volume of sample and filler to the level of the vessel's fill line.

3. Use a soft bristle brush to clean any sample material from the vessel threads.

4. Place the extraction vessel's cap on the extraction chamber without screwing in the threads.
5. Lightly lubricate the extraction vessel's cap threads using a PTFE spray lubricant, taking care not to contact the sample material with the lubricant.

   Requirement: Be sure to spray all of the cap threads.

   Warning: To avoid hand or foot injury caused by accidently dropping a vessel's cap, wear steel-toed shoes and use care when handling the cap. Depending on its size, the cap can weigh as much as 5 kg.

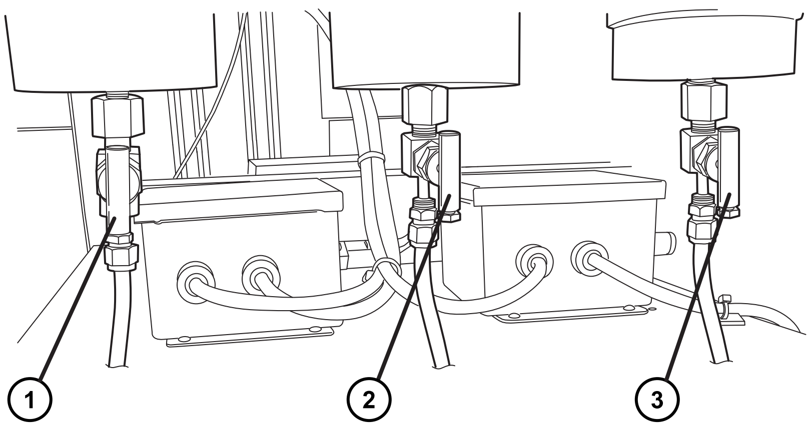
6. Hand-tighten the cap by rotating it clockwise onto the vessel.
7. Use the quick-disconnect fitting to reconnect the MV1–V1 tubing to the cap.

6.8.7 Verifying that the cyclone separator drain valves are closed

To verify that the cyclone separator drain valves are closed:

1. Locate the cyclone separator drain valves at the bottom of each cyclone separator vessel.

   Figure 6–21: Cyclone separator drain valves



   1 CS1 drain valve

   2 CS2 drain valve

   3 CS3 drain valve

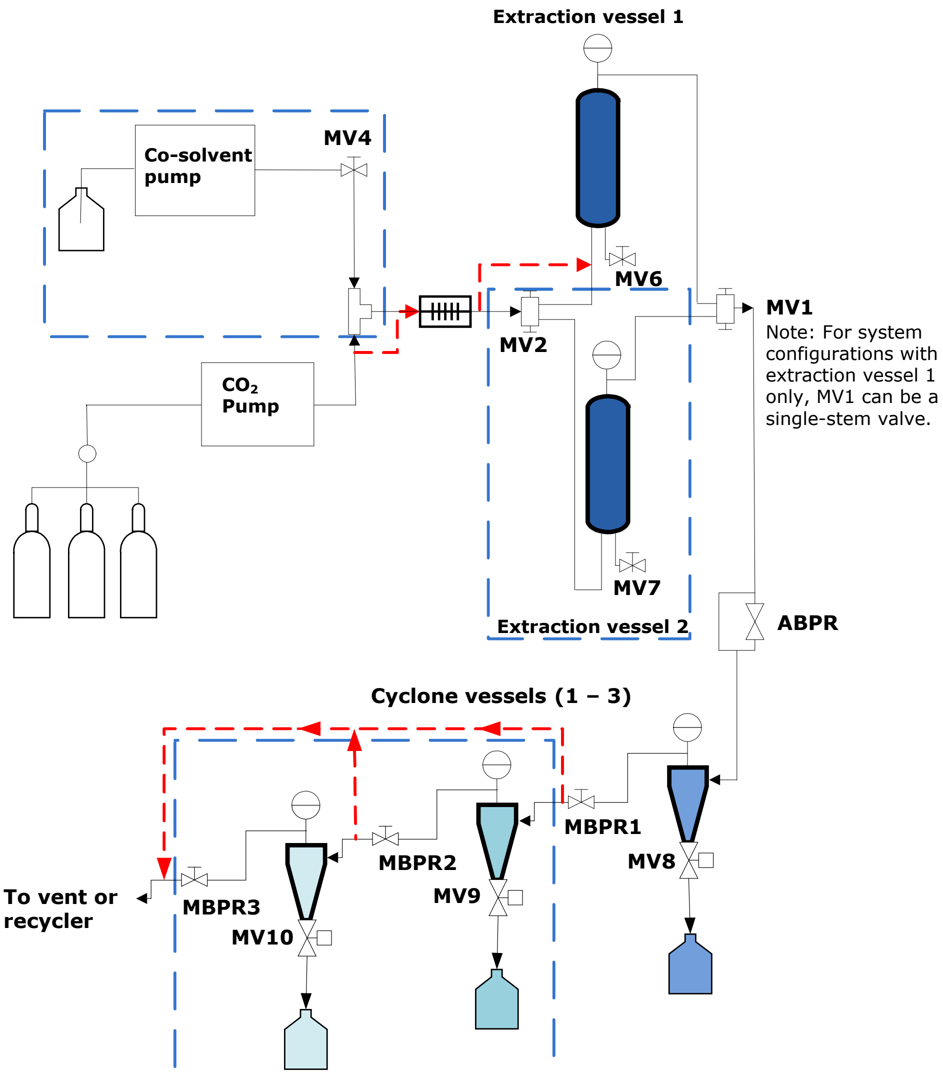
2. Ensure that the cyclone separator drain valve handles (MV8, MV9, and MV10) are closed.

6.9 Setting the system's manual valve positions for a run

The primary manual valves a system uses to direct flow and manipulate pressure vary according to the system's configuration. The following diagram identifies all of the manual valves (MV) and manual back-pressure regulator (MBPR) valves for a fully configured system. Before starting a run, verify the locations of the system's valves that you must manually operate. Ensure that the valves are positioned properly, referring to the table "MV and MBPR valve positions".

Note: For systems equipped with automated extraction-valve switching, the valves directing flow to and from extraction vessels are switched by the software according to which extraction vessel you select from the Status Control panel, or via a running sample list. These systems have no MV1 and MV2 valves to adjust.

Figure 6–22: Manual valves and manual back-pressure regulator valves in a fully configured system



Legend:

——————— (solid black line)	Main flow path
– – – – – (red dashed line)	Alternate main flow path for other system configurations
—— —— —— (blue dashed line)	Configuration-dependent components

Follow these guidelines for operating the system valves:

- All of the system's manual valves open and close in similar fashion. When facing the valve handle, rotate the handle counterclockwise to open the valve, or clockwise to close the valve.
- For systems with manual extraction-valve switching, the MV2 and MV1 valves direct flow to and from the extraction vessel used in the run. After loading the vessel with sample, verify that the MV2 and MV1 valves are positioned properly for the extraction vessel you intend to use for the run.
- For systems with manual extraction-valve switching that are equipped with the optional kit to plumb the extraction vessels in series, depending on which vessel is connected as inlet or outlet to the system, verify that the MV2 and MV1 valves are positioned accordingly.
- If the system is configured with an optional co-solvent pump, the MV4 valve is open to the system when pumping co-solvent, or closed when no co-solvent is being pumped.
- Each extraction and collection vessel is equipped with a valve at its base to enable draining of liquids and for depressurizing the vessel. During a run, the drain or pressure-relief valves (MV6, MV7, MV8, MV9, and MV10) are closed.
- The MBPR valves, (for example MBPR1, MBPR2, and MBPR3) serve to adjust the pressure in the cyclone separators (CS1, CS2, and CS3). You can adjust them only when the system is pressurized, as normal, during a run. If the MBPRs are not pre-adjusted to method conditions before you start a run, open them and adjust them to the prescribed method pressures when the system pressure has stabilized.

> **!** **Notice:** To avoid bursting the rupture-disc safety device for a cyclone separator, if the MBPR is not pre-adjusted to method conditions, before starting a run, fully open the MBPR by rotating its handle counterclockwise.

Table 6–1: MV and MBPR valve positions

Valve	Position at start of run	Operation during run
MV2	To open inflow to vessel 1: open MV2–V1; close MV2–V2 To open inflow to vessel 2: open MV2–V2; close MV2–V1	Remains open in initial position for duration of run with the designated extraction vessel.

Table 6–1: MV and MBPR valve positions (continued)

Valve	Position at start of run	Operation during run
MV1	To open outflow from extraction vessel 1, open MV1–V1; close MV1–V2 To open outflow from extraction vessel 2, open MV1–V2; close MV1–V1	Remains open in initial position, except for periods of static duration when you must close the outflow. To close outflow from vessel 1 for static duration: close MV1–V1; leaving MV1–V2 closed. To close outflow from vessel 2: close MV1–V2, leaving MV1–V1 closed. To return to dynamic duration, return the MV1 valve stems to their initial positions.
MV4	Open the right-hand (system) side of the valve if using the co-solvent pump to pump co-solvent; otherwise close the right-hand side of the valve. **Note:** If you must prime the co-solvent pump, see the instructions in the maintenance section.	Remains in initial system position for duration of run.
MV6 and MV7	Close the pressure relief valves for the extraction vessels designated for the run	Remains closed for duration of run.
MBPRs	Open fully, or leave set to the pre-adjusted positions for the run's method conditions	Adjust as needed during the run. **See also:** Adjusting the manual back-pressure regulator valves **!** **Notice:** To avoid bursting the rupture-disc safety device on cyclone separators, if the system is fitted with three such vessels, do not adjust the MBPR1 and MBPR2 valves so that pressure exceeds 34,474 kPa (345 bar, 5000 psi). In this configuration, both vessels' rupture disc is rated to burst at 41,369 kPa (414 bar, 6000 psi). You cannot adjust the MBPR for the last vessel above 13,790 kPa (138 bar, 2000 psi).
MV8, MV9, and MV10	Close the cyclone separator drain valves	Leave closed until you collect fractions.

6.10 Setting conditions for system operation

To set extraction-run conditions for flow, temperature, and pressure, and other run parameters, it is best to save the system settings in a method, and then run the method. For details explaining how to view and create methods, see the section "Methods for controlling system functions".

Alternatives:

- With the method queue empty, click **Start** to place the powered-on modules in their running state and to adopt the system default method parameters for flow, pressure, and temperature.

  #### Tips:

  - The settings for the system default method parameters are those that were changed via the Status Control panel or those that were in effect when you last used ChromScope.
  - This feature is useful for setting the systems's initial running conditions for verification, equilibration, or performing a basic extraction run with no run-time counter. A data-log file for the session is generated and stored under the Default Project node.

- From the Status Control panel, manually specify flow, pressure, and temperature settings.

  #### Tips:

  - The practice of manually operating the system is typically reserved for system equilibration or diagnostic testing.
  - When you do this to alter the observed conditions of a currently running method, the modified settings immediately take effect for the remainder of the run time in the method's current step.
  - During the time when the modified settings are in effect, you can optionally save the new settings by renaming the method and clicking **Save**.
  - Conversely, if you manually specify flow, pressure, or temperature settings when a method is not operating the system, upon exiting from ChromScope, the changes are automatically saved as the new settings for the system default method parameters.

Guidelines:

- When you click the Current Method under the Current Run panel, if no method is running, a message stating so appears, prompting you to load a method into the method queue.
- When operating the system via the default method parameters, you can proceed directly to method control without stopping the system by loading a method and clicking **Start** to run the method. When you do so, a new data-log file is initiated under the method's project node.
- If no method is running, after you load one or more methods into the queue, to run them, you must click **Start**.

- Before the last method ends and the queue empties, any additional methods that you load into the queue are automatically run in the order in which they are queued.
- If you add a method to the emptied queue while the system is in a pending-shutdown state (that is, before the specified period of inactivity expires), you must click **Start** to run the method.

To load a method:

1. In the Folder View panel for your project, click the **Method Files** node to display the method files in the File View panel that you want to load in preparation for operating the system.
2. For each method that you want to load, right-click the method and click **Open**.
3. In the method editor, click **Add to Queue**.

   Result: The methods appear in the method queue in the order in which you add them, and they can run sequentially in that order.

   Tip: You can use the **Up** and **Down** buttons in the method queue to reorder the methods in the queue. To remove a method from the queue, select it and click **Remove**.

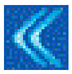
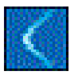
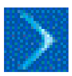
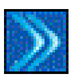
6.11 Starting and stopping a run

You can start a run or stop one before its specified end. You can also move forward and backward among functions within the list or method queue and view information about the system and a current run.

Table 6–2: Acquisition Control panel

Control	Function
Start	Start a currently loaded method, which appears at the top of the method queue. If the queue is empty, start the system default method parameters. **Result:** The modules are placed in their running state and adopt the method or system default parameters. A new data-log file is created.
Pause	Temporarily halt a running method. **Result:** The current method's run-time counter stops. Yet the current settings for temperature, pressure, and CO_2 and co-solvent flows remain unaltered.

Table 6–2: Acquisition Control panel (continued)

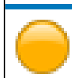
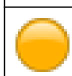
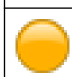
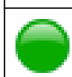
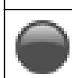
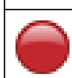
Control	Function
Stop 	Stop the run and immediately halt all system functions. **Result:** A currently running method stops. All system functions, including pumps, heat exchanger, and pressure regulator, are placed in the idle state.
Restart from beginning 	Click to restart the current method from the beginning.
Restart the current step 	Click to restart the current step, or run, in the method.
Skip to the next step 	Click to skip to the next step, or run, in the method.
Skip to the next method 	Click to skip to the next method in the queue.

Notes:

- You can also perform the Acquisition Control panel functions from the toolbar.
- If the icon for the acquisition control function is unavailable, the represented function is unavailable because of the run state.

The system status indicator in the ChromScope Acquisition Status panel shows the system's operating state.

Table 6–3: System operation statuses

Indicator	System's operating state
	Initializing system
	Equilibrating devices
	Current run is paused
	Running
	Idle
	Stopped

6.12 Verifying that the system run conditions are equilibrated

You must allow the system flow, pressure, and temperature readings to stabilize, and the system to equilibrate to these conditions, before you set the manual back-pressure regulators to their operating positions. The system flow, pressure, and temperature readings are stabilized, and the system equilibrated, when these events occur:

- The specified CO_2 flow, pressure, and temperature set points are achieved.
- The trend plots for flow, pressure, and temperature in the ChromScope Current Run tabbed view are stable.
- The pressure readings indicated by the extraction vessel's pressure gauge and the ABPR's pressure-regulator display in the ChromScope System Panel are approximately the same.

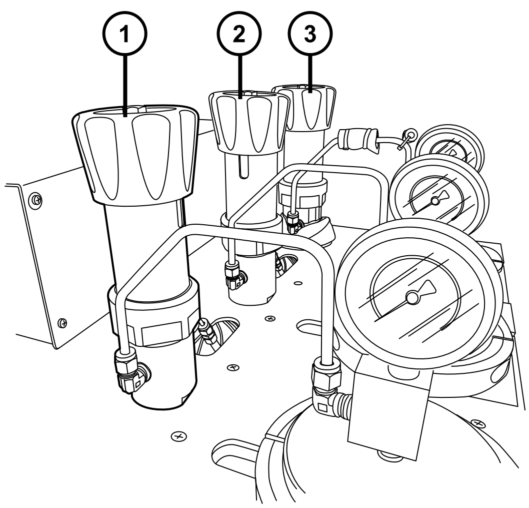
See also: Monitoring system and module status

6.13 Adjusting the manual back-pressure regulator valves

While monitoring a run's initial conditions, when the flow, temperature, and pressure of a system are equilibrated, you must adjust the manual back-pressure regulator (MBPR) valves to begin precipitating the extracted compounds out of solution and into the cyclone separators.

! **Notice:** To avoid bursting the rupture-disc safety device for the cyclone separators, before adjusting the MBPR valves, ensure that the system flow, temperature, and pressure conditions have equilibrated at their specified set points.

Figure 6–23: Adjusting the MBPR valves



1 MBPR1 for CS1

2 MBPR2 for CS2

3 MBPR3 for CS3

To adjust the MBPR valves:

Notice: To avoid bursting the rupture-disc safety device on cyclone separators, if the system is fitted with three such vessels, do not adjust the MBPR1 and MBPR2 valves so that pressure exceeds 34,474 kPa (345 bar, 5000 psi). In this configuration, both vessels' rupture disc is rated to burst at 41,369 kPa (414 bar, 6000 psi). You cannot adjust the MBPR for the last vessel above 13,790 kPa (138 bar, 2000 psi).

1. For MBPR1, associated with the first cyclone separator (CS1), adjust the pressure until the vessel's gauge reads between 7584 to 30,337 kPa (76 to 303 bar, 1100 to 4400 psi), according to the application.

   Tips:

   - When adjusting an MBPR valve, do so by incrementally turning the valve and waiting for the pressure to respond to the adjustment before turning the valve again. The valve-adjustment process requires approximately 20 seconds to complete.
   - The final adjustment should be in the positive pressure direction.
   - For general reference, the valve is fully open, or fully closed, in nine complete rotations.
   - If bleeding pressure from the vessel is necessary, do so by slowly opening the vessel's drain valve (MV8, MV9, or MV10) until sufficient pressure is relieved.

2. Referring to the table below, repeat the MBPR valve adjustment process for all cycles.

   See also: For more information on setting pressures, see the "Best practices" appendix.

Table 6–4: Adjusting the MBPR valves

Cyclone separator	MBPR valve	Pressure adjustment
CS1	MBPR1	7584 to 15,858 kPa (76 to 159 bar, 1100 to 2300 psi)
CS2	MBPR2	6895 to 12,411 kPa (69 to 124 bar, 1000 to 1800 psi)
CS3 (without CO_2 recycler)	MBPR3	207 to 689 kPa (2 to 7 bar, 30 to 100 psi)
CS3 (with CO_2 recycler)	MBPR3	4964 to 5171 kPa (50 to 52 bar, 720 to 750 psi)[1]

Table 6–4: Adjusting the MBPR valves (continued)

Cyclone separator	MBPR valve	Pressure adjustment
[1]If material carries over into the recycler storage tank, you must then drain the tank and refill it with fresh CO_2. See also: The *CO_2 Recycler Overview and Maintenance Guide*.		

6.14 Monitoring system and module status

When the system is equilibrating or running a method, you can, via the ChromScope Acquisition Status panel, determine system run status and monitor system performance. Additionally, both the System Status and Status Control panels provide indicators to monitor and report the real-time status of components like the CO_2 and co-solvent pump's flow rate. The status indicator within each module icon in the System Status panel shows the represented module's operating state. The status is "On" when the module is operating during a run, and "Idle" when the module is powered-on and in the ready state.

These are the system's other operational modes, as indicated by the run status color in the Acquisition Status panel:

- Green indicates that the loaded method is running.
- Black indicates that a method is not currently running.
- Red (Alarm) indicates that a system module is in an error state, requiring a reset.
- Yellow indicates that a system module is equilibrating or not ready.

The System Messages box in the ChromScope main window provides a running summary of system events, including system warnings and alarms.

Tips:

- When you move the pointer over each of the module representations in the System Status and Status Control panels, the System Messages box displays additional, module-specific status and parameter details.
- To scroll upward or downward through the status and system messages in the System Messages box, use the mouse's wheel.
- To halt the auto-scroll of system messages, click inside the System Messages box. You can then use the mouse's wheel to scroll upward to view earlier messages.

During a run, open the Current Run panel to view real-time plots for temperature, flow, and pressure.

6.14.1 Determining the operational status of the co-solvent and the CO_2 pump modules

Status or control	Indicator location
Power	The Power LED is on the lower left-hand side of the module's front panel. When the module is powered-on and working properly, the Power LED glows steady green. When the module is powered-off, the Power LED is dark (unlit).
Operating state	The Enabled LED is on the lower left-hand side of the module's front panel, above the Power LED. When the module is operating properly, it glows steady green. The Enabled LED's other operational modes are as follows: • Flashing green during operation indicates that the module is in an error state and requires a reset. • Unlit (black) indicates that the module is idle.
Flow rate set point	In the Status Control's CO2 and Co-Solvent Pump subpanels, the value of the setting in the box next to **Flow** shows the current CO_2 and co-solvent flow set points in mL/min. **Important:** From the Status Control panel, you can directly control the pump's flow rate by changing the value for the **Flow** set point. If you do this while a method is controlling the system, and the method has not fully run, any manual changes you initiate remain in effect only until the method's next step, when the conditions specified in the method are reinstated.
Current flow rate	In the System Status **Pump** icons and Status Control's CO2 Pump and Co-Solvent Pump subpanels, the **Flow** indicators report the current CO_2 and co-solvent flow rates, in g/min and mL/min, respectively, in the system.
Flow plot	The Current Run Flow plot shows the real-time flow plot for the current run. **Tip:** To display the data-point coordinates in an area of a plot, in the toolbar, click **Data Cursor**, and then move the pointer over the plot area to view the data coordinates. Click **Data Cursor** again to disable the function.
RPM	The **RPM** indicator in the System Status **Pump** icon and Status Control's CO2 Pump and Co-Solvent Pump subpanels, report the pump's current operating rate. **Important:** When a pump's check valve fails, the pump's efficiency declines, causing it to run faster to maintain a specified flow rate. A typical flow rate slightly exceeds a pump's RPM multiplied by a factor of four. If the RPM is significantly greater than four times the flow reading, than one or more check valves are likely defective. Other problems possibly responsible for this condition include improper cooling or circulation of coolant on the inlet, leaking piston seals, and low supply source.

Status or control	Indicator location
Pressure	The pump's pressure gauge, on the right-hand side of the module's front panel, shows the pressure, in bar and PSI units, of the co-solvent or CO_2 flow in the system. Also, in the System Status **Pump** icon and Status Control's CO2 Pump and Co-Solvent Pump subpanels, the **Pressure** indicator shows the current CO_2 and co-solvent flow pressure in the system in bars. During operation, the CO_2 and co-solvent pumps work with the ABPR to maintain the flow and system pressure at the specified back-pressure set point. **Rule:** For the pressure readings to be valid, the pump must be operating. **Tip:** When working properly, the readings from the pressure gauge and ChromScope pressure indicators must approximate one another. The readings can be slightly higher than the system back pressure, owing to the pressure drop across the extraction vessel.

6.14.2 Determining the operational status of the heater controller module and its controlled heating devices

Status	Indicator location
Heater controller module power	The Power LED is on the lower right-hand side of the module's front panel. When the module is powered-on and working properly, the Power LED glows steady green. When the module is powered off, the Power LED is dark (unlit).
Heater controller module operating state	The Enabled LEDs, Z1 through Z6, on the module's front panel glow steady green when the represented zone is actively controlling its designated heat-exchanger module. These are the Enabled LED's other operational modes: • Flashing green during operation indicates the module is working to maintain the temperature set point, alternately activating and deactivating the heat exchanger, as needed. • Unlit (black) indicates the zone is idle.
Temperature set point and read back for the inline heater[1]	In the Status Control's Inline Heater subpanel, the value of the setting in the box next to **Temperature (C)** shows the current temperature set point, in degrees Celsius, of the extracting solvent heat exchanger (HE2) that heats the extracting solvent. The box next to the temperature set point is the read back. This read back indicator also appears in the **Inline Heater** icon in the System Status panel.

Status	Indicator location
Temperature set point and read back for the electrical vessel heaters[1]	In the Status Control's Vessel Heater 1 and Vessel Heater 2 subpanels, the value of the setting in the box next to **Temperature (C)** shows the current temperature set point, in degrees Celsius, of the individual vessel heaters. The box next to the temperature set point is the read back. This read back indicator also appears in the **Vessel Heater** icon in the System Status panel.
Temperature set point and read back for the electrical cyclone heaters[1]	In the Status Control's Cyclone Heater 1, Cyclone Heater 2, and Cyclone Heater 3 subpanels (if configured), the value of the setting in the box next to **Temperature (C)** shows the current temperature set point, in degrees Celsius, of the individual cyclone heaters. The box next to the temperature set point is the read back. This read back indicator also appears in the **Cyclone Heater** icon in the System Status panel.
Temperature plot	The Current Run Temperature plot shows the real-time temperature plots for the current run. **Tip:** To display the data-point coordinates in an area of a plot, in the toolbar, click **Data Cursor**, and then move the pointer over the plot area to view the data coordinates. Click **Data Cursor** again to disable this function.

1

Important: From the Status Control panel, you can directly control the temperature set point by changing the setting in the **Temperature** box. If you do this while a method is controlling the system, and the method has not fully run, any manual changes that you initiate remain in effect only until the method's next step, at which point the conditions specified in the method are reinstated.

6.14.3 Determining the operational status of the vessel switcher and the automated CO_2 recycler

Status	Indicator location
Vessel switcher operating status and control[1]	For systems equipped with automation, find the current operating state of the vessel switcher valve configuration in the Status Control's Vessel Switcher subpanel. This status indication also appears in the **Vessel Switcher** icon in the System Status panel. In the Vessel Switcher subpanel's **Select Vessel** box, you can change the vessel switcher valve configuration by choosing any of the following selections: • **Extraction 1** – Sets the valves to extraction vessel 1. • **Extraction 2** – Sets the valves to extraction vessel 2. • **Vessel Equilibration** – Equalizes the pressures in extraction vessel 1 and extraction vessel 2. You typically use this setting as a transitional step to reclaim CO_2 and increase throughput by directing the system to siphon the CO_2 from the pressurized vessel into the unpressurized alternative vessel before the system switches to the alternative vessel in the next step. **Note:** You specify the duration for equilibration on the Vessel Switcher configuration subpanel located under the Device Configuration node. • **Series: 1 to 2** – Connects the outflow of extraction vessel 1 to the inflow of extraction vessel 2. • **Series: 2 to 1** – Connects the outflow of extraction vessel 2 to the inflow of extraction vessel 1. **Note:** Series configurations are limited to configurations with 5-liter extraction vessels equipped with the series configuration kit.

Status	Indicator location
Automated CO_2 recycler	For systems equipped with the automated CO_2 recycler, find the current pressure, in bars, of the CO_2 recycler in the Status Control's CO2 Recycler subpanel. **See also:** Specifying settings or viewing device status for the vessel switcher. **Note:** • If the pressure is less than 4,826 kPa (48 bar, 700 psi), increase the CO_2 recycler chiller temperature. • If the pressure is greater than 4,999 kPa (50 bar, 725 psi), decrease the CO_2 recycler chiller temperature. For details and operating instructions, refer to the *CO_2 Recycler Overview and Maintenance Guide* that accompanies the system.
1 **Important:** From the Status Control panel, you can control the vessel switcher valve configuration by changing the selection in the **Select Vessel** box. If you do this while a method is controlling the system, and the method has not fully run, any manual changes that you make remain in effect only until the method's next step, at which point the conditions specified in the method are reinstated.	

6.14.4 Determining the operational status of the ABPR module

Status	Indicator location
Power	The Power LED is on the left-hand side of the module's front panel. When the module is powered-on and working properly, the Power LED glows steady green. When the module is powered-off, the Power LED is dark (unlit).
Operating state	The Enabled LED appears on the left-hand side of the module's front panel, above the Power LED. When the module is powered-on and working properly, the Enabled LED glows steady green. The Enabled LED's other operational modes are as follows: • Flashing green during operation indicates that the module is in an error state and requires a reset. • Dark (unlit) indicates that the module is idle (no run in progress).

Status	Indicator location
Current system pressure	In the System Status **ABPR** icon and Status Control's ABPR subpanel, the **Pressure** indicator shows the current system pressure measured at the pressure regulator. During operation, the CO_2 and co-solvent pumps work together with the ABPR to maintain the flow and system pressure at the specified set points. **Note:** The ABPR does not regulate pressure below 6000 kPa (60 bar, 870 psi), the lower end of the system's operating range, because of the inlet pressure of the liquid CO_2.
Pressure set point	In the Status Control's ABPR subpanel, the value of the setting in the box next to Pressure (Bar) shows the pressure regulator's pressure set point. **Important:** From the Status Control's ABPR subpanel, you can directly control the pressure set point by changing the setting in the **Pressure (Bar)** box. If a method is controlling the system and the method has not fully run, any manual changes you initiate remain in effect only until the method's next step, when the conditions specified in the method are reinstated. **Rule:** When the system flow and pressure settings are equilibrated, and the pumps and pressure regulator are operating properly, the current pressure reading indicators for the ABPR match the setting in the **Pressure (Bar)** box, within ±200 kPa (±2 bar, ±29 psi), to account for normal operating fluctuation.
Pressure Drop	The **Pressure Drop** indicator in the Status Control's ABPR subpanel shows the difference in pressure measured before and after the extraction vessel.
Pressure plot	The Current Run Pressure plot shows the real-time pressure plot for the current run. **Tip:** To display the data-point coordinates in an area of a plot, in the toolbar, click **Data Cursor**, and then move the pointer over the plot area to view the data coordinates. Click **Data Cursor** again to disable this function.
Valve temperature	In the System Messages box, with the pointer over the System Status **ABPR** icon or Status Control's ABPR subpanel, the valve-temperature indicator reports the temperature, in degrees Celsius, of the ABPR valve.
External Heater Temperature	In the System Messages box, with the pointer over the System Status **ABPR** icon or Status Control's ABPR subpanel, the **External Heater Temperature** indicator reports the temperature, in degrees Celsius, of the optional external heater, if it is connected to the ABPR. (If an external heater is not connected, the reported temperature is 0 °C.)

Status	Indicator location
Needle Position	In the Status Control's ABPR subpanel, the **Needle Position** indicator for the ABPR module indicates the extent of the valve's open position. The software can automatically open the valve to a maximum needle count of 3000. To fully open the valve and depressurize the system, you can manually set a needle count of 6000. A needle count of 0 indicates that the ABPR is closed and pressurizing the extraction vessel. **Tip:** To fully open the valve and depressurize the system, when the ABPR is idle, from the Device Configuration's ABPR subpanel, use the **Set Needle Count** setting to manually set a needle count of 6000.

6.15 Collecting fractions

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye or facial injury, wear a face shield when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid personal injury caused by contact with splashing extracted compounds or solvent when collecting fractions, before opening a cyclone separator drain, ensure that the collection-drain splash guard is in its protective position above the drain opening and fraction-collection container.

Warning: To avoid injuries caused by shattering glass fragments, do not use laboratory glassware to collect fractions or waste components. When opening drain valves, a high-pressure release of solvent or extracted compounds can knock a glass container out of your hand and cause it to shatter.

Warning: To avoid spills, empty the collection container at regular intervals.

Tip: If your application calls for reducing front-end system pressure before collecting fractions, for best results, avoid lowering the pressure at too rapid a rate. If you are in the middle of a run, lower the automated back-pressure regulator (ABPR) set point incrementally, in 2500 kPa (25 bar, 363 psi) steps, until the desired end-pressure setting is achieved. If you are at the end of a run, use the automatic depressurization settings to lower the pressure.

Important: During depressurization, the system pressure does not always achieve the specified end-pressure setting because the ABPR cannot reduce the system pressure below the level established by the manual back-pressure regulator on cyclone separator 1 (MBPR1). In this situation, specifying an end-pressure below that set by the MBPR1 has no effect.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Face shield
- Lab coat
- Several non-glass containers appropriately sized for collecting fractions and capable of fitting beneath the collection-drain splash guard.

To collect fractions:

1. Power off the CO_2 pump.
2. If the system includes an optional co-solvent pump, verify that the status for that pump is 0 mL/min, or that the pump switched off.
3. Depressurize the system to less than 6895 kPa (69 bar, 1000 psi) by slowly opening MBPR1 and MBPR2.
4. Place a collection container under one of the cyclone separator's drain outlets, beneath the splash guard, and slowly open its drain valve to begin collecting the extracted material.

   Tips:

   - If you did not depressurize the system to less than 6895 kPa (69 bar, 1000 psi), you must begin collecting fractions at CS1, and continue to CS2, and then CS3.
   - To avoid a high-pressure release and resultant loss of extracted material, use care when opening any of the cyclone separator's drain valves. You must release pressure in the cyclone separators gradually.
   - Typically, the material you collect from CS1 is the most resinous or viscous and is only soluble at high pressures.
   - Typically, the material you collect from CS2 is more solid than liquid and is only soluble at intermediate pressures.
   - Typically, the material you collect from CS3 is liquid and is only soluble at low pressures.
5. When the flow of extracted material exiting the cyclone separator stops, close its drain valve.

6. For the next cyclone separator, place a collection container under the separator's drain outlet, beneath the splash guard, and slowly open its drain valve to begin collecting the extracted material.
7. When the flow of extracted material exiting the cyclone separator vessel stops, close its drain valve.
8. For systems equipped with three cyclone separators, place a collection container under the drain outlet of the next cyclone separator, beneath the splash guard, and slowly open its drain valve to begin collecting the extracted material; otherwise, skip this step.
9. When the flow of extracted material exiting the cyclone separator stops, close its drain valve.
10. If you are not preparing the system for a subsequent run, verify that the temperature settings for the heat exchanger, extraction vessel, and cyclone separators are switched off.

6.16 Preparing for the next extraction run

After an extraction run, you must prepare the system for the next run.

Warning: To avoid burn injuries, exercise care when handling the extraction vessels, separation cyclones, heat exchanger outlet tubing, or other components heated to high temperatures or cooled to low temperatures. Wait until the hot components have sufficiently cooled or the cold components have sufficiently warmed before you handle them.

Tip: For systems equipped with dual extraction vessels that you are using one at a time, switch the flow path, to allow cleaning and reloading of the previously used vessel, and to prepare for the next run using the alternative vessel.

To prepare for the next extraction run:

1. Empty the extraction vessel and its cap for sludge deposits. If you observe sludge deposits, clean or replace the cap frits (see Emptying and cleaning the extraction vessel).
2. Inspect the view cell. If you observe liquid CO_2 or dark material in the view cell lens, clean the view cell (see Cleaning the view cell).
3. If the view cell required cleaning, after you clean it, check the CO_2 recycler for carryover (see Checking the CO_2 recycler for carryover).
4. If you observe carryover from the CO_2 recycler, clean or replace the inlet filter (see Servicing the CO_2 inlet filter), and then clean the manual back-pressure regulators (see Cleaning the manual back-pressure regulators).
5. Verify that the CO_2 supply source is sufficient and that the tank pressure is greater than 5,861 kPa (59 bar, 850 psi).

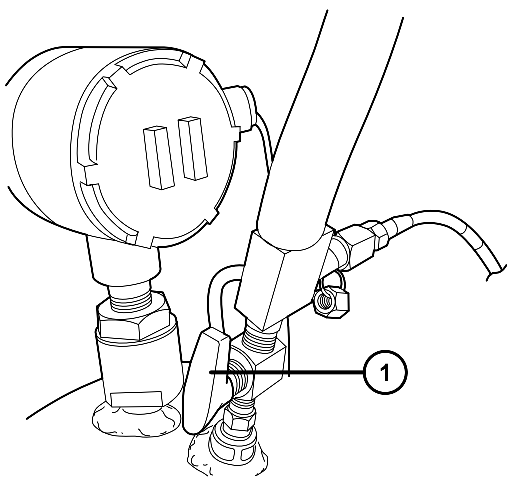
6. Ensure that the room temperature is 18 to 24 °C.

   Important: Thermal variations in excess of 2 °C per 1.5 hours can adversely affect system performance.

7. If the system is not running, ensure that the recirculation valve on the CO_2 recycler is in the open (vertical) position. When ready to run, turn the recirculation valve to the closed (horizontal) position.

   Note: The "open" recirculation valve position may also be referred to as the Standby position in other system documents.

Figure 6–24: CO_2 recycler recirculation valve handle in open (vertical) position



1 Recirculation valve handle in open (vertical) position

Tip: If the CO_2 tank is over pressure, turn the recirculation valve back to the open (vertical) position for one hour.

6.16.1 Emptying and cleaning the extraction vessel

After the extraction vessel is sufficiently cooled and switched out of the system flow, you can disconnect, empty, and clean it.

Warning: To avoid electric shock,

- ensure that the electrical power to the equipment is interrupted;
- when cleaning the surface of the equipment, apply water to a cloth, and then wipe the instrument or device. Do not spray or otherwise apply water directly onto any equipment surface.

Warning: To avoid personal injury, use eye and hand protection during the cleaning process.

Warning: To avoid burn injuries, exercise care when handling the extraction vessels, separation cyclones, heat exchanger outlet tubing, or other components heated to high temperatures or cooled to low temperatures. Wait until the hot components have sufficiently cooled or the cold components have sufficiently warmed before you handle them.

Required tools and materials

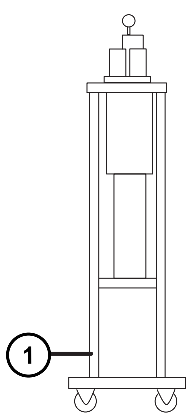
- Food-grade ethanol
- Lint-free cloth
- Mild detergent and water
- Plastic scoop or shop vacuum
- PTFE spray
- Soft bristle brush
- Steel-toed shoes
- Extraction vessel cap frit (if replacing)

To disconnect, empty, and clean the extraction vessel:

1. Ensure that the pumps and automated back-pressure regulator (ABPR) are switched off, and that the extraction vessel has cooled to a safe-handling temperature and is switched out of the system flow path.
2. Ensure that the extraction vessel's pressure gauge displays 0 kPa, (0 bar, 0 psi). If necessary, carefully drain any residual pressure from the extraction vessel by slowly opening its exhaust valve (MV6 or MV7).

   Important: For safety, this valve must be exhausted to an outside vent.

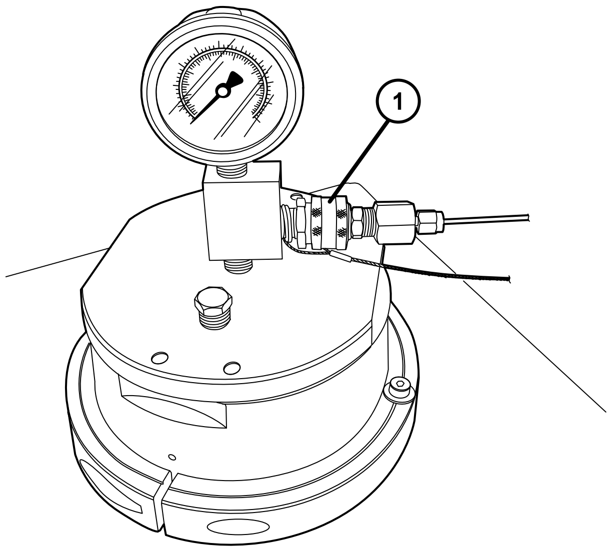
   Figure 6–25: Location of MV6 or MV7 valve on back side of extraction vessel



1 Location of MV6 or MV7 valve

3. Use the quick-disconnect fitting to disconnect the tubing connect to the extraction vessel's cap.

Figure 6–26: Quick-disconnect fitting on extraction vessel



(1) Quick-disconnect fitting

Warning: To avoid a high-pressure release of sample and solvent, or damage to the vessel's cap, never attempt to force open a vessel cap using a wrench. In the event that the vessel's pressure-escape vent becomes plugged, the vessel can remain under pressure indefinitely. If you cannot easily open the vessel's cap, allow the vessel to depressurize for at least fifteen minutes, and then try again to remove its cap.

Warning: To avoid hand or foot injury caused by accidently dropping a vessel's cap, wear steel-toed shoes and use care when handling the cap. Depending on its size, the cap can weigh as much as 5 kg.

4. Loosen and remove the extraction vessel's cap by turning it clockwise.

Notice: To avoid damaging the extraction vessel's sealing surface, do not empty the vessel using a metal implement.

5. Use a plastic scoop or shop vacuum to empty the vessel of any remaining sample material.

Notice: To avoid damaging the vessel's sealing surface, do not scrub the vessel with steel wool or a wire brush.

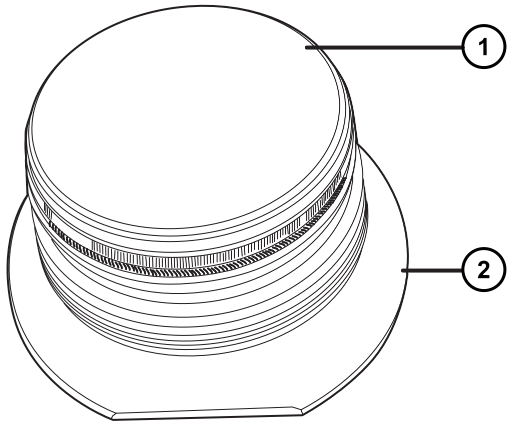
6. Optionally, spray the vessel's interior with 95% food-grade ethanol, and then wipe it dry with a clean, lint-free cloth.

7. Allow the vessel to completely air dry.

8. If you observe any sludge under the cap, do the following:

   a. Remove the frit from the cap by turning it clockwise.

   Figure 6–27: Extraction vessel cap with frit

   1 Frit

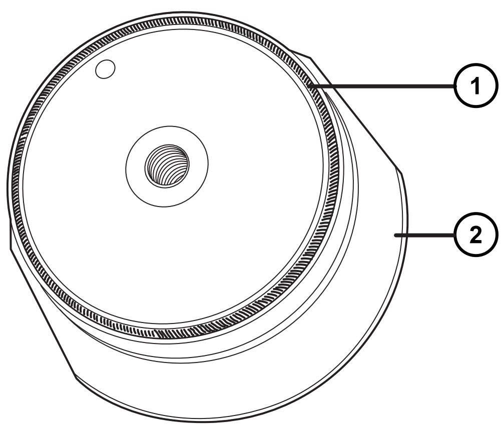
   2 Extraction vessel cap

   b. Clean the frit with 95% food-grade ethanol.

   c. If the frit is damaged, replace it.

   d. Inspect the spring in the seal and clean it with 95% food-grade ethanol, if needed.

   Figure 6–28: Extraction vessel cap with frit removed

   1 Spring in seal

   2 Extraction vessel cap

   e. Reinstall the frit on the cap.

9. Use a soft, lint-free cloth to clean the threads of the vessel's cap.

10. Apply a light coating of PTFE spray to the threads of the cap.
11. Reinstall the cap by hand-tightening it.
12. Use a cloth dampened with a solution of mild detergent and water to clean the vessel's exterior.

6.16.2 Reducing the CO_2 recycler pressure

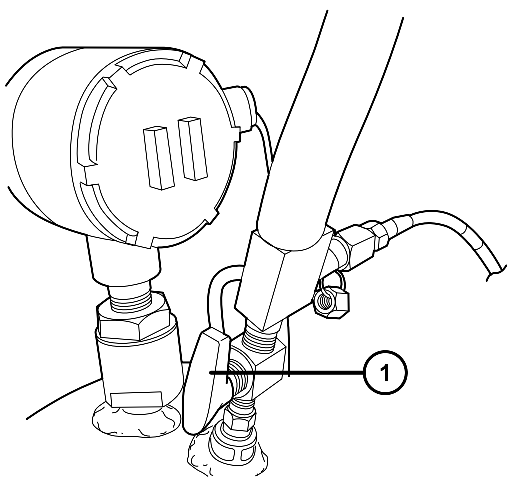
High CO_2 recycler pressure increases the risk of sample carryover to the recycler.

To reduce the CO_2 recycler pressure:

1. In the ChromScope Main window, click **Stop** to stop the system flow.
2. Set the recirculation valve on the CO_2 recycler in the open (vertical) position.

   Result: This forces gas pressure from the top portion of the recycler through the recycling cooling heat exchanger.

   Figure 6–29: CO_2 recycler recirculation valve handle in open (vertical) position

   (1) Recirculation valve handle in Open (vertical) position

3. Change the recycler chiller temperature from 11 °C to 6 °C.
4. Wait until the recycler pressure drops to approximately 4826 kPa (48 bar, 700 psi).

   Tip: The pressure drop takes approximately 30 to 90 minutes.

5. Reset the recycler chiller temperature back to the original 11 °C (or the original chiller conditions).

   Tip: 11 °C applies to 19 to 23 °C room temperature conditions.

6. Set the recirculation valve on the CO_2 recycler in the closed (horizontal) position.

7. Resume normal system operations.

6.16.3 Cleaning the view cell

Use the view cell to inspect for carryover from the cyclone separator or view cell to the CO_2 recycler. Liquid CO_2 or dark material in the view cell lens indicates carryover to the recycler. Clean the view cell, and then adjust the temperature and pressures on cyclone separator 3 (CS3) so that only CO_2 gas appears in the view cell lens.

Note: Generally, increasing the temperature or decreasing the pressure in CS3 will decrease the CO_2 density, decreasing its solvating power and causing material that was being carried over to the recycler to precipitate in CS3.

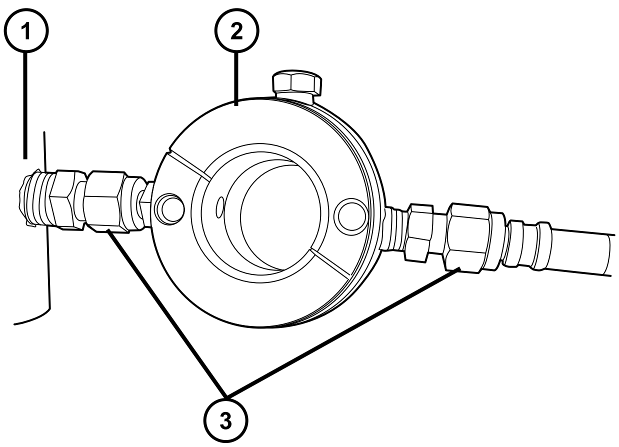
Required tools and materials

- 1-1/2-inch spanner wrench
- 9/16-inch open-end wrench
- Food-grade ethanol (for cleaning a view cell in a system extracting compounds from botanical products)
- Methanol (for cleaning a view cell in a system extracting compounds from non-botanical products)

To clean the view cell:

1. Depressurize the system and then wait 15 minutes to dissipate all pressure from the cyclone separators.
2. Use the 9/16-inch open-end wrench to loosen the two fittings that secure the view cell assembly to the back-side of the manual back-pressure regulator (MBPR) on CS3, and then remove the assembly.

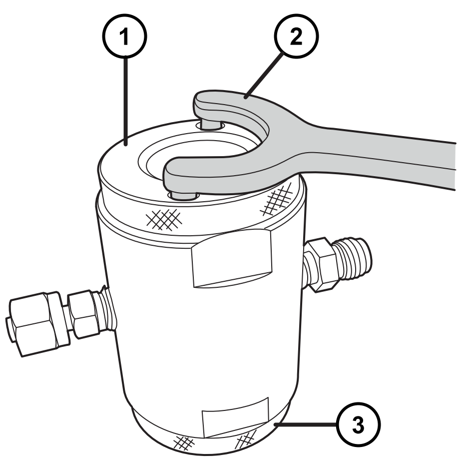
Figure 6–30: View cell installed in system



1. MBPR3
2. View cell assembly
3. Fittings

3. Use the 1-1/2-inch spanner wrench to loosen the view cell lens cover on each side of the view cell, and remove the covers.

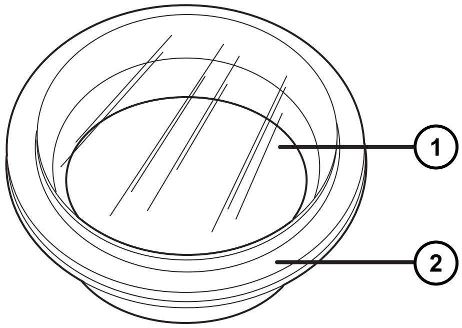
Figure 6–31: Spanner wrench on view cell lens cover



1. View cell lens cover
2. Spanner wrench
3. View cell lens cover

4. Remove both view cell lenses from the view cell and clean them with 95% food-grade ethanol.

Figure 6–32: View cell lens and O-ring



(1) Lens

(2) O-ring

5. Inspect the O-rings on each lens and replace them if necessary.
6. Reinstall the lenses and O-rings in the view cell.
7. Screw each lens cover onto the view cell, and then use the 1-1/2-inch spanner wrench to tighten them.
8. Use the 9/16-inch open-end wrench to reinstall the view cell assembly on the back-side of MBPR3.

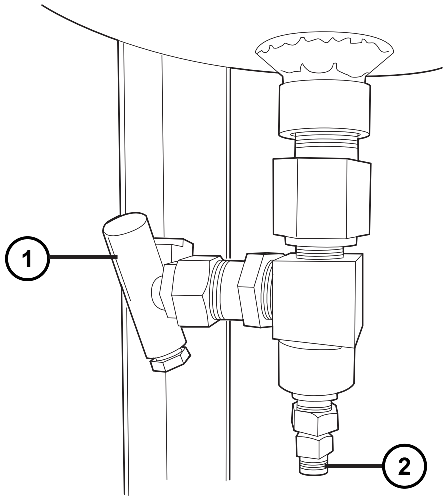
6.16.4 Checking the CO_2 recycler for carryover

Visible liquid CO_2 or dark material in the view cell lens indicates carryover to the CO_2 recycler. Clean the view cell, and then check the CO_2 recycler for carryover.

To check the CO_2 recycler for carryover:

1. Place a paper towel under the CO_2 recycler drain port, and then partially open and close its manual drain valve repeatedly to expel any sample carryover.

Figure 6–33: CO_2 recycler manual drain valve



1 Drain valve handle

2 Drain port

2. If sludge is visible on the paper towel, perform the Level 1 cleaning procedure (see Performing the Level 1 cleaning procedure).

6.16.5 Servicing the CO_2 inlet filter

An in-line filter in the CO_2 inlet line helps prevent particles from entering the system and damaging components such as check valves. A clogged filter restricts flow to the pump, diminishing its performance. Clean or replace the CO_2 inlet filter as part of a scheduled maintenance routine or whenever a clogged filter restricts CO_2 flow.

Warning: To avoid serious injury, including asphyxiation, that can result from disconnecting a pressurized CO_2 line, before disconnecting the line from the CO_2 source, stop the flow of CO_2 from the system, and shut the valve at the CO_2 supply.

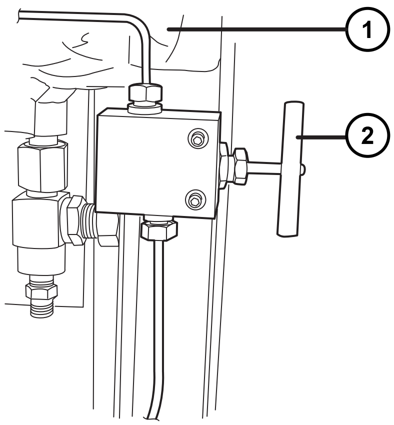
Required tools and materials

- 3/4-inch open-end wrench
- Food-grade ethanol (if cleaning)
- Ultrasonic bath
- CO_2 inlet filter (if replacing)
- CO_2 inlet filter seal (if replacing)

To service the inlet filter:

1. Close the CO_2 recycler outlet (isolation) valve.

   Figure 6–34: CO_2 recycler outlet (isolation) valve

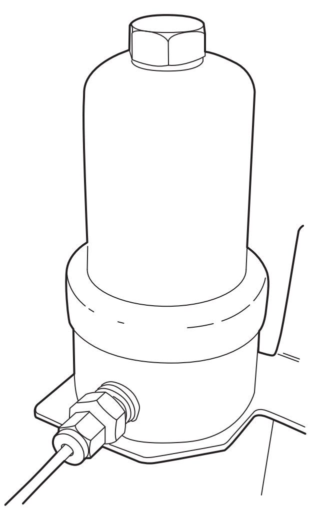
   1. Bottom of CO_2 recycler
   2. CO_2 recycler outlet (isolation) valve handle

2. On the ChromScope main page, set the CO_2 flow to `30 g/min` until it shuts down.

   Result: This will drain the CO_2 from the filter tubing and inlet.

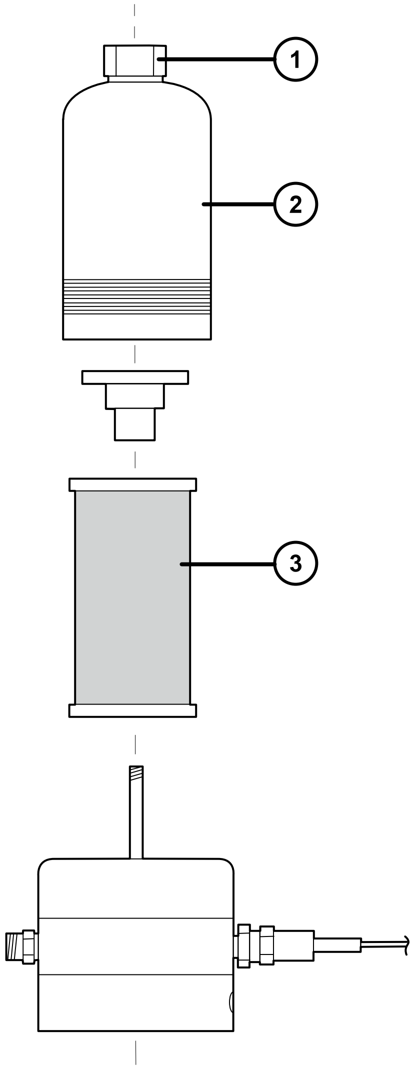
3. Locate the CO_2 inlet filter assembly where the CO_2 supply connects to the system.

   Figure 6–35: CO_2 inlet filter assembly

4. Use a 3/4-inch open-end wrench to loosen and remove the cover of the inlet filter.

Figure 6–36: CO_2 inlet filter assembly exploded view



1. Place the 3/4-inch open-end wrench here
2. Inlet filter cover
3. Inlet filter

5. Remove the inlet filter from the inlet filter housing, and then do one of the following:
   - If the filter is partially blocked, sonicate the filter in 95% food-grade ethanol for 20 minutes, and then reinstall it.
   - If the filter is completely blocked, replace the filter, and perform the level 3 cleaning procedure.

   Tip: A partially or completely blocked filter indicates that the CO_2 source might be contaminated with particulate matter.

6. Replace and tighten the inlet filter cover.
7. Open the valve to the CO_2 supply source, and inspect the inlet filter assembly for leaks.

6.17 Shutting down the system after a single extraction application

Follow this shutdown procedure when completing a single extraction or switching types of materials for extraction.

See also: For information on extracting material from the second extraction vessel, see Manually switching to the alternative extraction vessel.

Required tools and materials

- 9/16-inch open-end wrench
- 2-1/4-inch open-end wrench
- Food-grade ethanol
- Heat gun
- Shop vacuum
- Steel-toed shoes
- Soft bristle brush
- Ultrasonic bath
- Wash bottle

Shutting down the system after a single extraction application involves these steps:

1. Preparing the extraction vessel.
2. Cleaning the cyclone separators.
3. Opening the recirculation valve on the CO_2 recycler.

6.17.1 Preparing the extraction vessel

Required tools and materials

- Food-grade ethanol
- Shop vacuum
- Steel-toed shoes
- Ultrasonic bath

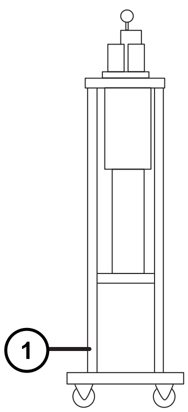
To prepare the extraction vessel:

1. Ensure that the extraction vessel being loaded has cooled to a safe handling temperature.
2. If the vessel is connected to the system, ensure that the pumps and ABPR are switched off, and that the vessel is switched out of the system flow path.
3. Depressurize the extraction vessel to at least the pressure of cyclone separator 1 (CS1).
4. Gradually open the MV6 exhaust valve to drain the CO_2 from the extraction vessel.

   Tip: The MV6 exhaust valve is located on the left, backside of the extraction vessel.

   Important: For safety, this valve must be exhausted to an outside vent.

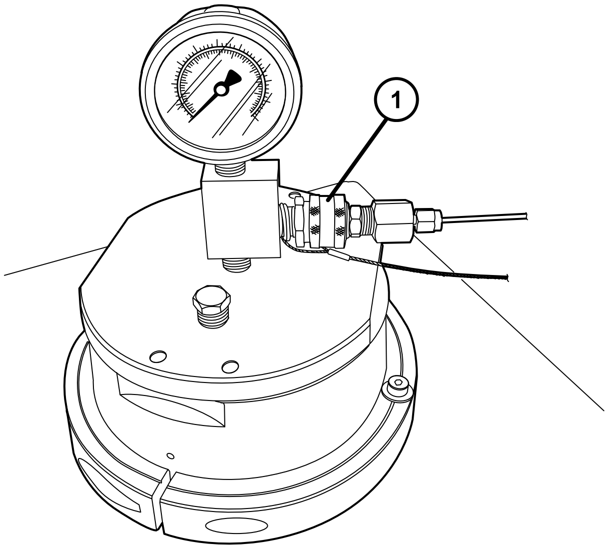
   Figure 6–37: Location of MV6 valve on back side of extraction vessel

   1 Location of MV6 valve

5. After draining the CO_2 from the extraction vessel, close the MV6 valve.
6. Confirm that the extraction vessel's pressure gauge displays 0 kPa, (0 bar, 0 psi) to ensure that all CO_2 has been drained from it.
7. Use the quick-disconnect fitting to disconnect the MV1–V1 tubing from the extraction vessel's cap.

Figure 6–38: Quick-disconnect fitting on extraction vessel



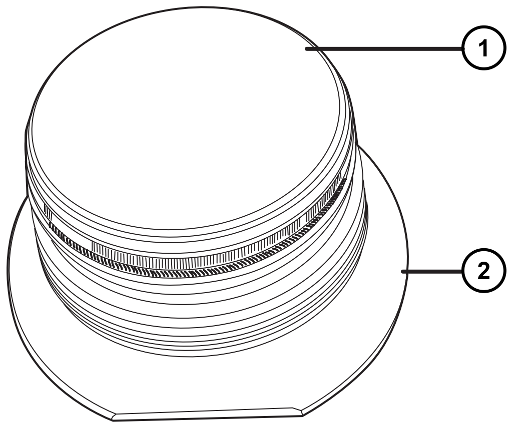
1 Quick-disconnect fitting

Warning: To avoid a high-pressure release of sample and solvent, or damage to the vessel's cap, never attempt to force open a vessel cap using a wrench. In the event that the vessel's pressure-escape vent becomes plugged, the vessel can remain under pressure indefinitely. If you cannot easily open the vessel's cap, allow the vessel to depressurize for at least fifteen minutes, and then try again to remove its cap.

Warning: To avoid hand or foot injury caused by accidently dropping a vessel's cap, wear steel-toed shoes and use care when handling the cap. Depending on its size, the cap can weigh as much as 5 kg.

8. Loosen and remove the extraction vessel's cap by turning it counterclockwise.
9. Use the shop vacuum to remove any dry waste from the extraction vessel.
10. If you observe any sludge under the cap, do the following:
    a. Remove the frit from the cap by turning it clockwise.

Figure 6–39: Extraction vessel cap with frit



1 Frit

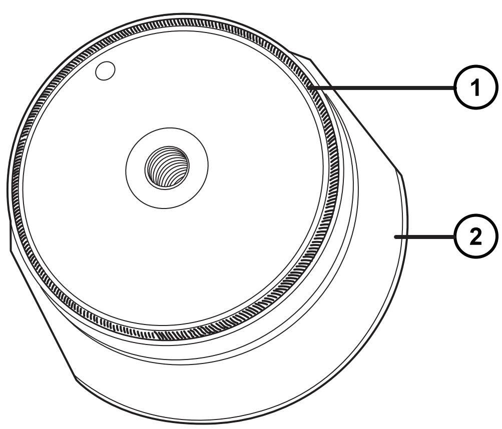
2 Extraction vessel cap

b. Inspect the frit and clean it with 95% food-grade ethanol, if needed.

c. If the frit is damaged, replace it.

d. Inspect the spring in the seal and sonicate it with 95% food-grade ethanol, if clogged.

Figure 6–40: Extraction vessel cap with frit removed



1 Spring in seal

2 Extraction vessel cap

e. If the spring is damaged, replace it.

f. Reinstall the frit on the cap.

11. Repeat the steps above to prepare a second extraction vessel.

6.17.2 Cleaning the cyclone separators and tubing

Required tools and materials

- 9/16-inch open-end wrench
- Food-grade ethanol
- Heat gun
- 2-1/4-inch open-end wrench
- Soft bristle brush with 3-inches bristles
- Ultrasonic bath (optional)
- Wash bottle

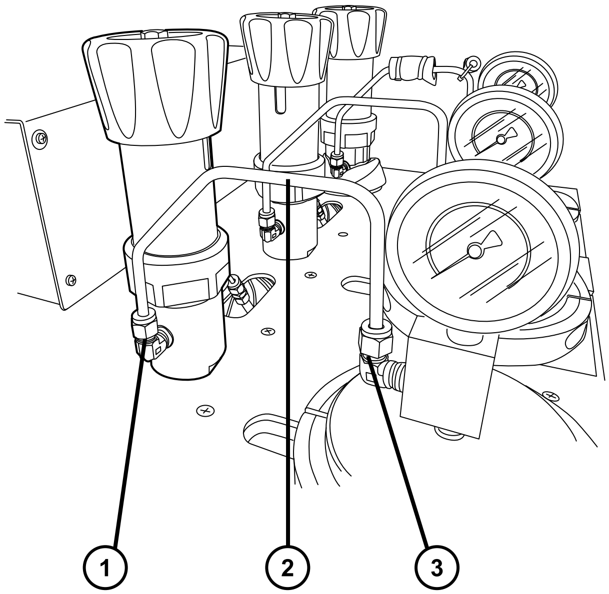
To clean the cyclone separators and tubing:

1. Confirm the system is at atmospheric pressure and that the CO_2 pump and co-solvent pump (if present) are powered-off.

   Tip: When the system reaches atmospheric pressure, the pressure reading on cyclone separator 3 (CS3) is zero.

2. In the ChromScope software, set the temperature of each cyclone separator to `40 °C`.
3. Label the tubing and each cyclone separator cap as CS1, CS2, and CS3.
4. Use the 9/16-inch open-end wrench to slowly remove the 1/4-inch tubing that connects each cyclone separator outlet to its respective manual back-pressure regulator (MBPR) inlet.

Figure 6–41: Manual back-pressure regulator inlet, tubing, and cyclone separator outlet



1 Manual back-pressure regulator inlet

2 Tubing

3 Cyclone separator outlet

5. Use a wash bottle of 95% food-grade ethanol to clean all of the tubing.

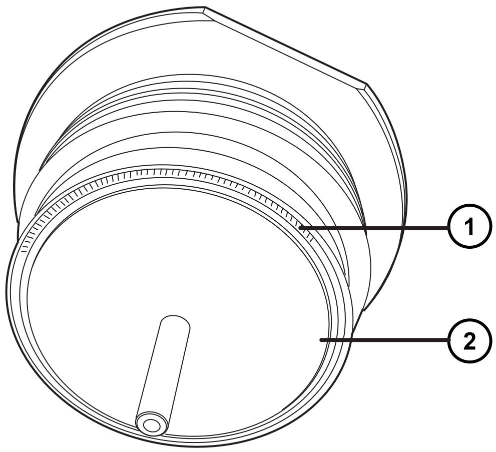
   Tip: If the tubing is severely clogged, sonicate it with 95% food-grade ethanol.

6. Remove the cyclone separator caps.

   Tip: If the cyclone separator cap will not turn, use the 2-1/4-inch open-end wrench to loosen it.

7. Inspect the O-ring on each cap and replace it if necessary.

Figure 6–42: Underside of cyclone separator cap and O-ring

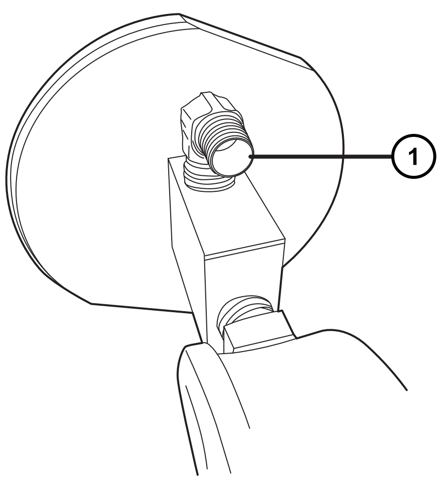


(1) O-ring

(2) Underside of cap

8. Clean the underside of each cap with the wash bottle and rag (or paper towels).
9. Squirt 95% food-grade ethanol into the outlet fittings, located on the top of each cyclone separator cap.

Figure 6–43: Location of outlet fitting on cyclone separator cap

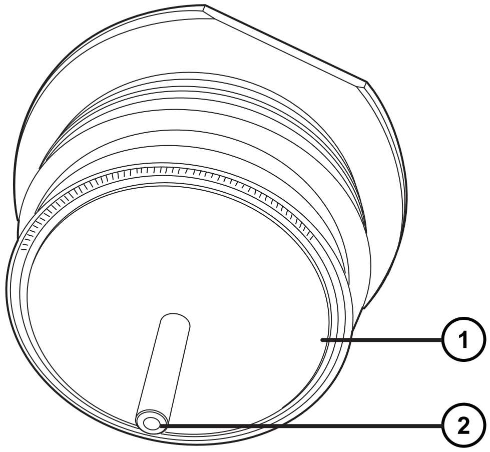


(1) Outlet fitting

10. Close each cyclone separator drain valve.

Note: The ethanol exits out of the tube on the underside of the cap.

Figure 6–44: Tube on underside of cyclone separator cap

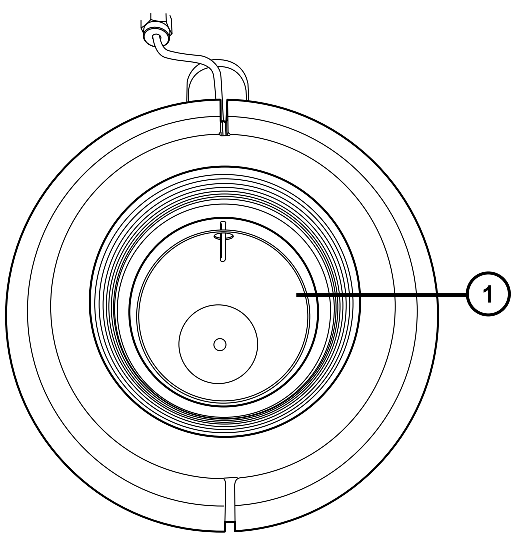


1 Underside of cap

2 Tube

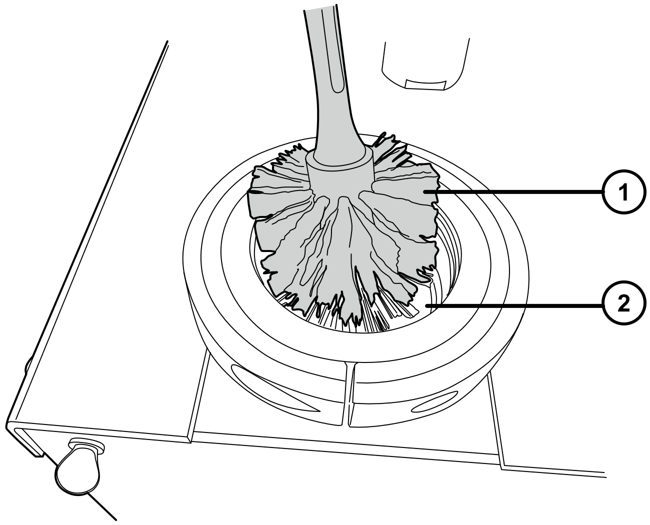
11. Place a beaker under each of the cyclone separator drain lines.
12. Squirt approximately 300 mL of 95% food-grade ethanol along the interior walls in each cyclone separator.

Figure 6–45: Cyclone separator interior wall



1 Interior wall

13. Scrub the interior walls of each cyclone separator using a soft-bristle brush.



1 Soft-bristle brush

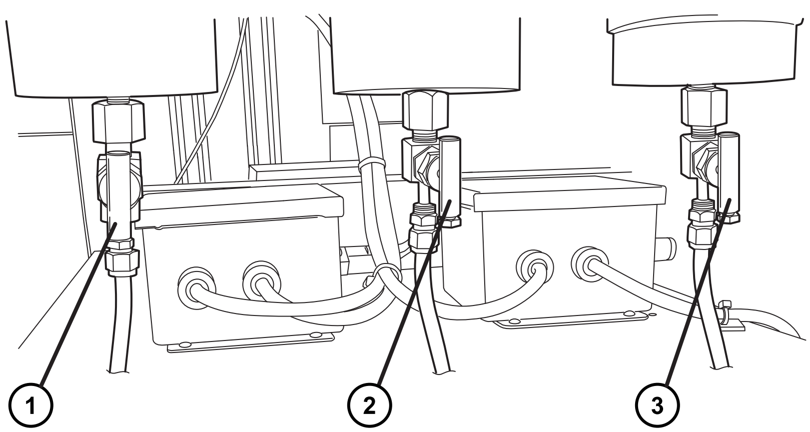
2 Interior wall

14. After approximately 5 to 10 minutes of soaking, open each cyclone separator drain valve.

    Tip: If there is wax in the cyclone separator drain valve at the beginning of the cleaning process, use a heat gun to melt it.

15. When the ethanol begins to drain, continuously squirt 95% food-grade ethanol along the interior walls of each cyclone separator until it runs clear.
16. Turn off the cyclone separator heaters.
17. Close each cyclone separator drain valve.

Figure 6–46: Cyclone separator drain valves



① CS1 drain valve

② CS2 drain valve

③ CS3 drain valve

18. Reinstall the cyclone separator caps and tubing to their labeled positions.

    Requirement: Set all the MBPRs to their original values at system startup.

19. Reconnect the stainless steel flex line from the CO_2 recycler.

6.17.3 Opening the recirculation valve on the CO_2 recycler

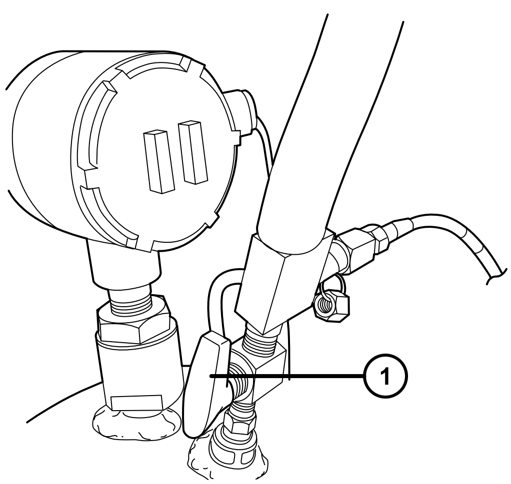
To open the recirculation valve on the CO_2 recycler:

1. Place the recirculation valve handle on the CO_2 recycler in the open (vertical) position.

   Tips:

   - Opening the CO_2 recycler's recirculation valve prevents overheating and overpressure issues.
   - The "open" recirculation valve position may also be referred to as the Standby position in other system documents.

Figure 6–47: CO_2 recycler recirculation valve handle in open position (manual recycler shown)



① Recirculation valve handle in open (vertical) position

6.18 Switching extraction vessels in a system using manual valves

After a current run ends on systems equipped with manually operated valves used to switch flow to the designated extraction vessel, to perform the next run using the alternative extraction vessel, you must stop the system flow before switching the flow path to the alternative extraction vessel (for example, switching the flow path from extraction vessel 1 to extraction vessel 2). If the system is configured with automated valve switching, these tasks are unnecessary.

Warning: To avoid burn injuries, exercise care when handling the extraction vessels, separation cyclones, heat exchanger outlet tubing, or other components heated to high temperatures or cooled to low temperatures. Wait until the hot components have sufficiently cooled or the cold components have sufficiently warmed before you handle them.

To switch extraction vessels in a system using manual valves:

1. If the pumps and ABPR remain in operation, in the ChromScope Main window, click **Stop** to switch off the system pumps, ABPR, and heaters.

   Note: This will also cancel the method.

2. To reclaim CO_2 remaining in the preceding run's extraction vessel, depressurize the system to less than 6,895 kPa (69 bar, 1,000 psi) by slowly opening MBPR1 and MBPR2.

   Tip: Depressurizing the system reduces CO_2 waste and ensures safe switching between extraction vessels.

3. Equalize the pressure between the two extraction vessels by slowly opening MV1-V2 while keeping MV1-V1 open.

4. Wait until the pressures on both vessels' gauges equalize, and then, depending on which extraction vessel you are switching to for the next run, complete one of these actions:

   - If switching from extraction vessel 1 to extraction vessel 2, keep MV1-V2 and MV2-V2 open, and close MV1-V1 and MV2-V1.
   - If switching from extraction vessel 2 to extraction vessel 1, keep MV1-V1 and MV2-V1 open, and close MV1-V2 and MV2-V2.

   Important: You must release the pressure remaining in the preceding run's extraction vessel before disconnecting the tubing from the vessel's cap and emptying, cleaning, and loading the vessel for the next run.

5. Perform the next run using the alternative extraction vessel, referring to the procedures in System startup and extraction workflow.

6.19 Switching extraction vessels in a system with an automation module

Bio-Botanical Extraction Systems with an automation module can automatically switch between extraction vessel 1 (EV1) and extraction vessel 2 (EV2). The following table shows the valid extraction selections for systems with an automation module.

Table 6–5: Extraction selections for systems with an automation module

Extraction selection	Requirements	Details
Extraction 1	Automation module	Changes the automation valves to EV1
Extraction 2	Automation module	Changes the automation valves to EV2
Vessel equilibration	Automation module	• Opens both automation valves for EV1 and EV2. • Equalizes the pressures between EV1 and EV2. This uses half of the volume of CO_2 in EV1 to fill EV2.
Series: 1 to 2	Bio-Botanical Extraction System in-line serial kit, 5-liter only	Connects the outflow of EV1 to the inflow of EV2.
Series: 2 to 1	Bio-Botanical Extraction System in-line serial kit, 5-liter only	Connects the outflow of EV2 to the inflow of EV1.

Bio-Botanical Extraction Systems that have 2-liter cyclone separators can delay collection (pulling) of the extract until after both extraction vessels have been run. Although this saves time, it does not allow volatile components (terpenes) to be collected.

Extraction sequence for collecting terpenes and oils in both 1- and 2-liter cyclone separators:

1. Extract terpenes from EV1 for approximately 60 minutes.
2. Collect terpenes in cyclone separator 3 (CS3).
3. Extract oils from EV1 for approximately 180 minutes.
4. Collect oils in CS2 and some CS1 (also waxes).
5. Reduce the flow pressure and flow rate.
6. Vessel equilibration switching EV1 to EV2 for approximately six minutes.
7. Extract terpenes from EV2 for approximately 60 minutes.
8. Collect terpenes in cyclone separator 3 (CS3).

9. Extract oils from EV2 for approximately 180 minutes.
10. Collect oils in CS2 and some CS1 (also waxes).
11. Reduce the flow pressure.
12. Power-down the system.

Extraction sequence for collecting oils in 2-liter cyclone separators:

1. Extract oils from EV1 for approximately 180 minutes or more.
2. Reduce the flow pressure and flow rate.
3. Vessel equilibration switching from EV1 to EV2 for approximately six minutes.
4. Extract oils from EV2 for approximately 180 minutes.
5. Collect oils in CS2 and some CS1 (also waxes) or more.
6. Reduce the flow pressure.
7. Power-down the system.

Extraction sequence for collecting oils in 1-liter cyclone separators:

1. Extract oils from EV1 for approximately 180 minutes or more.
2. Collect oils in CS2 and some CS1 (also waxes) or more.
3. Reduce the flow pressure and flow rate.
4. Vessel equilibration switching from EV1 to EV2 for approximately six minutes.
5. Extract oils from EV2 for approximately 180 minutes.
6. Collect oils in CS2 and some CS1 (also waxes) or more.
7. Reduce the flow pressure.
8. Power-down the system.

7 Data analysis

7.1 Data-log files

ChromScope automatically logs data about each extraction run and stores it in data-log files assigned the file extension (*.tta). These files appear in the Project's `Data Files` folder. If you specified a subfolder naming convention when setting up the project, the data-log files are organized by date, sample name, or user name, and labeled with a time stamp indicating when the run ended.

7.1.1 Viewing data-log files

Observe these guidelines when opening and viewing data-log files:

- Upon opening a data-log file, view the file's details in the tabbed area of the data grid.
- Specify a file-open mode preference for opening your data-log files according to how you want to view them.
- In the opened data-log file, right-click to select plots that you want to view in overlay.

You can export any of the plot images shown in the data-log file by creating a document and then copying and pasting the plots into it. To copy and paste a plot, right-click it and click **Save**.

Alternatively, from the toolbar, click **Copy to Clipboard** . You can then paste the plot into your document.

To view a run's data-log file:

Click the file name.

Result: The data-log file opens in the selected data-file folder. Click the tabs on the opened file to view the run's temperature, flow rate, and pressure plots. You can also click the General Info and Method Info tabs to view a record of the run's general and method information.

7.1.1.1 Setting a preference for file-open mode

You can select from three file-open modes (**New Tab**, **Current Tab**, or **Current Tab Overlay**) according to how you want to open and view data-log files. When you select a mode, all data-log files you open remain open in that mode until you select another mode. The following table describes each of the three modes and its affect on currently opened data-log files when you use it to open a new data file.

File-open mode	File tab caption upon opening a new data-log file	Effect on currently opened data-log files
New Tab	A new file (outer) tab, which displays the file's name, is created for each data-log file that you open.	All opened data-log files remain open.
Current Tab	The data-log file is opened in the currently selected tab, which displays the file's name.	The data-log file that was previously opened in the current tab is closed. Other data-log files opened in other tabs remain open.
Current Tab Overlay	The data-log file is opened in the currently selected tab, which displays the file's name.	All opened data-log files in the current tab remain open. The tab displays the name of the last opened file.

To set the file-open mode:

From the **File** menu, click **Data File Open Mode** > **New Tab**, **Current Tab**, or **Current Tab Overlay**.

Alternative: From the toolbar, click **New Tab**, **Current Tab**, or **Current Tab Overlay**.

Rules:

- For **New Tab** and **Current Tab**, the information in the data grid pertains only to the single, opened data-log file.
- For **Current Tab Overlay**, the information in the data grid encompasses all of the data-log files opened in the current tab.

7.1.2 Viewing plots

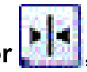
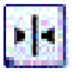
ChromScope software provides options for viewing plots that display acquired data so that you can analyze them. You can open a single plot or all of the plots for a selected data file in an expanded-view window, where you perform reporting tasks.

You can choose between the following two views of displaying the plots in the Expanded View window.

Current Graph Expanded View	Displays an enlarged view of a current plot from the open data-log file in the View window. This view is useful when you want only to analyze and work with a single plot.
All Graphs Expanded View	Displays all of the plots from the open data-log file in the View window. This view is useful when you want to generate reports of multiple plots from the same data-log file.

Tips:

- To simply view the plots in a separate window that does not include the expanded toolbar, from the **View** menu, click **Show** > **Current Graph** or **All Graphs in File**.

- To display data point coordinates in an area of a plot, in the toolbar, click **Data Cursor** , and then move the pointer over the plot area to view the data coordinates. Click **Data Cursor**  again to disable this function.

7.1.2.1 Opening a single plot in expanded view

To open a single plot in expanded view:

1. From the open data file, select the tab of the plot that you want to expand.
2. From the **View** menu, click **Expand** > **Current Graph**.

   Alternative: Double-click the file tab to view the expanded graph in a new window.

   Result: The plot appears in a new window, which automatically closes when you select another plot or data-log file.

7.1.2.2 Opening all of the plots in the expanded view

To open all of the plots in expanded view:

1. Select the tab of the opened data-log file that you want to expand.
2. From the **View** menu, click **Expand** > **All Graphs in File**.

   Alternative: Double-click the file tab to view the expanded graph in a new window.

   Result: All of the file's plots appear in a new window, which automatically closes when you select another data-log file.

7.1.2.3 Displaying the expanded view toolbar

To display the expanded view toolbar:

1. Open a plot or plots in expanded view.
2. In the expanded view window, select the control that you want from the toolbar.

7.2 Configuring your display preferences

In the Display Options panel, you can specify and save your preferences for viewing plots and their associated data in the data-log file's data grid. Use these preferences to show or hide various elements of the data-log file's raw data.

Rule: The raw data contained in the data-log file remains unchanged by these settings. Consequently, you can cancel or change your display preferences to show or hide elements that were previously hidden or displayed in the opened data-log file.

You can set display options to show or hide these elements:

- Run information that appears in the data grid
- Graph components, including individual plots such as Flow or Pressure, and their properties such as Duration Marks and Grid lines
- Options for applying actual or normalized scales to files displayed in overlay mode

You can also set display options to control the characteristics of these elements:

- General preferences for determining how flow is displayed
- Zooming controls that determine the displayed chart's viewable boundaries for all plots or just the currently active plot
- Axis offset in overlay
- Annotation orientation for duration information

7.2.1 Setting display preference for flow

You can set a preference for how you want pump flow to appear in charts.

To set display preference for flow:

1. From the **View** menu, click **Display Options** to open the Display Options panel.

   Alternative: In the toolbar, click **Display Options**  .

2. In the General Options subpanel, select **CO2 and CoSolvent Flow Values** to show both of these values in the Flow Plot, or **CoSolvent%** to show only the co-solvent percentage in the Flow Plot.
3. Click **OK** to save your display settings.

7.2.2 Setting zooming options and parameters

The zooming values determine the displayed chart's viewable boundaries.

To set zooming options and parameters:

1. From the **View** menu, click **Display Options** to open the Display Options panel.

   Alternative: In the toolbar, click **Display Options**  .

2. In the Zoom Options subpanel, select the Zooming option **Use Global Zoom** to apply the zooming controls to all plots, or **Use Local Zoom** to apply the zooming controls only to the currently active plot.
3. Specify the values for the **X Min**, **Y Min**, **X Span**, and **Y Span** parameters.

   Tip: To set a floating zoom capability that corresponds to the full data range of the displayed chart, specify `0` for the X Min, Y Min, X Span, and Y Span values.

   Table 7–1: Example

Value	Result in the displayed chart
X Min = 1	The start value for the *x* axis is 1.
Y Min = 2.5	The end value for the *y* axis is 3 (X Min + X Span).
X Span = 2	The start value for the *y* axis is 2.5.
X Span = 2	The end value for the *y* axis is 3 (Y Min + Y Span).

4. Click **OK** to save your display settings.

7.2.3 Setting run information display options

The Run Information settings affect only the data grid. Chart and reports remain unaffected.

Note: The Run Information fields provide extraction run details, such as vessel and cyclone temperatures. In the data grid, this information appears on the **Method Info** tab.

The table at the end of this procedure shows all of the Run Information options that are available to display on the **Method Info** tab.

To set run information display options:

1. From the **View** menu, click **Display Options** to open the Display Options panel.

   Alternative: In the toolbar, click **Display Options**  .

2. In the Run Information subpanel, select the individual run information options that you want to appear in the data grid, or click **Select All** to select all of the available run information options.

Tip: To hide all run information, clear the **Select All** check box and the check boxes for the individual run information options.

3. Click **OK** to save your display settings.

Table 7–2: Run information options for Method Info

Run Information options
Date Time Stamp
Method
Method Version
Sample
Extraction Vessel
Extraction Vessel 1 Temperature (C)
Extraction Vessel 2 Temperature (C)
Cyclone Vessel 1 Temperature (C)
Cyclone Vessel 2 Temperature (C)
Cyclone Vessel 3 Temperature (C)
Inline Heater Temperature (C)
Inline Collection Heater Temperature (C)
Dynamic Duration 1 (min)
Static Duration (min)
Dynamic Duration 2 (min)
CO2 Flow Rate
Co-Solvent Flow Rate
Front Pressure
Back Pressure
Pressure Drop

7.2.4 Setting annotation display options and orientation

Selected annotations appear in both charts and reports.

To set annotations display options and orientation:

1. From the **View** menu, click **Display Options** to open the Display Options panel.

Alternative: In the toolbar, click **Display Options**  .

2. In the Annotations subpanel, select the **Duration Marks Label** to display duration marks in your plots and reports.
3. Specify the orientation, in degrees, for positioning the annotations on the chart.

   Range: `0` to `180`
4. Click **Set Font** and **Set Font Color** to revise Annotation text size, font, font style, and color.
5. Click **OK** to save your display settings.

7.2.5 Setting graph display options

Graph components include individual plots, such as Flow or Pressure, and their properties such as Duration Marks and Grid Lines.

The table, at the end of this procedure, lists the graph components in the Display Options panel, which appear in the opened data-log file. Depending on a system's configuration, the temperatures for some vessels (for example Cyc Vessel 3 Temperature) may not apply.

To set graph display options:

1. From the **View** menu, click **Display Options** to open the Display Options panel.

   Alternative: In the toolbar, click **Display Options**  .
2. In the Graph Display Options subpanel, select the individual graph components that you want to appear in the data-log file and its plots, or click **Select All** to select all of the available graph components.

   Tip: To hide all graph components, clear the **Select All** check box and the check boxes for the individual graph components.
3. Click **OK** to save your display settings.

Table 7–3: Graph display options for charts

Graph display options
Temperature Plot
Flow Plot
Pressure Plot
Duration Marks
Show Grid Lines
Ext Vessel 1 Temperature

Table 7–3: Graph display options for charts (continued)

Graph display options
Ext Vessel 2 Temperature
Ext Vessel 1 Band 2 Temperature
Ext Vessel 2 Band 2 Temperature
Cyc Vessel 1 Temperature
Cyc Vessel 2 Temperature
Cyc Vessel 3 Temperature
Inline Heater Temperature
Inline Collection Heater Temperature
Ext Vessel 1 Internal Temperature
Ext Vessel 2 Internal Temperature

7.2.6 Setting overlay, axis offsets in overlay, and axis scale options

You can specify options for applying actual or normalized scales to the files displayed in overlay mode.

To set overlay options and axis offsets in overlay:

1. From the **View** menu, click **Display Options** to open the Display Options panel.

   Alternative: In the toolbar, click **Display Options** .

2. In the Overlay Options subpanel, select **Actual Overlay** to display your plots on the actual scale on the *y* axis, or select **Normalized OverLay** to display them on a normalized scale.

   Rules:

   - Normalization has no affect on data-log files that are not opened in overlay mode.
   - Normalization applies to flow and pressure plots.

3. To manually align adjacent, overlaid plots, set the offsets affecting the time (*x* axis) and the signal (*y* axis) that you require by specifying values for **X Offset** and **Y Offset** parameters.

   Result: For flow and pressure plots, an X Offset value shifts the time for each plot horizontally on the *x* axis, while a Y Offset value shifts the signal for each plot vertically on the *y* axis.

   Rules:

- Axis offset values affect only the data-log file's plots. The stored data and calculations in the data grid remain unchanged.
- When you select **Normalized Overlay**, the value for Y Offset is defined as a percentage of the normalized Y scale.

4. For the Current Run Axis Scale Options, select the appropriate run axis scale option to give **Real Time Axis Scale** or **Static Axis Scale**.

   Tip: Static Axis Scale enables manual setting of scale values, which is helpful when you want a predetermined scale.

5. Click **OK** to save your display settings.

8 Data management and reporting

8.1 Data-log files

8.1.1 Exporting a set of data-log files

You can export data-log files by group—as Microsoft Excel or CSV file formats—for use with document-processing applications and other data-control software. When you do so, the exported files are created with the name "<sourcefilename>.xls" or "<sourcefilename>.csv" and saved at a location of your choice.

To export a set of data-log files:

1. From the **Tools** menu, click **Export** > **To Excel or To CSV** > **Browse for File(s)**.
2. In the open window, select the data-log files for export.

   Tip: Use Shift or Ctrl keys to select multiple files.
3. Click **Open**.

8.1.2 Exporting individual data-log files

You can export data-log files individually as Microsoft Excel or CSV file formats for use with document processing applications and other data control software. When you do so, the exported files are created with the name "<sourcefilename>.xls" or "<sourcefilename>.csv" and saved at a location of your choice.

Rules:

- If no data-log file is open, Export > Current File is disabled.
- If a set of files is open in overlay mode, Export > Current File works only with the last opened data-log file.

To Export a currently opened data-log file:

1. Open the data-log file.
2. From the **Tools** menu, click **Export** > **To Excel or To CSV** > **Current File**.
3. Click **Open**.

8.1.3 Submitting data-log files for batch processing

You can use batch processing to process a group of data-log files sequentially, making these actions possible:

- Selecting which report templates to process in the data-log file.
- Printing batch reports on the default printer or to a PDF file.

To submit data-log files for batch processing:

1. From the **Tools** menu, click **Batch Process**.
2. In the Batch Processing window, identify the location (the system and project) containing the files that you want to process.
3. To select a common report template for all of the files you are processing, select the template from the Report Template list; otherwise, leave the Report Template box blank.

   Rule: If you leave the Report Template box blank, each data stream of the files you are processing must be reported according to the report template associated with the log files when you created the methods or the template selected during offline analysis using the Choose Report Template option.
4. To add files to the batch, select the data folder, select the files that you want to process from the Available Files list, and then click **Add files**  to move them into the Batch Process Files List.

   Tips:

   - Use the `Shift` or `Ctrl` keys to select multiple files.
   - To remove files from the Batch Process File List, select the files and click **Remove** .
5. Select **PDF** or **Default printer** for the batch report.
6. Click **Start**.

   Results:

   - Batch reports of the processed data-log files are printed on a default printer or saved as PDF files.
   - If saved as PDF files, the file names are auto-generated, and the files are saved in the defined location.

8.2 Quick reports

Quick Report is an integrated reporting utility that presents data about each run in a predefined format. You can generate a quick report from an open data-log file by saving it to a PDF file or by sending it to a printer that is connected to the ChromScope workstation.

ChromScope includes a predefined Quick Report Template, located in the `Default Project's Report Templates` folder. You can use this default template or select your own Quick Report template from the available custom report templates.

Tip: You can create a custom report template and assign it as a Quick Report template. For details, see Choosing a Quick Report Template.

See also: Custom report templates.

8.2.1 Choosing a quick report template

For open data-log files where you want to use the Quick Report printing options, you can use the default project's Quick Report Template, or select a custom report template from any of the project folders, and assign it as the Quick Report Template.

To choose a Quick Report Template:

1. From the **Tools** menu, click **Choose Quick Report Template**.
2. Select the Project folder and report template, and then click **Apply**.

   Result: The template you selected is used the next time you use the **Print Quick Report To PDF** or **Print Quick Report To Printer** options from the **File** menu.

8.2.2 Printing a quick report to a PDF file

To print a quick report to a PDF file:

1. Open the data-log file.
2. Click the tab to display the plot that you want to capture in the report.
3. From the **File** menu, click **Print Quick Report To PDF**.

   Alternative: From the toolbar, click **Print Quick Report To PDF**  .

4. In the Save dialog box, type a *name* for the PDF file or accept the default name, and click **OK**.

   Tip: The default name for the PDF file is the data-log file name appended with the date and time stamp.

Result: The file is saved under the project's `Reports` folder.

8.2.3 Printing a quick report to a printer

Requirement: You must power-on a printer connected to the ChromScope workstation.

To print a quick report to a printer:

1. Open the data-log file.
2. Click the tab to display the plot that you want to appear in the report.
3. From the **File** menu, click **Print Quick Report To Printer**, and select the printer.

   Alternative: From the toolbar, click **Print Quick Report To Printer**  to print the report on the default printer.

   Restriction: The message `Printer not available` appears when the printer is not connected properly and powered-on.

8.3 Custom report templates

You can define and save a set of custom report templates for each project. Each report template stores the information used to generate a custom report. This information includes the report's displayed information content, the order of this content, the header and footer layout, and page setup. Later, you can associate this template with a data-log file by attaching it to the opened file and use it to generate, preview, and print a custom report.

The topics in this section describe the steps for creating custom report templates and attaching them to data-log files.

8.3.1 Creating a report template

1. In the Folder View panel for the project, right-click the **Report Templates** subnode and select **Add new template**.

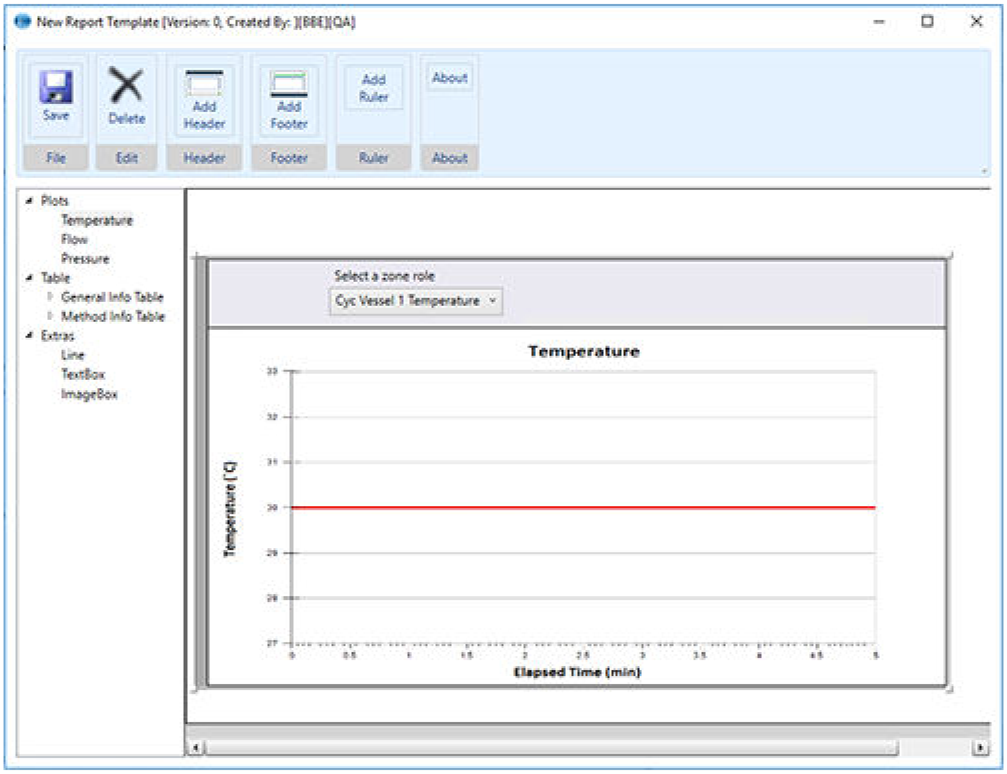
   Alternative: From the **File** menu, click **Create new project files** > ***project name*** > **Create Report Template**.
2. Define the template layout referring to the section "Report Template editor".
3. When you finish defining your report template, click **Save** to save your report template settings.

   Result: The new template file is saved under the project's Reports Template subnode.

8.3.1.1 Report template editor

To create a new report template, or open an existing one, you use the Report Template editor, which opens in the Report Template window. The Report Template window comprises three main areas: a toolbar at the top, an object navigation tree on the left-hand side, and the report canvas on the right-hand side.

Figure 8–1: Example Report Template editor window



The Report Template canvas is where you define the actual layout of the report template by dragging and dropping objects from the object navigation tree. You can then use the toolbar controls to manipulate these objects and define the page setup.

The following table describes all of the various ways that you can manipulate objects on the report canvas.

To add an object to the canvas	Click the object in the object tree, and drag the object onto the canvas. Release the button when the object is in the desired position. You can drag and drop these items: • Plots (temperature, flow, and pressure) • Tables containing the data-log file's general information and and sample list information • Individual fields from the tables • Lines, text boxes, and image boxes You can drag and drop an object multiple times. You can also insert a table with limited fields, or columns, by dragging one field at a time to the table being constructed. Alternatively, you can drag the whole table onto the canvas, and then remove the unwanted fields. **Restriction:** You cannot create a table by mixing fields from the data-log file's General Info and Method Info.
To resize an object on the canvas	Select the object and move the resize cursor on its border to stretch or reduce the object's size. For a table field, select the field-separator line, and when the cursor shape becomes a bidirectional arrow, move it to increase or decrease the width of the field.
To remove an object	Select the object and click **Delete** . To delete columns in a table, right-click the shaded region above the column header and click **Delete Column**. **Tip:** If the table has only one column, to delete it, you must select it and click **Delete** .
To reposition an object	Move the mouse pointer over the object's shaded region, and when it becomes a cross hair, click and drag the object to its new position. To reposition fields, or columns, click the shaded region above the column header and drag the field to its new position in the table.
To select multiple objects	While holding the `Ctrl` or `Shift` keys, click the objects you want selected. **Tip:** To select all of the objects on the canvas, right-click an empty place on the canvas and click **Select All**.

8.3.1.1.1 Adding and formatting text

In a text box, you can specify the font, size and style of text. You can insert system variables such as page number, total pages, and time of report generation. You can also insert variables for commonly used fields such as file name, method name, or log author.

To specify text properties:

1. Right-click the shaded region of the text box, and select **Properties**.
2. In the Text Properties window, choose how you want to specify text properties referring to the following table.

To format text characters	In the Properties window, select the Font Family, Font Size, Font Effects (Underline, Strikethrough, or None), and style (Font Bold and Font Italic) you want for the text box.
To insert system variables	In the Properties window, click **System Variable**, and select Page Number, Total Pages, or Time from the list.
To insert a data-log file field	In the Properties window, click **System Variable**, and select the field from the **Fields** sublist.
To format system variables	You format system variables in two steps: 1. Set the text format for the text box, as described above, and save your settings. 2. Reopen the Properties window, and select the variables you need.

8.3.1.1.2 Adding headers and footers

You can add footers and headers to your custom template, to display information at the top and bottom of every page on a custom report.

You can add these elements to a header or footer:

- Page number
- Total pages
- Report generation time
- Frequently used fields, including "log author", "report by", "file name", and "method name"
- Graphics, including lines and company logo images

To add a header, click the **Add Header** control. To add a text box or graphic to the header, drag and drop those objects, which appear in the object navigation tree, into the header area.

Tip: When you add a header, the **Add Header** control changes to Remove Header, which you can then click to remove the header.

To add a footer, click the **Add Footer** control. To add a text box or graphic to the footer, drag and drop those objects, which appear in the object navigation tree, into the footer area.

Tip: When you add a footer, the **Add Footer** control changes to **Remove Footer**, which you can then click to remove the footer.

8.3.2 Attaching report templates to data-log files

Observe these guidelines when attaching your report templates:

- If you assign a report template to a method step and run the method, a custom report of each plot is automatically printed in a "run and report" queue according to the assigned report template.
- You can attach a report template to an opened data-log file and associate it with the currently displayed plot.
- You can change what report templates are assigned to a data-log file at any time.
- After you attach a report template to a data-log file, you can display the custom report in a print preview window.

To attach a report template to the data-log file:

1. Open the data-log file.
2. From the **File** menu, click **Choose Report Template**.
3. Select the report template from the list of available templates.
4. Click **Apply** to attach the selected template to the opened data-log file.

   Result: The template is attached to the data-log file, so you can generate and print a custom report for the opened data-log file.

   Rules:

   - At any time, you can remove or change the report template attached to the opened data-log file.
   - Using the Report Template editor, you can modify the report template currently attached to a data-log file. After you do so, any newly generated custom reports for the file reflect the template's changes.

8.3.3 Exporting a report template

You can export a report template from one project to another.

To export a report template:

1. Click the **Report template** node, to display the currently stored instrument methods.
2. Right-click the file that you want to export, and select **Export To** and the destination project from the list.

   Result: The report template appears in the new project location.

8.4 Custom reports

After you define a custom report template and attach it to a data-log file, you can generate a custom report from the opened data-log file by saving the report to a PDF file or sending it to a printer connected to the ChromScope workstation. You can also preview a custom report of the opened data-log file in a Report Viewer window.

Alternative: You can associate a report template with the steps of a method, and specify a custom report of each plot automatically printed in a "run and report" queue, according to the assigned report template.

Depending on what input boxes and components are defined for the report template used for the custom report, the report can comprise these main elements:

- Header on each page
- Plots:
  - Flow, showing the CO_2 and co-solvent flow rates during the run
  - Pressure, showing the head-pressure and back-pressure rates during the run
  - Temperature, showing the baseline of the mobile-phase temperature during the run
- General information, or details, about the data-log file
- Method information, or details, about the run's extraction parameters
- Footer on each page

8.4.1 Previewing a custom report

To preview a custom report:

1. Open the data-log file.
2. From the **File** menu, click **Preview Custom Report**, and then click **OK**.

   Alternative: From the toolbar, click **Preview Custom Report**  .

   Result: The custom report opens in the Report Viewer window.

8.4.2 Printing a custom report to a PDF file

Requirement: A report template must be attached to the data-log file that you want captured in the report. If a template was not previously attached, the Choose Report Template dialog box appears.

To print a custom report to a PDF file:

1. Open the data-log file.
2. Click the tab to display the plot that you want to capture in the report.
3. From the **File** menu, click **Print Custom Report To PDF**.

   Alternative: From the toolbar, click **Print Custom Report To PDF** .
4. In the Save dialog box, type a *name* for the PDF file or accept the default name, and click **OK**.

   Tip: The default name for the PDF file is the data-log file name appended with the date and time stamp.

   Result: The file is saved under the project's `Reports` folder.

8.4.3 Printing a custom report to a printer

Requirements:

- You must power-on a printer connected to the ChromScope workstation.
- A report template must be attached to the data-log file that you want captured in the report. If one was not previously attached, the Choose Report Template dialog box appears.

To print a custom report to a printer:

1. Open the data-log file.
2. From the **File** menu, click **Print Custom Report To Printer** and select the printer.

   Alternative: From the toolbar, click **Print Custom Report To Printer**  to print the report on the default printer.

   Important: The message `Printer not available` appears if the printer is not connected properly and powered-on.

8.5 Configuring auto report mail

You can configure an option to automatically distribute reports to a list of email recipients as part of a method's batch "run and report" queue. The reports are based on the Report Template settings in the method.

To configure the auto report mail option:

1. From the **Configure** menu, click **Configure Auto Report Mail**.
2. In the Configure Mail window, for each recipient, type the recipient's email address and click **Add to Recipient** to add the recipient's name to the "To:" list box.

   Tip: To remove a recipient's name from the "To:" list box, select the name and click **Remove** .
3. To set a default mail subject line, type the text for your subject in the **Set Mail Subject** text box, and then select the **Apply Default Mail Subject** check box.

   Requirement: To edit subject text in the **Set Mail Subject** text box, you must first clear the **Apply Default Mail Subject** check box.
4. To set a default mail body text, type the text for your mail in the **Set Mail Body** text box, and then select the **Apply Default Mail Body** check box.

   Requirement: To edit mail body text in the **Set Mail Body** text box, you must first clear the **Apply Default Mail Body** check box.
5. Consult your company's IT department to specify the remaining settings for **Sender Email Address**, **Sender Email Password**, and the **Mail Server Address** and **Mail Server Port** settings.
6. Click **OK** to save your settings.

9 System maintenance

9.1 Cleaning guidelines for all configurations

Use these tables as a guide for cleaning the Bio-Botanical Extraction System. When cleaning the system, always observe Good Laboratory Practice (GLP) guidelines and your laboratory's standard operating procedures.

Table 9–1: Cleaning level descriptions

Cleaning level	Cleaning description
1	Flush the system
2	• Clean the manual back-pressure regulators (MBPR) • Check and clean the view cell • Check the CO_2 recycler for carryover • Check and clean the inlet filter
3	Clean the CO_2 cooling heat exchanger (HE1) tubing, inlet tubing, and flow meter
4	Clean the CO_2 recycler

Table 9–2: Cleaning guidelines

When to clean	Level 1 cleaning	Level 2 cleaning	Level 3 cleaning	Level 4 cleaning
After every use	x	x		
Before running a material that differs substantially from the last material run	x	x	x	x
After every 6 extractions of heavy-wax material			x	
After every 10 extractions of low-wax material			x	
After running any material suspected of being contaminated with pesticides or mold	x	x	x	x

Table 9–2: Cleaning guidelines (continued)

When to clean	Level 1 cleaning	Level 2 cleaning	Level 3 cleaning	Level 4 cleaning
When the recycler tank pressure drops too low				x
When the CO_2 inlet density is too low (less than 0.87 g/cm³), but the CO_2 recycler pressure is within an acceptable range of 4654 to 5171 kPa (47 to 52 bar, 675 to 750 psi)			x	x

9.2 Maintenance schedule

Use this table as a guide for planning system maintenance. When performing service or maintenance tasks, always observe Good Laboratory Practice (GLP) guidelines and your laboratory's standard operating procedures.

Table 9–3: System maintenance tasks

Task	Component	Maintenance interval
Clean the view cell	Cyclone separator 3 (CS3) tubing	Once a week or when liquid CO_2 or dark material appears in the view cell lens.
Clean the manual back-pressure regulators	Manual back-pressure regulators	Once a year or as necessary.
Service the CO_2 filter	CO_2 filter	Replace every six months, or when a clogged filter impedes CO_2 flow.
Replace the CO_2 pump's inlet and outlet check valves	CO_2 pump	When pump performance degrades.
Replace the CO_2 pump's piston seals	CO_2 pump	When pump performance degrades.
Replace the CO_2 pump's rupture disc	CO_2 pump	As necessary.
Replace the co-solvent pump's inlet and outlet check valves	Co-solvent pump	When pump performance degrades.
Replace the co-solvent pump's piston seals	Co-solvent pump	When pump performance degrades.

Table 9–3: System maintenance tasks (continued)

Task	Component	Maintenance interval
Replace the co-solvent pump's rupture disc	Co-solvent pump	Once a year or as necessary.
Replace the vessel's rupture discs	Extraction vessels and cyclone separators	Once a year or as necessary.
Replace fuses in rear of modules	CO_2 pump, automated back-pressure regulator, heat exchanger, and six-zone heater controller modules	Replace upon failure.
Clean extraction and collection vessel surfaces	Extraction and collection vessels	Clean exterior surfaces as needed. Clean interior surfaces after each run, or as needed.

9.3 Spare parts

To ensure that your system operates as designed, use only Waters Quality Parts. Visit www.waters.com/wqp for information about Waters Quality Parts, including how to order them.

9.4 Performing the Level 1 cleaning procedure

Perform the Level 1 cleaning procedure after every use and before you run a material that significantly differs from the last material run.

Warning: To avoid eye or facial injury, wear a face shield when performing this procedure.

Required tools and materials

- Face shield
- Thermally insulated gloves
- Large beaker (polypropylene or metal, 1 L)

To perform the Level 1 cleaning procedure:

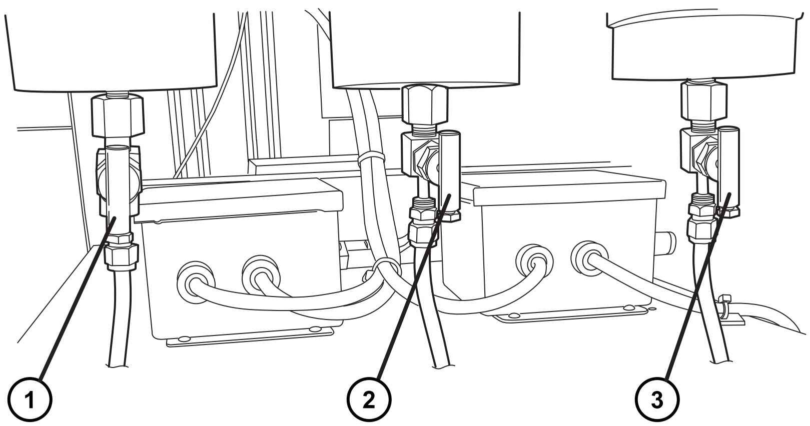
1. Ensure that there is no botanical material in the extraction vessels.

Note: You can use marbles or other inert material to fill the void in the extraction vessels. Doing so fills the system with CO_2 faster.

2. In the ChromScope software, set the CO_2 flow to 50 g/min and the automated back-pressure regulator (ABPR) to 1551 kPa (15.5 bar, 225 psi).
3. Specify normal pressures and temperatures for the cyclone separators.
4. Allow the system to run for a minimum of 1 hour at normal operating pressure.

   Tip: If possible, allow the system to run overnight.
5. Open the drain valve on cyclone separator 3 (CS3), then cyclone separator 2 (CS2), and then cyclone separator 1 (CS1).

   Figure 9–1: Cyclone separator drain valves

   (1) CS1 drain valve

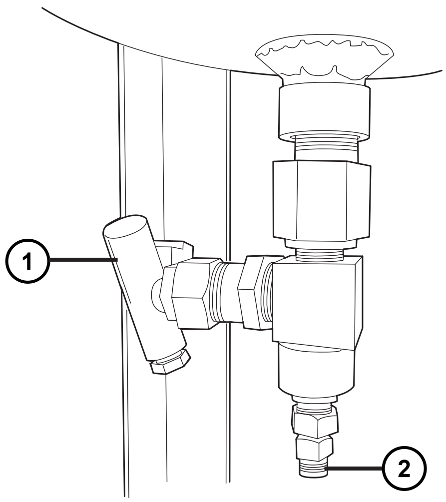
   (2) CS2 drain valve

   (3) CS3 drain valve
6. Close the CO_2 inlet valve to the recycle tank, and then close the CO_2 outlet valve from the recycle tank to the mass flow controller.

   Tip: This isolates the CO_2 recycler tank.

   Warning: To avoid injuries caused by shattering glass fragments, do not use laboratory glassware to collect fractions or waste components. When opening drain valves, a high-pressure release of solvent or extracted compounds can knock a glass container out of your hand and cause it to shatter.
7. Place a beaker under the CO_2 recycler drain outlet.
8. Slowly open the CO_2 recycler manual drain valve to inspect for oils that carried over to the recycler.

Figure 9–2: Drain valve handle and outlet on bottom of CO_2 recycling vessel



1 Drain valve handle

2 Drain outlet

Warning: To avoid freeze burns when closing the CO_2 recycler manual drain valve, wear thermally insulated gloves. During draining, the drain valve can freeze due to Joule-Thompson cooling, and its temperature can fall as low as -78 °C.

Warning: To avoid eye or facial injury, wear a face shield when closing the CO_2 recycler manual drain valve. As the drain valve begins to warm, frozen CO_2 blocking the outlet could be explosively expelled.

9. When you see no more oils and only CO_2, close the CO_2 recycler manual drain valve.

   Tip: The CO_2 recycler tank can take several hours to drain, depending on the amount of CO_2 in the tank. When draining, the drain valve may freeze due to Joule-Thompson cooling and clog the drain outlet with frozen CO_2. As the drain outlet begins to warm, the frozen CO_2 may be explosively expelled causing a loud sound and an explosive release of CO_2. Wear a face shield and thermally insulated gloves to protect from freeze burns. The drain valve on the recycle tank will go to as low as -78 °C during draining.

9.5 Performing the Level 2 cleaning procedure on a system with a co-solvent pump

Perform the Level 2 cleaning procedure after every use and before you run a material that significantly differs from the last material run.

Tip: Ensure that you have all of the required tools and materials in stock before performing this procedure.

Required tools and materials

- 9/16-inch open-end wrench
- 2-1/4-inch open-end wrench
- Compressed air
- Food-grade lubricant
- Food-grade ethanol (1 L)
- Heat gun
- Soft bristle brush
- Ultrasonic bath
- Wash bottle

Performing the Level 2 cleaning procedure on a system with a co-solvent pump involves these steps:

1. Preparing the system and priming the co-solvent pump.
2. Flushing the system.
3. Cleaning the cyclone separators and their tubing.
4. Cleaning the manual back-pressure regulators.
5. Cleaning the view cell.
6. Checking the CO_2 recycler for carryover.
7. Servicing the CO_2 filter.

9.5.1 Preparing a system with a co-solvent pump for cleaning

Required tools and materials

- 9/16-inch open-end wrench
- Food-grade ethanol (1 L)

To prepare a system with a co-solvent pump for cleaning:

1. Prepare a solvent reservoir of 1 L 95% food-grade ethanol and place the pump siphon tube into it.
2. Prime the co-solvent pump.

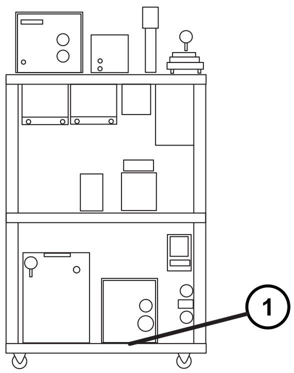
a. In the ChromScope software, stop the operation of the co-solvent pump.

b. Place the solvent reservoir from which you are drawing solvent at the same level as the pump or higher.

c. Ensure that the weighted, solvent-filter inlet is fully submerged in the solvent reservoir.

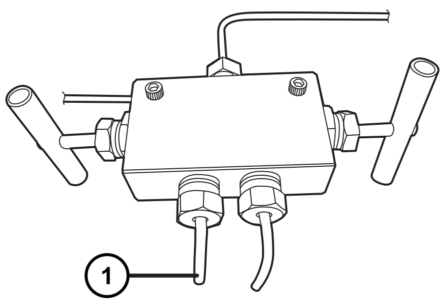
d. Place a waste receptacle beneath the MV4 priming outlet to collect the solvent.

Figure 9–3: Location of MV4 valve on module cart



1 Location of MV4 valve

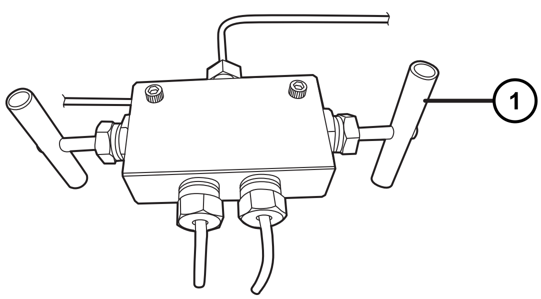
Figure 9–4: MV4 priming outlet



1 MV4 priming outlet

e. Close the MV4 system valve, which directs flow to the system.

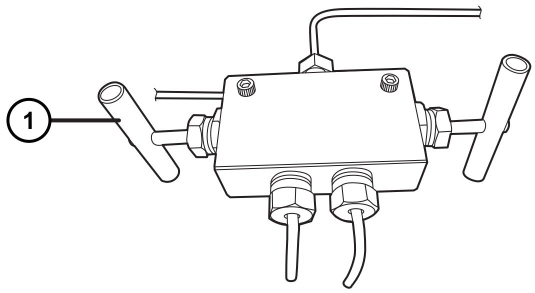
Figure 9–5: MV4 system valve handle



1 MV4 system valve

f. Slowly open the MV4 priming valve, which directs flow to the priming outlet.

Figure 9–6: MV4 priming valve handle



1 MV4 priming valve

Tip: Open the priming valve slowly. Any pressure remaining in the pump escapes through the valve.

g. In the ChromScope software, start the solvent flow.

h. Observe the flow until a steady stream exits the outlet of the priming valve, and then stop the operation.

Notice: To avoid a high-pressure burst of the pump's rupture disc, always leave one side open on the MV4 valve. Operating the pump with both sides of the MV4 valve closed will cause the rupture disc to immediately burst.

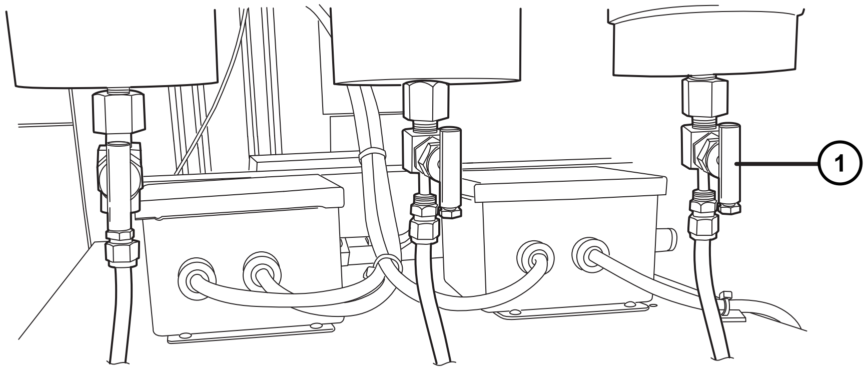
i. To complete the priming operation, close the MV4 prime valve, which closes the flow path to the priming valve, and then open the MV4 system valve, which opens the flow path to the system.

3. Ensure that there is no botanical material in the extraction vessels.

   Note: You can use marbles or other inert material to fill the void in the extraction vessels. Doing so fills the system with CO_2 faster.

4. Slowly open the drain valve on cyclone separator 3 (CS3) to bleed off the residual CO_2.

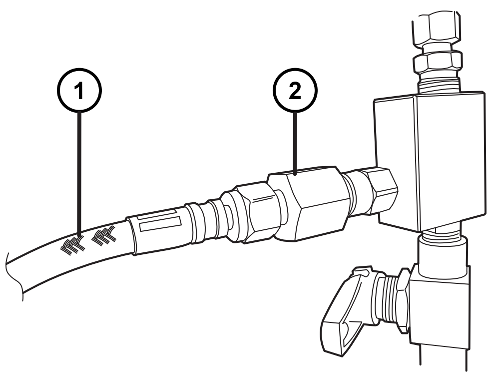
Figure 9–7: Location of CS3 drain valve



① CS3 drain valve

5. Use the 9/16-inch open-end wrench to slowly disconnect the stainless steel flex line from the exhaust inlet atop the CO_2 recycler to prevent co-solvent from getting into the recycler.

Figure 9–8: Stainless steel flex line and CO_2 recycler exhaust inlet



① Stainless steel flex line

② Exhaust inlet atop CO_2 recycler

6. Route the stainless steel flex line to a suitable exhaust system.

9.5.2 Flushing the system

To flush the system:

1. In the ChromScope software, set the CO_2 flow to 80 g/min.

   Note: You might need to fill the CO_2 recycler during this procedure.

2. If your system has a co-solvent pump, set the flow to 20 mL/min.

3. Set the automated back-pressure regulator (ABPR) to 19,995 kPa (200 bar, 2900 psi).

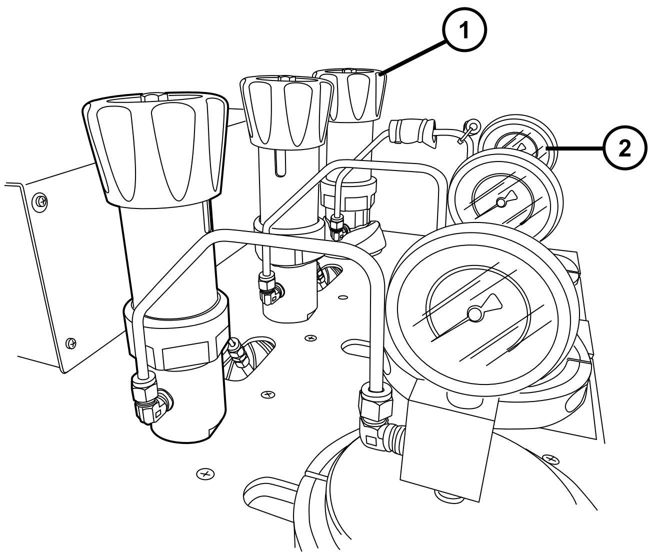
4. Set the extracting solvent heat exchanger (HE2) temperature to 70 °C.

5. Set the extraction vessel temperature to 60 °C.

6. Set the cyclone separator 3 (CS3) manual back-pressure regulator (MBPR) to 2068 kPa (21 bar, 300 psi).

   Important: Do not change the other cyclone separator settings.

   Figure 9–9: Location of CS3 MBPR and pressure gauge

   1 CS3 MBPR

   2 CS3 pressure gauge

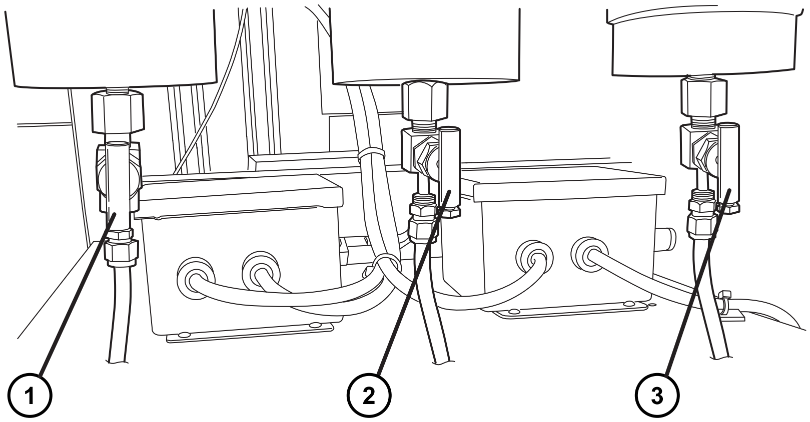
7. Ensure that the cyclone separator temperature settings are specified as follows:
   - CS1: 60 °C
   - CS2: 60 °C
   - CS3: 35 °C

8. If your system has a co-solvent pump:
   a. Start the CO_2 and co-solvent pumps and allow the flow to continue for approximately 25 minutes.
   b. After 25 minutes, stop the co-solvent pump and continue the CO_2 flow for 1 hour, to flush any residual ethanol out of the lines.

9. If your system does not have a co-solvent pump, start the CO_2 pump and allow the flow to continue for 85 minutes.

10. Open the drain valves on the cyclone separators (CS1, CS2, and CS3) slowly, to allow the ethanol to drain completely.

Figure 9–10: Cyclone separator drain valves



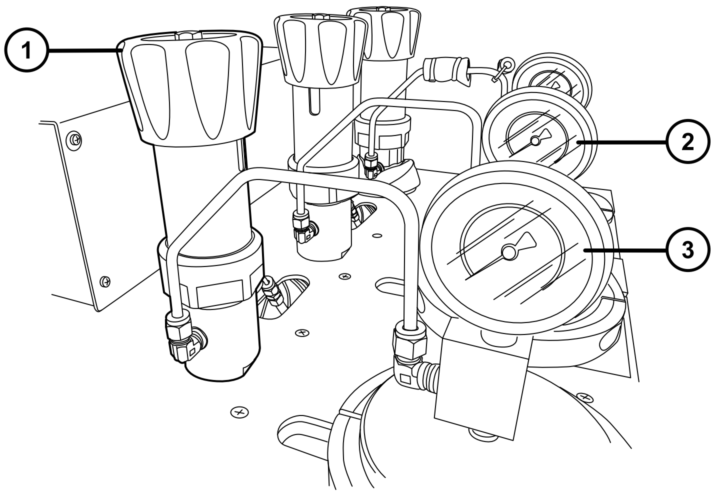
1 CS1 drain valve

2 CS2 drain valve

3 CS3 drain valve

11. After the ethanol drains, increase the pressure on CS3 to 2068 kPa (21 bar, 300 psi).
12. Depressurize the extraction vessel in 1999 kPa/min (20 bar/min, 290 psi/min) increments, to the pressure of CS1.
13. Slowly re-open the drain valves on CS1, CS2, and CS3 to verify that all the ethanol is drained from the system.
14. After the ethanol drains completely, close the drain valves on CS1, CS2, and CS3.
15. Stop the CO_2 pump, power-off the heaters, and bleed off the extraction vessel.
16. Set the temperature of CS1, CS2, and CS3 to 45 °C.
17. Slowly reduce the pressure (counterclockwise) on CS1 MBPR at a rate of 1999 kPa/min (20 bar/min, 290 psi/min), until it reaches the pressure of CS2.

Figure 9–11: CS1 MBPR and pressure gauges



1	CS1 MBPR
2	CS2 pressure gauge
3	CS1 pressure gauge

18. Slowly reduce the pressure on CS2 MBPR until it reaches the pressure of CS3.
19. Slowly open the drain valve on CS3 and continue to remove the residual CO_2.
20. While the CS3 is draining, open the MBPRs on CS1 and CS2 completely.

9.5.3 Cleaning the cyclone separators and tubing

Required tools and materials

- 9/16-inch open-end wrench
- Food-grade ethanol
- Heat gun
- 2-1/4-inch open-end wrench
- Soft bristle brush with 3-inches bristles
- Ultrasonic bath (optional)
- Wash bottle

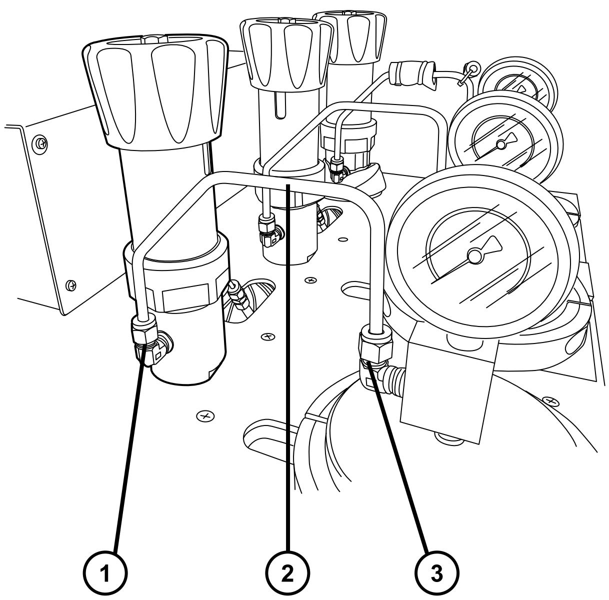
To clean the cyclone separators and tubing:

1. Confirm the system is at atmospheric pressure and that the CO_2 pump and co-solvent pump (if present) are powered-off.

   Tip: When the system reaches atmospheric pressure, the pressure reading on cyclone separator 3 (CS3) is zero.

2. In the ChromScope software, set the temperature of each cyclone separator to `40 °C`.
3. Label the tubing and each cyclone separator cap as CS1, CS2, and CS3.
4. Use the 9/16-inch open-end wrench to slowly remove the 1/4-inch tubing that connects each cyclone separator outlet to its respective manual back-pressure regulator (MBPR) inlet.

   Figure 9–12: Manual back-pressure regulator inlet, tubing, and cyclone separator outlet

   1. Manual back-pressure regulator inlet
   2. Tubing
   3. Cyclone separator outlet

5. Use a wash bottle of 95% food-grade ethanol to clean all of the tubing.

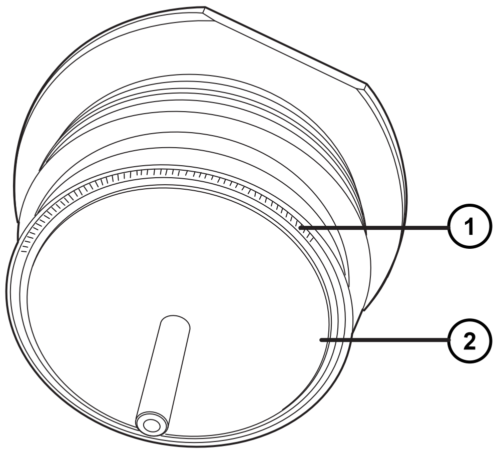
   Tip: If the tubing is severely clogged, sonicate it with 95% food-grade ethanol.

6. Remove the cyclone separator caps.

   Tip: If the cyclone separator cap will not turn, use the 2-1/4-inch open-end wrench to loosen it.

7. Inspect the O-ring on each cap and replace it if necessary.

Figure 9–13: Underside of cyclone separator cap and O-ring

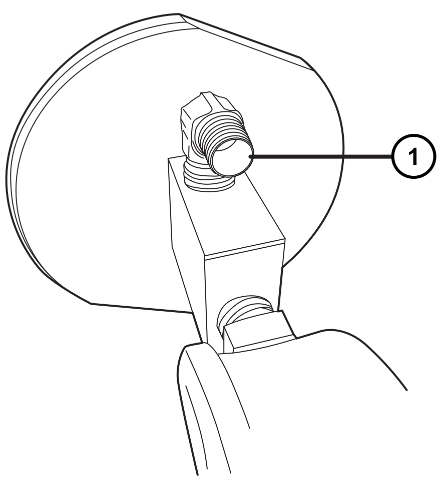


1 O-ring

2 Underside of cap

8. Clean the underside of each cap with the wash bottle and rag (or paper towels).
9. Squirt 95% food-grade ethanol into the outlet fittings, located on the top of each cyclone separator cap.

Figure 9–14: Location of outlet fitting on cyclone separator cap

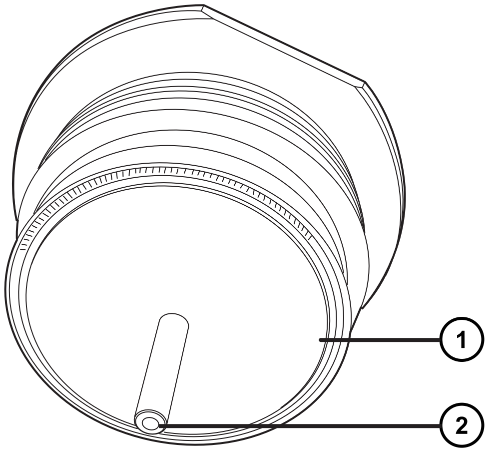


1 Outlet fitting

10. Close each cyclone separator drain valve.

Note: The ethanol exits out of the tube on the underside of the cap.

Figure 9–15: Tube on underside of cyclone separator cap

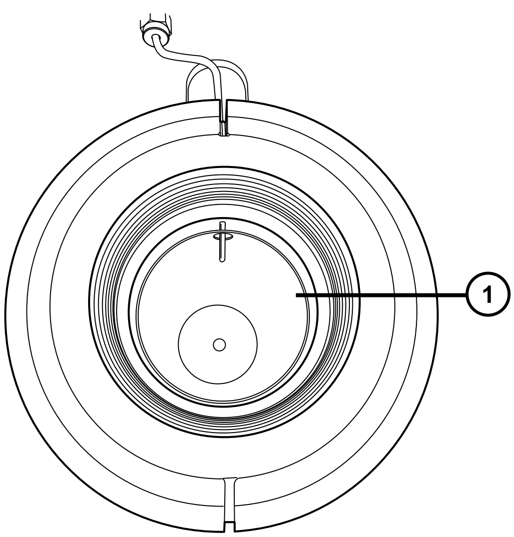


1 Underside of cap

2 Tube

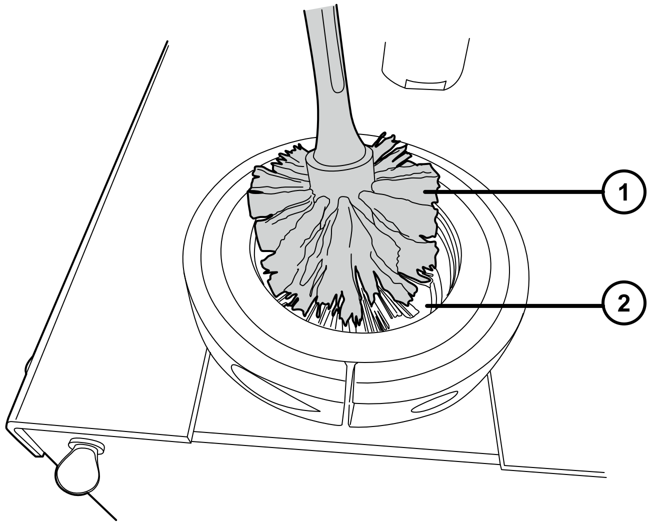
11. Place a beaker under each of the cyclone separator drain lines.
12. Squirt approximately 300 mL of 95% food-grade ethanol along the interior walls in each cyclone separator.

Figure 9–16: Cyclone separator interior wall



1 Interior wall

13. Scrub the interior walls of each cyclone separator using a soft-bristle brush.



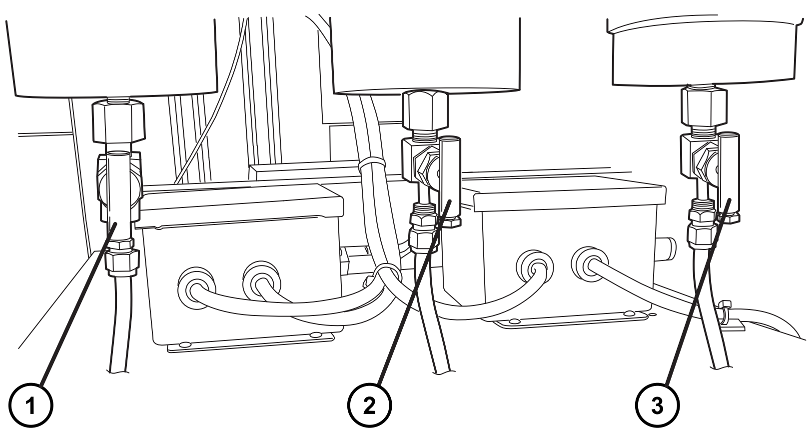
1. Soft-bristle brush
2. Interior wall

14. After approximately 5 to 10 minutes of soaking, open each cyclone separator drain valve.

    Tip: If there is wax in the cyclone separator drain valve at the beginning of the cleaning process, use a heat gun to melt it.

15. When the ethanol begins to drain, continuously squirt 95% food-grade ethanol along the interior walls of each cyclone separator until it runs clear.
16. Turn off the cyclone separator heaters.
17. Close each cyclone separator drain valve.

Figure 9–17: Cyclone separator drain valves



1 CS1 drain valve

2 CS2 drain valve

3 CS3 drain valve

18. Reinstall the cyclone separator caps and tubing to their labeled positions.

    Requirement: Set all the MBPRs to their original values at system startup.

19. Reconnect the stainless steel flex line from the CO_2 recycler.

9.5.4 Cleaning the manual back-pressure regulators

Clean the manual back-pressure regulators (MBPRs) whenever carryover is observed in the CO_2 recycler.

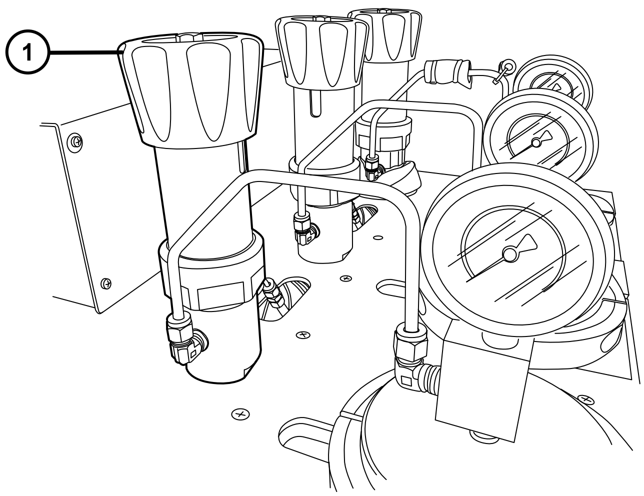
Required tools and materials

- 2-1/4-inch open-end wrench
- Compressed air
- Food-grade ethanol
- Food-grade lubricant
- 13,790 kPa (138 bar, 2000 psi) or 41,369 kPa (414 bar, 6000 psi) MBPR rebuild kit

To clean the manual back-pressure regulators:

1. Relieve all pressure from the system.
2. Turn the large, blue knob on the top of the MBPR fully counterclockwise to reduce the pressure.

Figure 9–18: Blue knob on MBPR

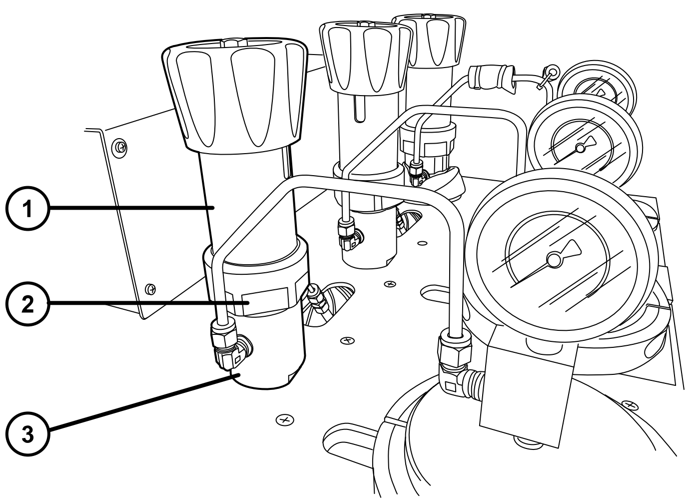


① Blue knob

3. Use the 2-1/4-inch open-end wrench to loosen and remove the spring housing from the regulator body.

   Note: The range spring will fall out of the spring housing when the housing is removed from the regulator body.

Figure 9–19: Location of wrench placement on MBPR body



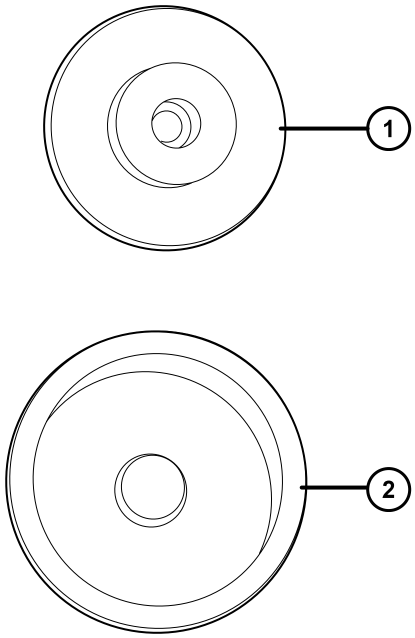
① Spring housing

② Place the 2-1/4-inch open-end wrench here

③ Regulator body

4. Remove the lower spring guide and piston/cylinder assembly.

Figure 9–20: Lower spring guide and piston/cylinder assembly

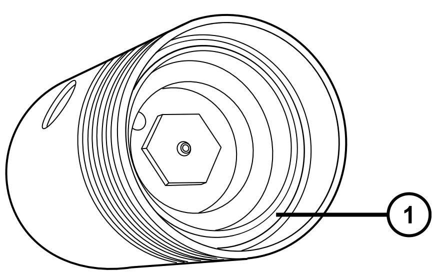


1 Lower spring guide

2 Piston/cylinder assembly

5. Carefully remove the cavity O-ring from the regulator body and discard it.

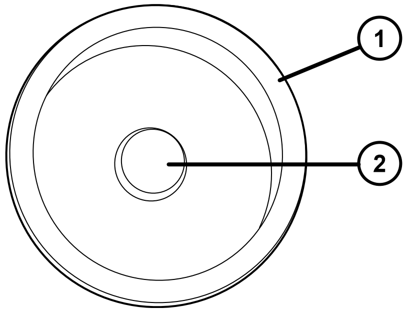
Figure 9–21: Location of cavity O-ring



1 Cavity O-ring

6. Thoroughly clean the cavity body with 95% food-grade ethanol.
7. Blow out any remaining solvent with clean, dry, compressed air.
8. Install a new cavity O-ring in the groove inside the regulator body.
9. Use your thumb to press the piston assembly out of the cylinder.

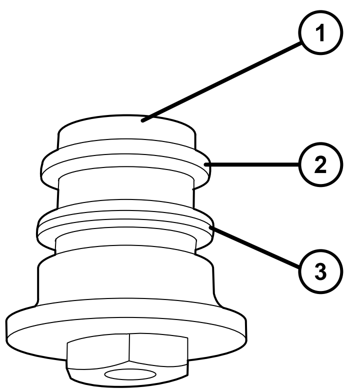
Figure 9–22: Location of thumb placement for piston assembly removal



1. Cylinder
2. Press thumb here

10. Remove the O-ring and backup ring from the piston assembly and discard them.

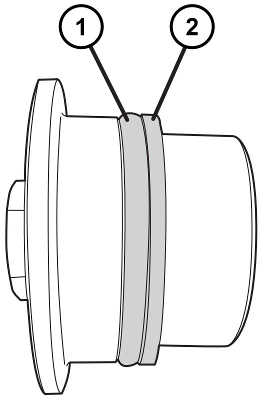
Figure 9–23: Piston assembly backup ring and O-ring



1. Piston assembly
2. Backup ring
3. O-ring

11. Install the small O-ring over the piston assembly.
12. Install the backup ring over the piston assembly by sliding it all the way against the O-ring. Ensure that the O-ring and backup ring are oriented correctly, with the O-ring on first, and the backup ring on second.

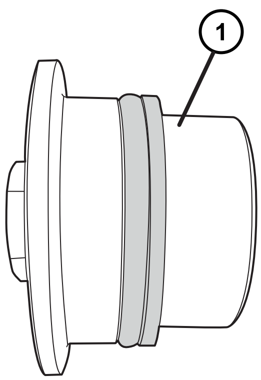
Figure 9–24: O-ring and backup ring installed on piston



1. O-ring
2. Backup ring

13. Apply the food-grade lubricant to the small outside diameter of the piston.

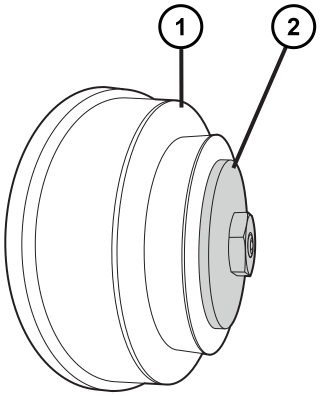
Figure 9–25: Location of lubricant application



1. Apply food-grade lubricant here

14. Install the piston into the cylinder, and then place the piston/cylinder assembly into the regulator cavity.

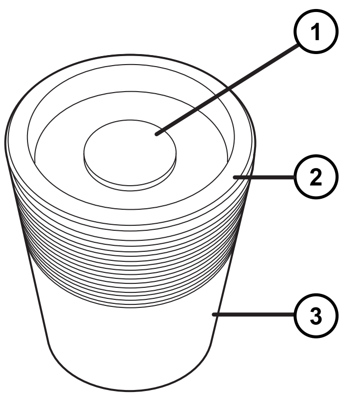
Figure 9–26: Piston installed in cylinder



1 Cylinder

2 Piston

Figure 9–27: Piston/cylinder installed in MBPR cavity



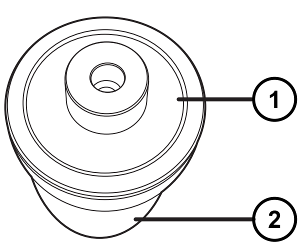
1 Piston assembly

2 Cylinder

3 MBPR body

15. Place the lower spring guide onto the end of the piston.

Figure 9–28: Lower spring guide on end of piston

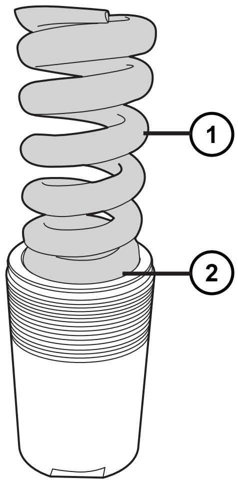


1 Lower spring guide

2 MBPR body

16. Place the range spring on the lower spring guide.

Figure 9–29: Range spring on lower spring guide



1 Range spring

2 Location of lower spring guide

17. Place a small amount of food-grade lubricant on the outer threads of the regulator body.
18. Place the spring housing assembly over the regulator body and engage the threads by hand. Hand-tighten the spring housing assembly.

   Notice: To avoid shearing off the bolts that secure the manual back-pressure regulator body to the shelf, do not overtighten the spring housing assembly.

19. Use the 2-1/4-inch open-end wrench to tighten the spring housing assembly an additional 1/2-turn.
20. Apply pressure and check for leaks.

   Tips:

   - If you detect a fitting leak, tighten the leaking fittings as necessary.
   - If you detect a CO_2 leak on the MBPR, this indicates that the O-ring or backup ring is damaged. To repair the leak, repeat the MBPR rebuild procedure.

9.5.5 Cleaning the view cell

Use the view cell to inspect for carryover from the cyclone separator or view cell to the CO_2 recycler. Liquid CO_2 or dark material in the view cell lens indicates carryover to the recycler. Clean the view cell, and then adjust the temperature and pressures on cyclone separator 3 (CS3) so that only CO_2 gas appears in the view cell lens.

Note: Generally, increasing the temperature or decreasing the pressure in CS3 will decrease the CO_2 density, decreasing its solvating power and causing material that was being carried over to the recycler to precipitate in CS3.

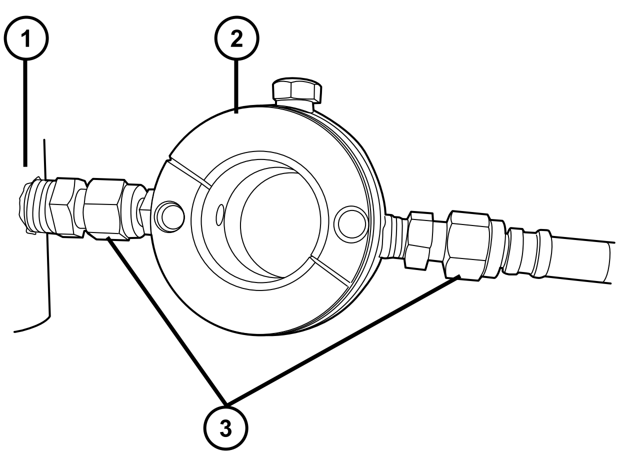
Required tools and materials

- 1-1/2-inch spanner wrench
- 9/16-inch open-end wrench
- Food-grade ethanol (for cleaning a view cell in a system extracting compounds from botanical products)
- Methanol (for cleaning a view cell in a system extracting compounds from non-botanical products)

To clean the view cell:

1. Depressurize the system and then wait 15 minutes to dissipate all pressure from the cyclone separators.
2. Use the 9/16-inch open-end wrench to loosen the two fittings that secure the view cell assembly to the back-side of the manual back-pressure regulator (MBPR) on CS3, and then remove the assembly.

Figure 9–30: View cell installed in system



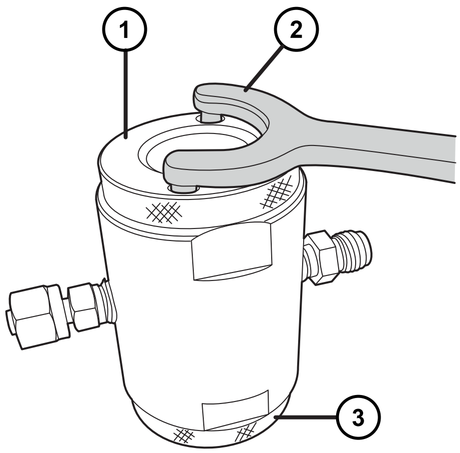
1 MBPR3

2 View cell assembly

3 Fittings

3. Use the 1-1/2-inch spanner wrench to loosen the view cell lens cover on each side of the view cell, and remove the covers.

Figure 9–31: Spanner wrench on view cell lens cover



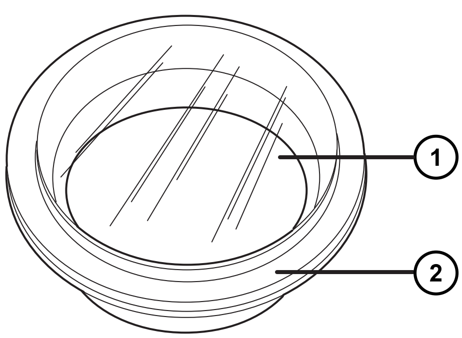
1 View cell lens cover

2 Spanner wrench

3 View cell lens cover

4. Remove both view cell lenses from the view cell and clean them with 95% food-grade ethanol.

Figure 9–32: View cell lens and O-ring



1 Lens

2 O-ring

5. Inspect the O-rings on each lens and replace them if necessary.
6. Reinstall the lenses and O-rings in the view cell.
7. Screw each lens cover onto the view cell, and then use the 1-1/2-inch spanner wrench to tighten them.
8. Use the 9/16-inch open-end wrench to reinstall the view cell assembly on the back-side of MBPR3.

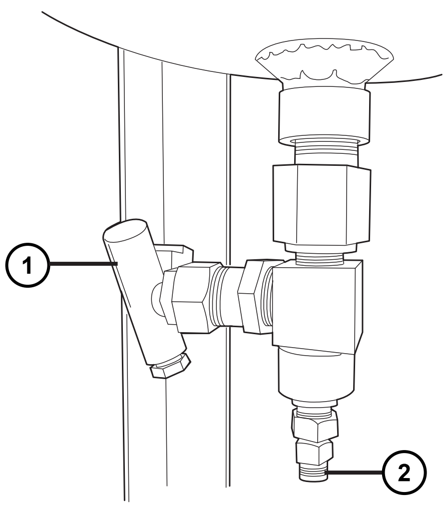
9.5.6 Checking the CO_2 recycler for carryover

Visible liquid CO_2 or dark material in the view cell lens indicates carryover to the CO_2 recycler. Clean the view cell, and then check the CO_2 recycler for carryover.

To check the CO_2 recycler for carryover:

1. Place a paper towel under the CO_2 recycler drain port, and then partially open and close its manual drain valve repeatedly to expel any sample carryover.

Figure 9–33: CO_2 recycler manual drain valve



(1) Drain valve handle

(2) Drain port

2. If sludge is visible on the paper towel, perform the Level 1 cleaning procedure (see Performing the Level 1 cleaning procedure).

9.5.7 Servicing the CO_2 inlet filter

An in-line filter in the CO_2 inlet line helps prevent particles from entering the system and damaging components such as check valves. A clogged filter restricts flow to the pump, diminishing its performance. Clean or replace the CO_2 inlet filter as part of a scheduled maintenance routine or whenever a clogged filter restricts CO_2 flow.

Warning: To avoid serious injury, including asphyxiation, that can result from disconnecting a pressurized CO_2 line, before disconnecting the line from the CO_2 source, stop the flow of CO_2 from the system, and shut the valve at the CO_2 supply.

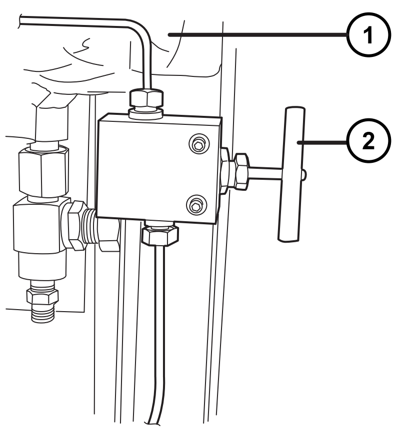
Required tools and materials

- 3/4-inch open-end wrench
- Food-grade ethanol (if cleaning)
- Ultrasonic bath
- CO_2 inlet filter (if replacing)
- CO_2 inlet filter seal (if replacing)

To service the inlet filter:

1. Close the CO_2 recycler outlet (isolation) valve.

   Figure 9–34: CO_2 recycler outlet (isolation) valve



   (1) Bottom of CO_2 recycler

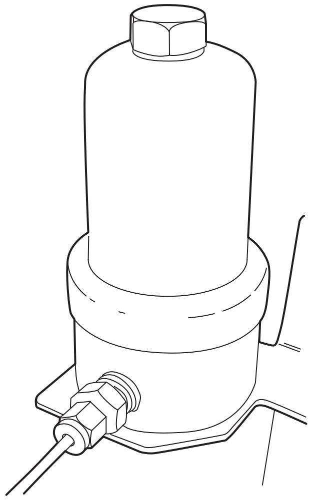
   (2) CO_2 recycler outlet (isolation) valve handle

2. On the ChromScope main page, set the CO_2 flow to `30 g/min` until it shuts down.

   Result: This will drain the CO_2 from the filter tubing and inlet.

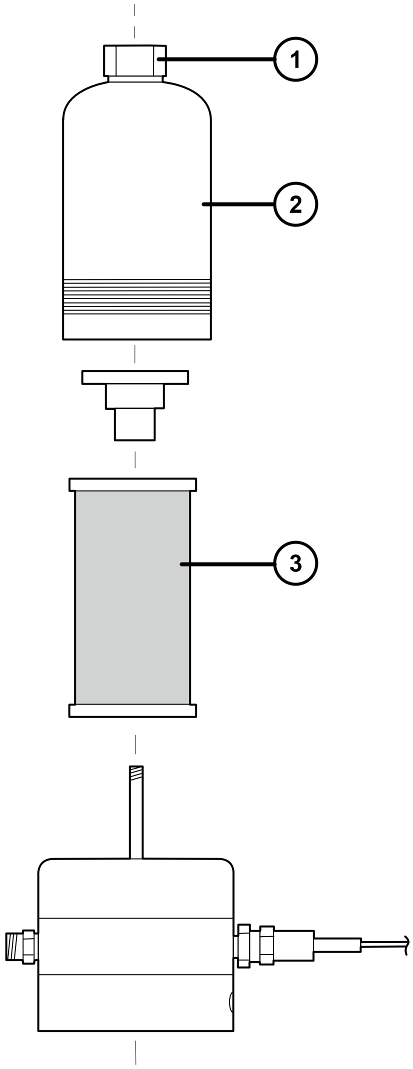
3. Locate the CO_2 inlet filter assembly where the CO_2 supply connects to the system.

Figure 9–35: CO_2 inlet filter assembly



4. Use a 3/4-inch open-end wrench to loosen and remove the cover of the inlet filter.

Figure 9–36: CO_2 inlet filter assembly exploded view



1 Place the 3/4-inch open-end wrench here

2 Inlet filter cover

3 Inlet filter

5. Remove the inlet filter from the inlet filter housing, and then do one of the following:
   - If the filter is partially blocked, sonicate the filter in 95% food-grade ethanol for 20 minutes, and then reinstall it.
   - If the filter is completely blocked, replace the filter, and perform the level 3 cleaning procedure.

   Tip: A partially or completely blocked filter indicates that the CO_2 source might be contaminated with particulate matter.

6. Replace and tighten the inlet filter cover.
7. Open the valve to the CO_2 supply source, and inspect the inlet filter assembly for leaks.

9.6 Performing the Level 2 cleaning procedure on a system without a co-solvent pump

Perform the Level 2 cleaning procedure after every use and before you run a material that significantly differs from the last material run.

Tip: Ensure that you have all of the required tools and materials in stock before performing this procedure.

Required tools and materials

- 9/16-inch open-end wrench
- Compressed air
- Food-grade ethanol (1 L)
- Heat gun
- Large, adjustable wrench
- Soft bristle brush
- Ultrasonic bath
- Wash bottle

Performing the Level 2 cleaning procedure on a system without a co-solvent pump involves these steps:

1. Preparing the system.
2. Flushing the system.
3. Cleaning the cyclone separators and their tubing.
4. Cleaning the manual back-pressure regulators.
5. Cleaning the view cell.
6. Checking the CO_2 recycler for carryover.
7. Servicing the CO_2 filter.

9.6.1 Preparing a system without a co-solvent pump for cleaning

Required tools and materials

- 9/16-inch open-end wrench
- Food-grade ethanol (500 mL)

To prepare a system without a co-solvent pump for cleaning:

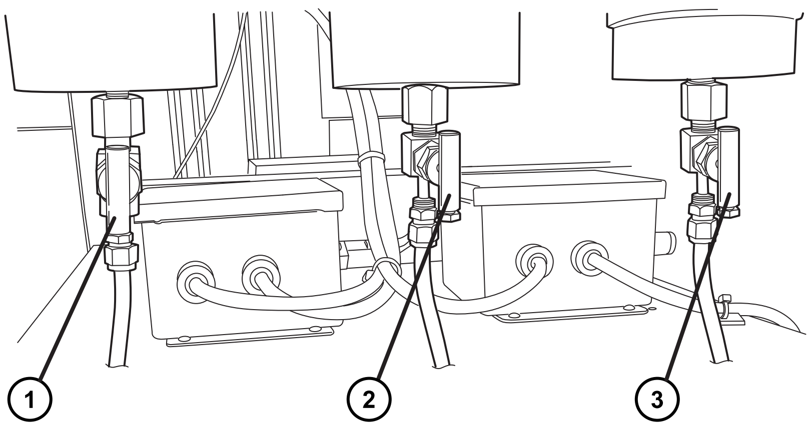
1. Prepare a solvent reservoir of 500 mL 95% food-grade ethanol and place the pump siphon tube into it.

2. Ensure that there is no botanical material in the extraction vessels.

   Note: You can use marbles or other inert material to fill the void in the extraction vessels. Doing so fills the system with CO_2 faster.

3. Slowly open the drain valves in the order of cyclone separator 3 (CS3), cyclone separator 2 (CS2) and cyclone separator 1 (CS1), to bleed off residual CO_2.

   Figure 9–37: Cyclone separator drain valves

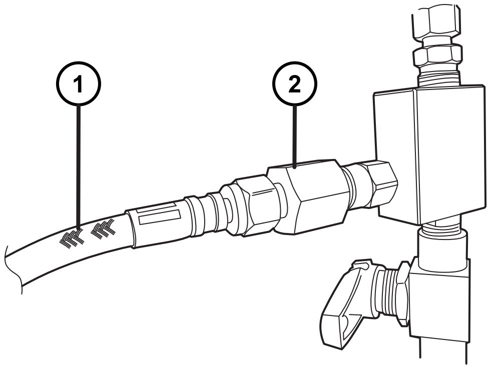
   1 CS1 drain valve

   2 CS2 drain valve

   3 CS3 drain valve

4. Use the 9/16-inch open-end wrench to slowly disconnect the stainless steel flex line from the CO_2 recycler to prevent co-solvent from getting into the recycler.

Figure 9–38: Stainless steel flex line and CO_2 recycler exhaust inlet



1. Stainless steel flex line
2. Exhaust inlet atop CO_2 recycler

5. Route the stainless steel flex line to a suitable exhaust system.

9.6.2 Flushing the system

To flush the system:

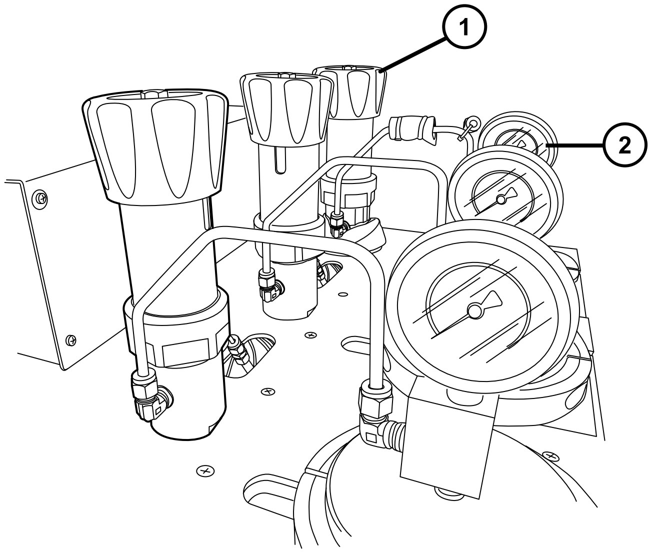
1. In the ChromScope software, set the CO_2 flow to 80 g/min.

   Note: You might need to fill the CO_2 recycler during this procedure.

2. If your system has a co-solvent pump, set the flow to 20 mL/min.
3. Set the automated back-pressure regulator (ABPR) to 19,995 kPa (200 bar, 2900 psi).
4. Set the extracting solvent heat exchanger (HE2) temperature to 70 °C.
5. Set the extraction vessel temperature to 60 °C.
6. Set the cyclone separator 3 (CS3) manual back-pressure regulator (MBPR) to 2068 kPa (21 bar, 300 psi).

   Important: Do not change the other cyclone separator settings.

Figure 9–39: Location of CS3 MBPR and pressure gauge

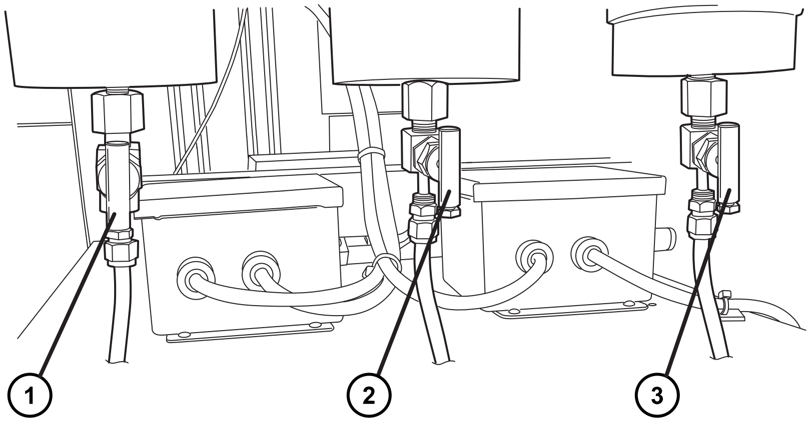


1 CS3 MBPR

2 CS3 pressure gauge

7. Ensure that the cyclone separator temperature settings are specified as follows:
   - CS1: 60 °C
   - CS2: 60 °C
   - CS3: 35 °C
8. If your system has a co-solvent pump:
   a. Start the CO_2 and co-solvent pumps and allow the flow to continue for approximately 25 minutes.
   b. After 25 minutes, stop the co-solvent pump and continue the CO_2 flow for 1 hour, to flush any residual ethanol out of the lines.
9. If your system does not have a co-solvent pump, start the CO_2 pump and allow the flow to continue for 85 minutes.
10. Open the drain valves on the cyclone separators (CS1, CS2, and CS3) slowly, to allow the ethanol to drain completely.

Figure 9–40: Cyclone separator drain valves



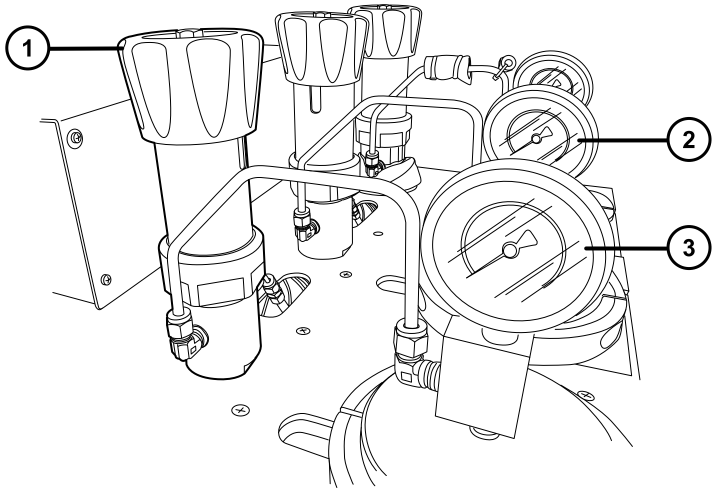
1 CS1 drain valve

2 CS2 drain valve

3 CS3 drain valve

11. After the ethanol drains, increase the pressure on CS3 to 2068 kPa (21 bar, 300 psi).
12. Depressurize the extraction vessel in 1999 kPa/min (20 bar/min, 290 psi/min) increments, to the pressure of CS1.
13. Slowly re-open the drain valves on CS1, CS2, and CS3 to verify that all the ethanol is drained from the system.
14. After the ethanol drains completely, close the drain valves on CS1, CS2, and CS3.
15. Stop the CO_2 pump, power-off the heaters, and bleed off the extraction vessel.
16. Set the temperature of CS1, CS2, and CS3 to 45 °C.
17. Slowly reduce the pressure (counterclockwise) on CS1 MBPR at a rate of 1999 kPa/min (20 bar/min, 290 psi/min), until it reaches the pressure of CS2.

Figure 9–41: CS1 MBPR and pressure gauges



1 CS1 MBPR

2 CS2 pressure gauge

3 CS1 pressure gauge

18. Slowly reduce the pressure on CS2 MBPR until it reaches the pressure of CS3.
19. Slowly open the drain valve on CS3 and continue to remove the residual CO_2.
20. While the CS3 is draining, open the MBPRs on CS1 and CS2 completely.

9.6.3 Cleaning the cyclone separators and tubing

Required tools and materials

- 9/16-inch open-end wrench
- Food-grade ethanol
- Heat gun
- 2-1/4-inch open-end wrench
- Soft bristle brush with 3-inches bristles
- Ultrasonic bath (optional)
- Wash bottle

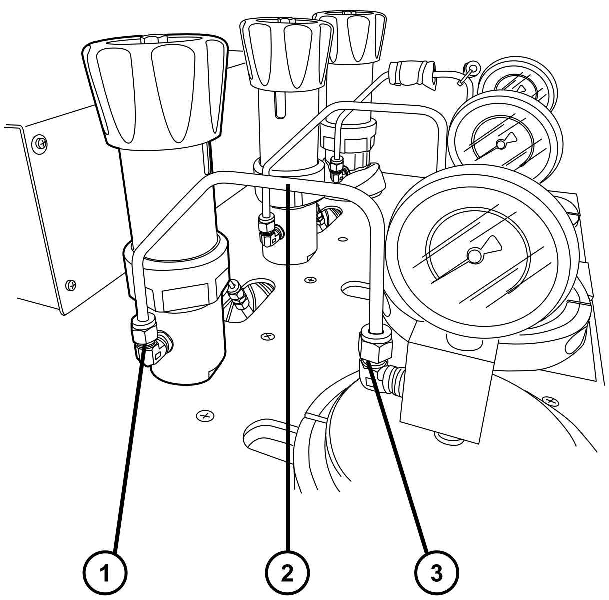
To clean the cyclone separators and tubing:

1. Confirm the system is at atmospheric pressure and that the CO_2 pump and co-solvent pump (if present) are powered-off.

   Tip: When the system reaches atmospheric pressure, the pressure reading on cyclone separator 3 (CS3) is zero.

2. In the ChromScope software, set the temperature of each cyclone separator to `40 °C`.
3. Label the tubing and each cyclone separator cap as CS1, CS2, and CS3.
4. Use the 9/16-inch open-end wrench to slowly remove the 1/4-inch tubing that connects each cyclone separator outlet to its respective manual back-pressure regulator (MBPR) inlet.

   Figure 9–42: Manual back-pressure regulator inlet, tubing, and cyclone separator outlet

   1. Manual back-pressure regulator inlet
   2. Tubing
   3. Cyclone separator outlet

5. Use a wash bottle of 95% food-grade ethanol to clean all of the tubing.

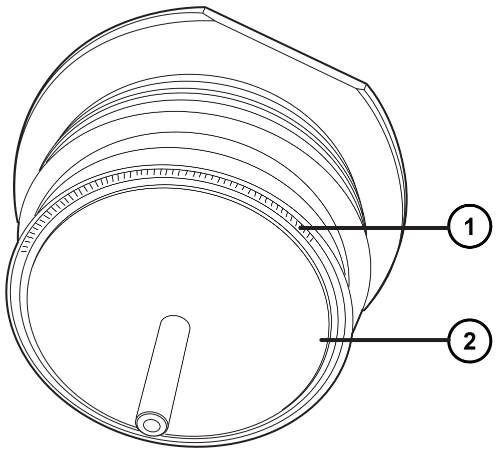
   Tip: If the tubing is severely clogged, sonicate it with 95% food-grade ethanol.

6. Remove the cyclone separator caps.

   Tip: If the cyclone separator cap will not turn, use the 2-1/4-inch open-end wrench to loosen it.

7. Inspect the O-ring on each cap and replace it if necessary.

Figure 9–43: Underside of cyclone separator cap and O-ring

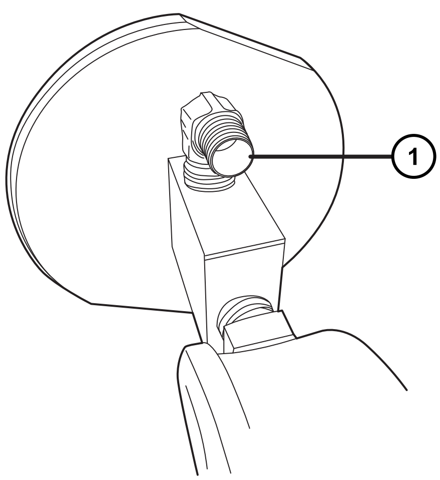


(1) O-ring

(2) Underside of cap

8. Clean the underside of each cap with the wash bottle and rag (or paper towels).
9. Squirt 95% food-grade ethanol into the outlet fittings, located on the top of each cyclone separator cap.

Figure 9–44: Location of outlet fitting on cyclone separator cap

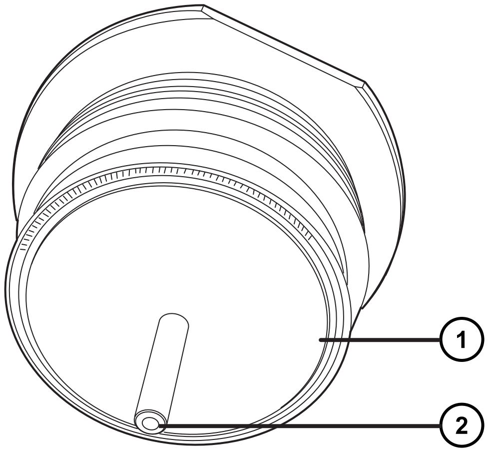


(1) Outlet fitting

10. Close each cyclone separator drain valve.

Note: The ethanol exits out of the tube on the underside of the cap.

Figure 9–45: Tube on underside of cyclone separator cap

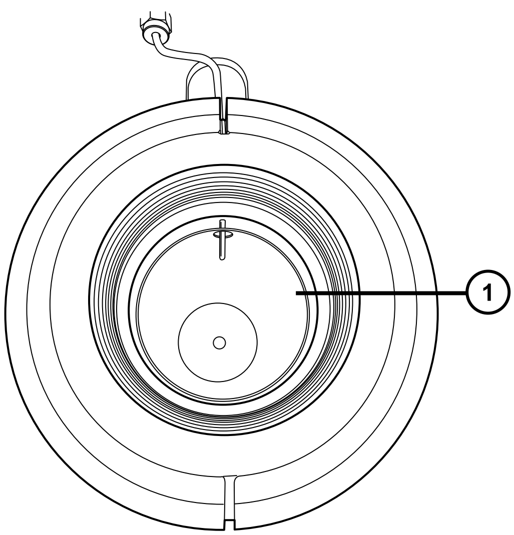


1 Underside of cap

2 Tube

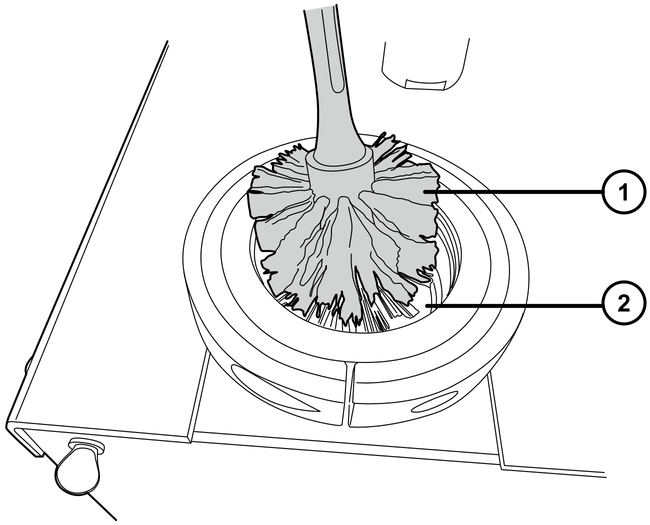
11. Place a beaker under each of the cyclone separator drain lines.
12. Squirt approximately 300 mL of 95% food-grade ethanol along the interior walls in each cyclone separator.

Figure 9–46: Cyclone separator interior wall



1 Interior wall

13. Scrub the interior walls of each cyclone separator using a soft-bristle brush.



1 Soft-bristle brush

2 Interior wall

14. After approximately 5 to 10 minutes of soaking, open each cyclone separator drain valve.

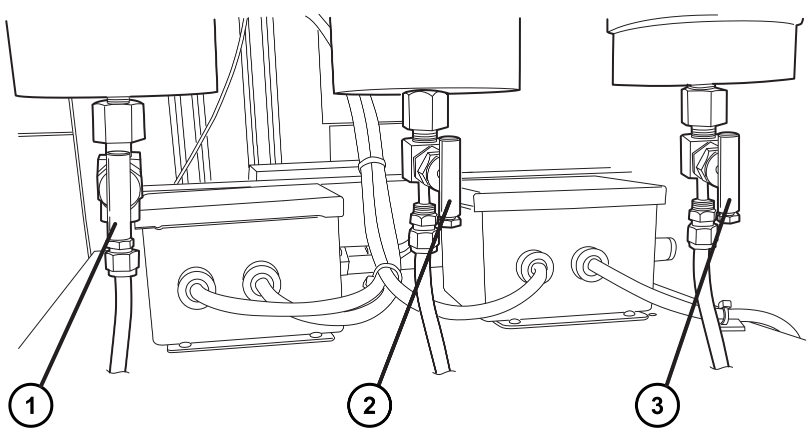
    Tip: If there is wax in the cyclone separator drain valve at the beginning of the cleaning process, use a heat gun to melt it.

15. When the ethanol begins to drain, continuously squirt 95% food-grade ethanol along the interior walls of each cyclone separator until it runs clear.

16. Turn off the cyclone separator heaters.

17. Close each cyclone separator drain valve.

Figure 9–47: Cyclone separator drain valves



1 CS1 drain valve

2 CS2 drain valve

3 CS3 drain valve

18. Reinstall the cyclone separator caps and tubing to their labeled positions.

    Requirement: Set all the MBPRs to their original values at system startup.

19. Reconnect the stainless steel flex line from the CO_2 recycler.

9.6.4 Cleaning the manual back-pressure regulators

Clean the manual back-pressure regulators (MBPRs) whenever carryover is observed in the CO_2 recycler.

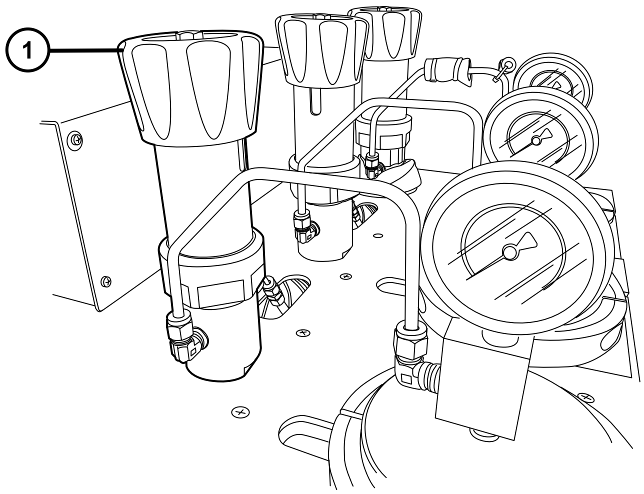
Required tools and materials

- 2-1/4-inch open-end wrench
- Compressed air
- Food-grade ethanol
- Food-grade lubricant
- 13,790 kPa (138 bar, 2000 psi) or 41,369 kPa (414 bar, 6000 psi) MBPR rebuild kit

To clean the manual back-pressure regulators:

1. Relieve all pressure from the system.
2. Turn the large, blue knob on the top of the MBPR fully counterclockwise to reduce the pressure.

Figure 9–48: Blue knob on MBPR

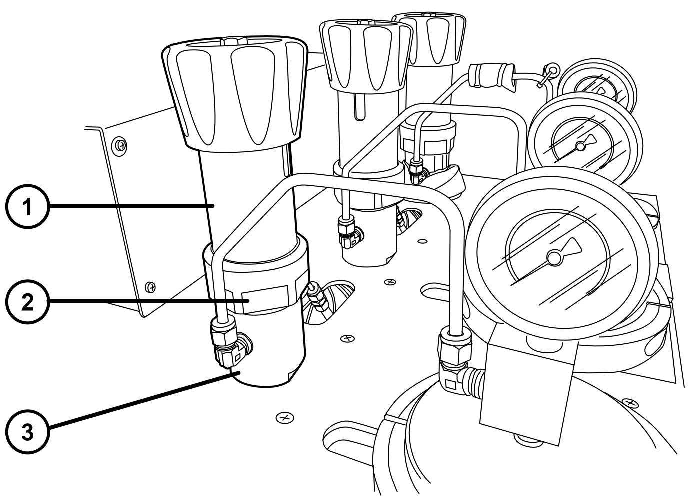


① Blue knob

3. Use the 2-1/4-inch open-end wrench to loosen and remove the spring housing from the regulator body.

   Note: The range spring will fall out of the spring housing when the housing is removed from the regulator body.

Figure 9–49: Location of wrench placement on MBPR body



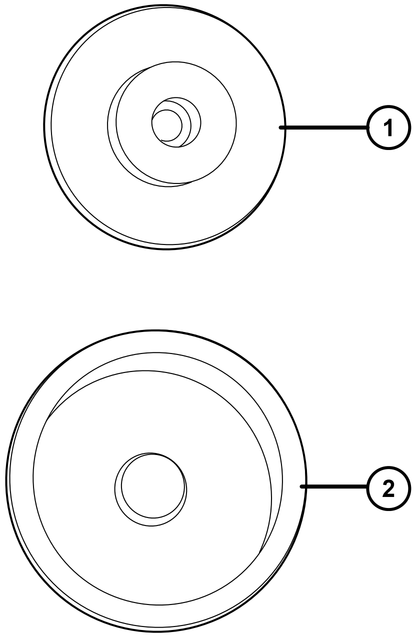
① Spring housing

② Place the 2-1/4-inch open-end wrench here

③ Regulator body

4. Remove the lower spring guide and piston/cylinder assembly.

Figure 9–50: Lower spring guide and piston/cylinder assembly

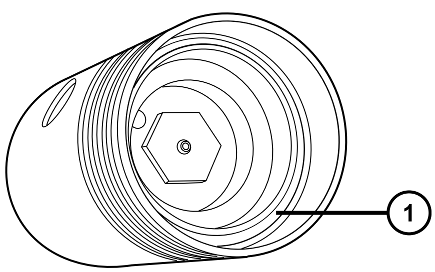


1 Lower spring guide

2 Piston/cylinder assembly

5. Carefully remove the cavity O-ring from the regulator body and discard it.

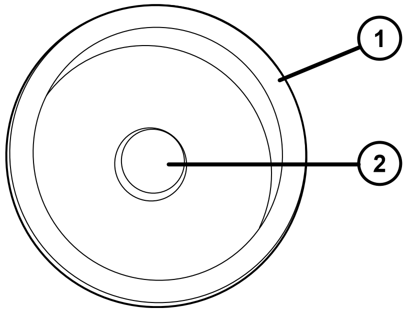
Figure 9–51: Location of cavity O-ring



1 Cavity O-ring

6. Thoroughly clean the cavity body with 95% food-grade ethanol.
7. Blow out any remaining solvent with clean, dry, compressed air.
8. Install a new cavity O-ring in the groove inside the regulator body.
9. Use your thumb to press the piston assembly out of the cylinder.

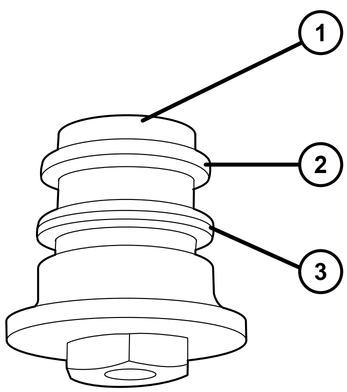
Figure 9–52: Location of thumb placement for piston assembly removal



1. Cylinder
2. Press thumb here

10. Remove the O-ring and backup ring from the piston assembly and discard them.

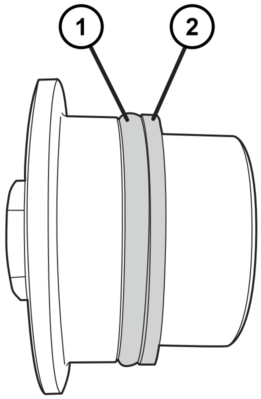
Figure 9–53: Piston assembly backup ring and O-ring



1. Piston assembly
2. Backup ring
3. O-ring

11. Install the small O-ring over the piston assembly.
12. Install the backup ring over the piston assembly by sliding it all the way against the O-ring. Ensure that the O-ring and backup ring are oriented correctly, with the O-ring on first, and the backup ring on second.

Figure 9–54: O-ring and backup ring installed on piston

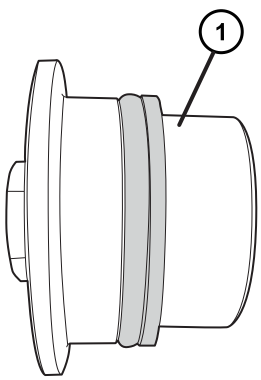


1 O-ring

2 Backup ring

13. Apply the food-grade lubricant to the small outside diameter of the piston.

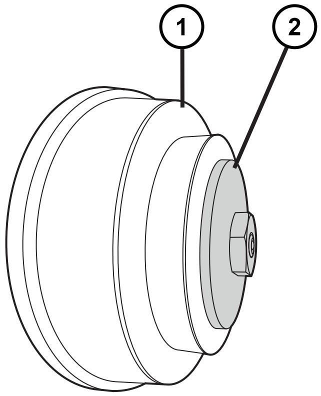
Figure 9–55: Location of lubricant application



1 Apply food-grade lubricant here

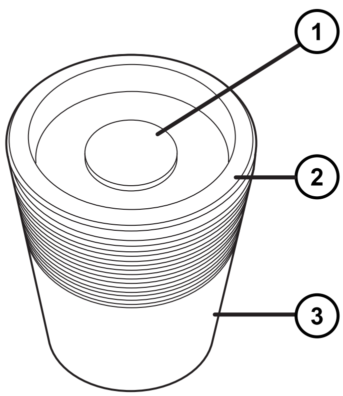
14. Install the piston into the cylinder, and then place the piston/cylinder assembly into the regulator cavity.

Figure 9–56: Piston installed in cylinder



1. Cylinder
2. Piston

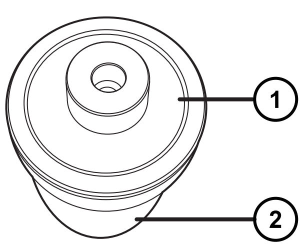
Figure 9–57: Piston/cylinder installed in MBPR cavity



1. Piston assembly
2. Cylinder
3. MBPR body

15. Place the lower spring guide onto the end of the piston.

Figure 9–58: Lower spring guide on end of piston

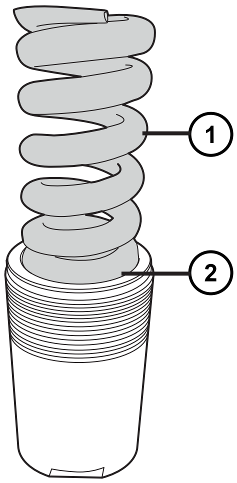


1 Lower spring guide

2 MBPR body

16. Place the range spring on the lower spring guide.

Figure 9–59: Range spring on lower spring guide



1 Range spring

2 Location of lower spring guide

17. Place a small amount of food-grade lubricant on the outer threads of the regulator body.
18. Place the spring housing assembly over the regulator body and engage the threads by hand. Hand-tighten the spring housing assembly.

   Notice: To avoid shearing off the bolts that secure the manual back-pressure regulator body to the shelf, do not overtighten the spring housing assembly.

19. Use the 2-1/4-inch open-end wrench to tighten the spring housing assembly an additional 1/2-turn.
20. Apply pressure and check for leaks.

   Tips:

   - If you detect a fitting leak, tighten the leaking fittings as necessary.
   - If you detect a CO_2 leak on the MBPR, this indicates that the O-ring or backup ring is damaged. To repair the leak, repeat the MBPR rebuild procedure.

9.6.5 Cleaning the view cell

Use the view cell to inspect for carryover from the cyclone separator or view cell to the CO_2 recycler. Liquid CO_2 or dark material in the view cell lens indicates carryover to the recycler. Clean the view cell, and then adjust the temperature and pressures on cyclone separator 3 (CS3) so that only CO_2 gas appears in the view cell lens.

Note: Generally, increasing the temperature or decreasing the pressure in CS3 will decrease the CO_2 density, decreasing its solvating power and causing material that was being carried over to the recycler to precipitate in CS3.

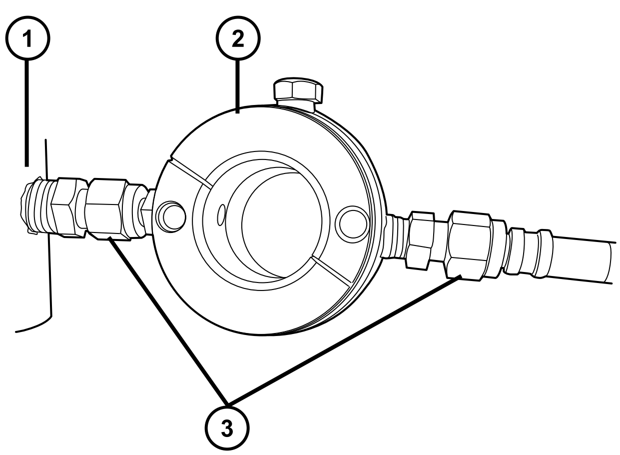
Required tools and materials

- 1-1/2-inch spanner wrench
- 9/16-inch open-end wrench
- Food-grade ethanol (for cleaning a view cell in a system extracting compounds from botanical products)
- Methanol (for cleaning a view cell in a system extracting compounds from non-botanical products)

To clean the view cell:

1. Depressurize the system and then wait 15 minutes to dissipate all pressure from the cyclone separators.
2. Use the 9/16-inch open-end wrench to loosen the two fittings that secure the view cell assembly to the back-side of the manual back-pressure regulator (MBPR) on CS3, and then remove the assembly.

   Figure 9–60: View cell installed in system



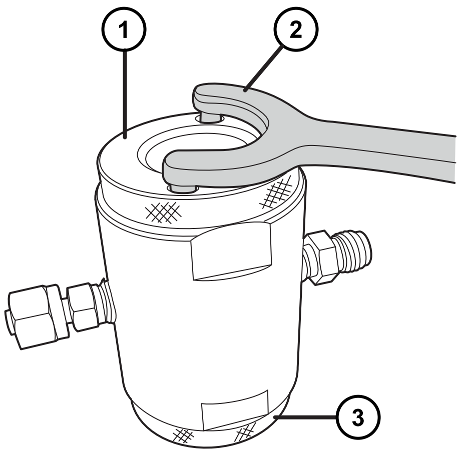
1 MBPR3

2 View cell assembly

3 Fittings

3. Use the 1-1/2-inch spanner wrench to loosen the view cell lens cover on each side of the view cell, and remove the covers.

Figure 9–61: Spanner wrench on view cell lens cover



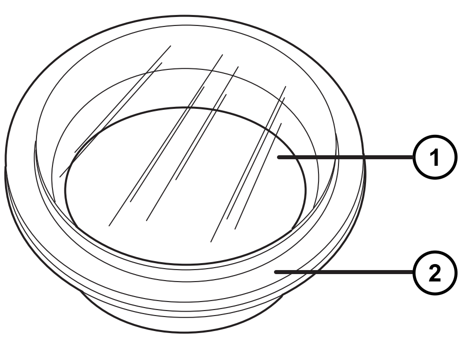
1 View cell lens cover

2 Spanner wrench

3 View cell lens cover

4. Remove both view cell lenses from the view cell and clean them with 95% food-grade ethanol.

Figure 9–62: View cell lens and O-ring



1 Lens

2 O-ring

5. Inspect the O-rings on each lens and replace them if necessary.
6. Reinstall the lenses and O-rings in the view cell.
7. Screw each lens cover onto the view cell, and then use the 1-1/2-inch spanner wrench to tighten them.
8. Use the 9/16-inch open-end wrench to reinstall the view cell assembly on the back-side of MBPR3.

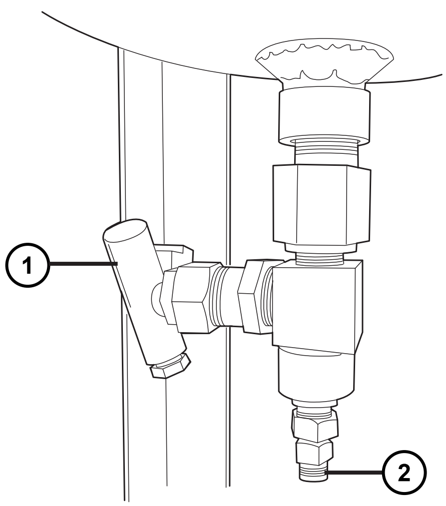
9.6.6 Checking the CO_2 recycler for carryover

Visible liquid CO_2 or dark material in the view cell lens indicates carryover to the CO_2 recycler. Clean the view cell, and then check the CO_2 recycler for carryover.

To check the CO_2 recycler for carryover:

1. Place a paper towel under the CO_2 recycler drain port, and then partially open and close its manual drain valve repeatedly to expel any sample carryover.

   Figure 9–63: CO_2 recycler manual drain valve

   (1) Drain valve handle

   (2) Drain port

2. If sludge is visible on the paper towel, perform the Level 1 cleaning procedure (see Performing the Level 1 cleaning procedure).

9.6.7 Servicing the CO_2 inlet filter

An in-line filter in the CO_2 inlet line helps prevent particles from entering the system and damaging components such as check valves. A clogged filter restricts flow to the pump, diminishing its performance. Clean or replace the CO_2 inlet filter as part of a scheduled maintenance routine or whenever a clogged filter restricts CO_2 flow.

Warning: To avoid serious injury, including asphyxiation, that can result from disconnecting a pressurized CO_2 line, before disconnecting the line from the CO_2 source, stop the flow of CO_2 from the system, and shut the valve at the CO_2 supply.

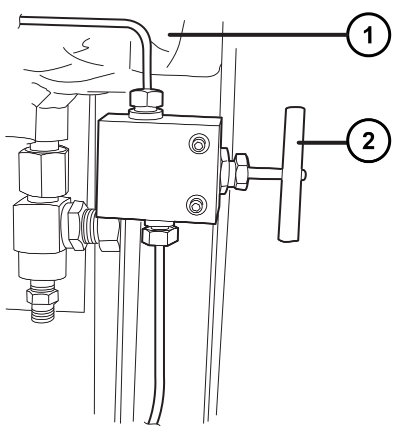
Required tools and materials

- 3/4-inch open-end wrench
- Food-grade ethanol (if cleaning)
- Ultrasonic bath
- CO_2 inlet filter (if replacing)
- CO_2 inlet filter seal (if replacing)

To service the inlet filter:

1. Close the CO_2 recycler outlet (isolation) valve.

   Figure 9–64: CO_2 recycler outlet (isolation) valve

   1 Bottom of CO_2 recycler

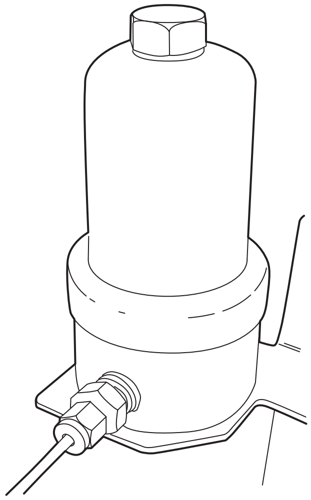
   2 CO_2 recycler outlet (isolation) valve handle

2. On the ChromScope main page, set the CO_2 flow to `30 g/min` until it shuts down.

   Result: This will drain the CO_2 from the filter tubing and inlet.

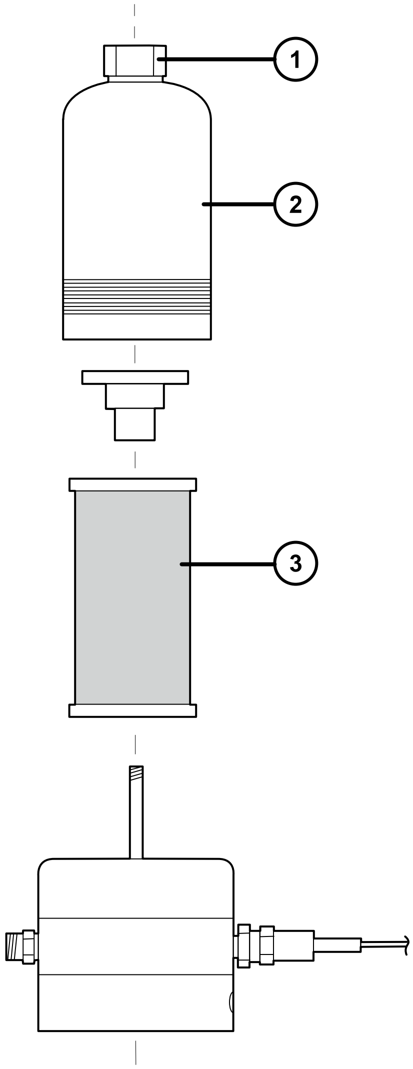
3. Locate the CO_2 inlet filter assembly where the CO_2 supply connects to the system.

Figure 9–65: CO_2 inlet filter assembly



4. Use a 3/4-inch open-end wrench to loosen and remove the cover of the inlet filter.

Figure 9–66: CO_2 inlet filter assembly exploded view



1 Place the 3/4-inch open-end wrench here

2 Inlet filter cover

3 Inlet filter

5. Remove the inlet filter from the inlet filter housing, and then do one of the following:
    - If the filter is partially blocked, sonicate the filter in 95% food-grade ethanol for 20 minutes, and then reinstall it.
    - If the filter is completely blocked, replace the filter, and perform the level 3 cleaning procedure.

    Tip: A partially or completely blocked filter indicates that the CO_2 source might be contaminated with particulate matter.

6. Replace and tighten the inlet filter cover.
7. Open the valve to the CO_2 supply source, and inspect the inlet filter assembly for leaks.

9.7 Performing the Level 3 cleaning procedure on a system with an automation module

This procedure, which involves cleaning the CO_2 cooling heat exchanger (HE1), the extracting solvent heat exchanger (HE2), the inlet tubing, and the flow meter (FM), is intended for systems that include an automation module, but do not include a co-solvent pump.

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid electrical shock, power-off and unplug each module before performing any maintenance operation on it.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Perform the Level 3 cleaning procedure:

- After every six extractions of heavy-wax material
- After every ten extractions of low-wax material
- Before running a material that differs substantially from the last material run
- After running any material suspected of being contaminated with pesticides or mold
- When the CO_2 inlet density is too low (less than 0.87 g/cm^3), but the CO_2 recycler pressure is within an acceptable range of 4654 to 5171 kPa (47 to 52 bar, 675 to 750 psi)

Tip: Ensure that you have all of the required tools and materials in stock before performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 3/8-inch open-end wrench
- 7/16-inch open-end wrench
- 1/2-inch open-end wrench
- 1/8-inch Stainless steel tubing cutter
- Handheld terminal (included in system startup kit)
- RS-232 adapter (included in system startup kit)
- 1/8-inch compression fitting nut
- 1/8-inch compression fitting ferrule set
- 1/8-inch tubing, Teflon inlet with filter, and 1/8-inch Valco type fitting
- 1/8-inch union
- Food-grade ethanol or 50:50 Food-grade ethanol/Water (4 L)
- 4-L bottle (2)

Performing the Level 3 cleaning procedure involves these steps:

1. Preparing the system for cleaning.
2. Cleaning the flow path.
3. Verifying the system.

9.7.1 Preparing a system with an automation module for cleaning

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

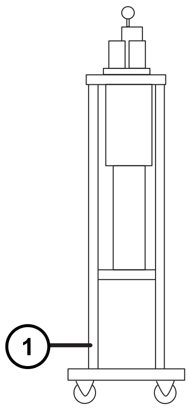
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 3/8-inch open-end wrench
- 7/16-inch open-end wrench
- Food-grade ethanol
- Handheld terminal (included in system startup kit)
- RS-232 adapter (included in system startup kit)

To prepare the system for cleaning:

1. Turn off the CO_2 supply by closing the manual CO_2 supply valve on the CO_2 recycler, the CO_2 tank valve, or the external CO_2 supply valve.
2. Ensure the extraction vessel 1 (EV1) pressure gauge displays 0 kPa, (0 bar, 0 psi). If necessary, carefully drain any residual pressure from the extraction vessel by slowly opening its exhaust valve (MV6).

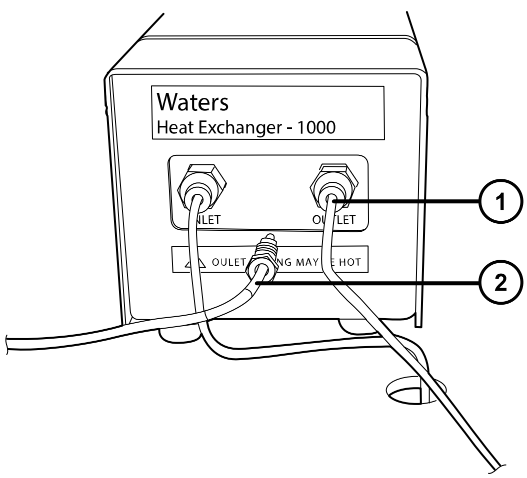
Figure 9–67: Location of MV6 valve on back side of extraction vessel



1 Location of MV6 valve

3. Use the 3/8-inch open-end wrench to disconnect the outlet tubing from the extracting solvent heat exchanger 2 (HE2).
4. Connect a length of 1/8-inch tubing with a 3/8-inch fitting to the HE2 outlet, and then tighten the fitting with the 3/8-inch open-end wrench.

Figure 9–68: Tubing in HE2 outlet

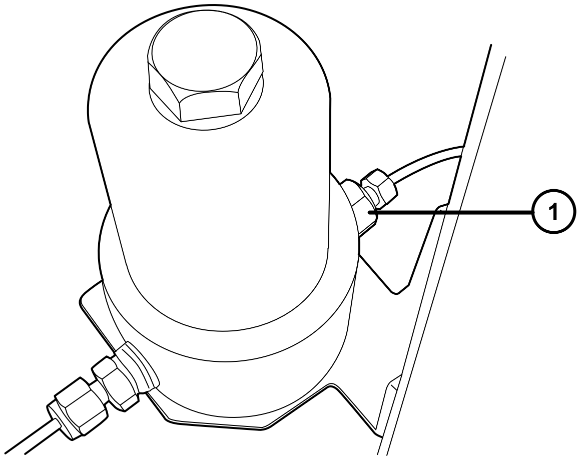


1 1/8-inch tubing with 3/8-inch fitting in HE2 outlet

2 Disconnected HE2 outlet tubing

5. Use the 3/8-inch open-end wrench to connect the other end of the tubing to the CO_2 inlet at the rear of the cart.

Figure 9–69: Location of CO_2 inlet



1 CO_2 inlet

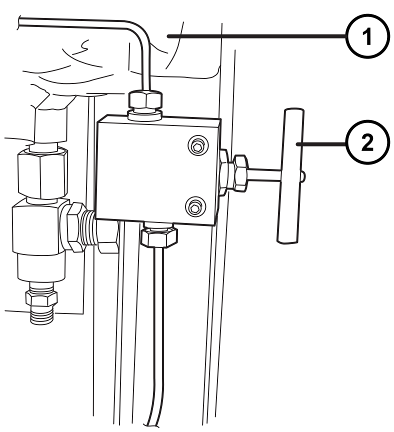
6. Set the chiller temperature to 38 °C.

   Note: The chiller is used with the CO_2 pump and CO_2 cooling heat exchanger 1 (HE1).

   Tip: Allow a minimum of 20 minutes for the chiller to reach the set temperature. To set the temperature, see the manufacturer instructions that were shipped with the chiller.

7. Close the CO_2 recycler outlet (isolation) valve to isolate the recycler from the system.

   Figure 9–70: CO_2 recycler outlet (isolation) valve

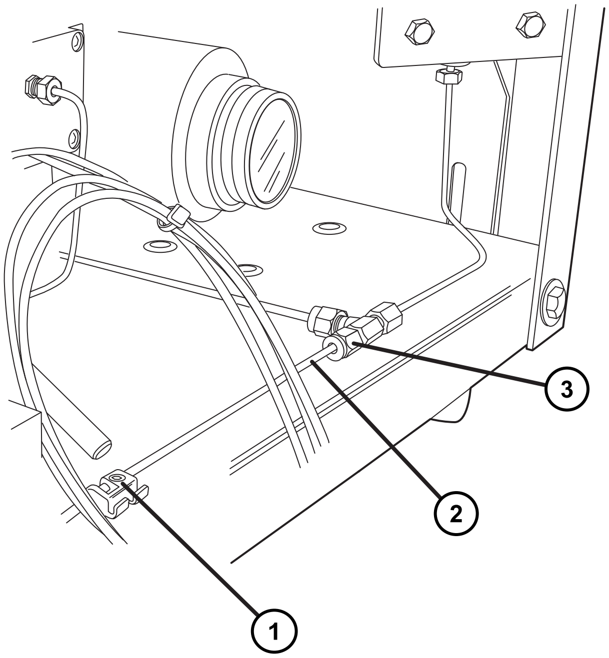


1 Bottom of CO_2 recycler

2 CO_2 recycler outlet (isolation) valve handle

8. Exit ChromScope.
9. At the CO_2 pump inlet tee, use the 7/16-inch open end wrench to loosen the nut on the left side of the tee, and then remove the tubing clamp to allow the pump inlet tubing to rotate.

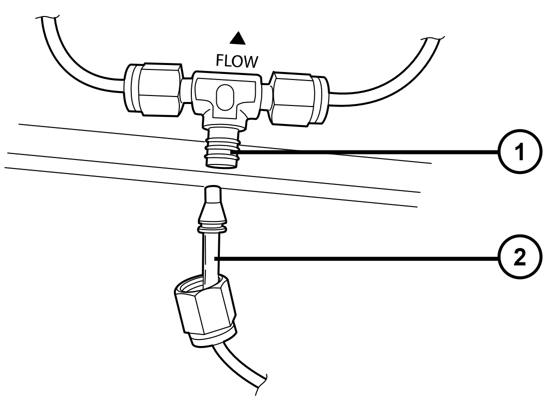
Figure 9–71: Location of tubing clamp and nut on left side of CO_2 pump inlet tee



1. Tubing clamp
2. CO_2 pump inlet tubing
3. Left-side nut

10. At the CO_2 pump inlet tee, remove the inlet tube, and then rotate the line and place it in an empty beaker to collect the solvent.

Figure 9–72: Removing inlet tube from CO_2 pump inlet tee



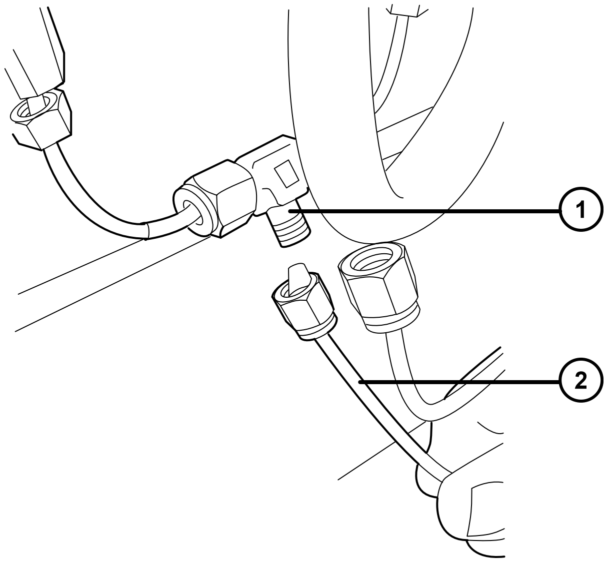
1. CO_2 pump inlet tee
2. Inlet tube

11. Re-tighten the nut on the left side of the CO_2 pump inlet tee.

Warning: To avoid injuries arising from contact with spilled solvent, ensure that the solvent bottle is stable.

12. Connect a small, 1/8-inch tube to the CO_2 pump inlet tee, and then insert the other end of the tube into a 4-L bottle of 95% food-grade ethanol.

Figure 9–73: Removing inlet tube from CO_2 pump inlet tee



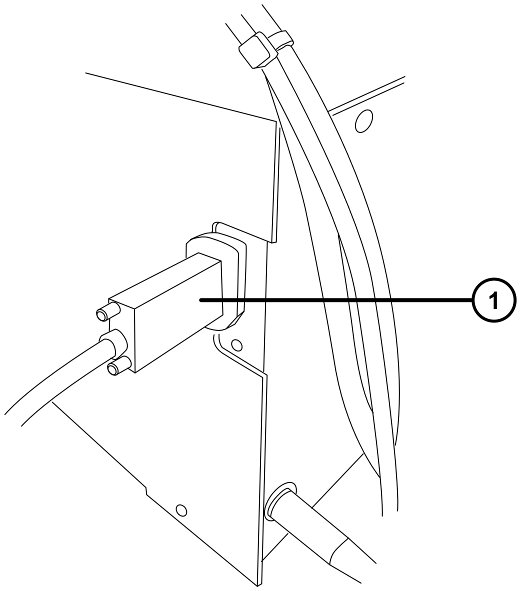
1 CO_2 pump inlet tee

2 Small, 1/8-inch tube

13. Disconnect the RS-232 cable (marked P0) from the port on the right-side, rear of the CO_2 pump, and then connect the handheld terminal cable to this port.

Requirement: If this is the first time using the handheld terminal, you must connect the RS-232 adapter (included in the system startup kit).

Figure 9–74: Handheld terminal cable in RS-232 port



(1) Handheld terminal cable

14. Power-on the CO_2 pump by pressing the switch at the rear of the pump.

    Result: The pump emits a beep, verifying that it recognizes the handheld terminal.

15. Verify that the chiller is at the set temperature of 38 °C.

    Requirement: Do not proceed to the next step until the chiller temperature is 38 °C.

16. On the handheld terminal, press `F1`, and then enter an alarm value of `200`.

17. Move the handheld terminal to a location near the extraction vessel inlet valve (MV2), and then prime the pump:

    a. On the handheld terminal, press `F2`, and then press `E`.

    b. Enter a flow rate of `25` ml, and then press `ENTER`.

    Warning: To avoid injuries arising from contact with spilled solvent, use a cloth to collect the solvent as it begins to exit the MV2 port that was formerly attached to extraction vessel 1.

18. When ethanol begins to exit the extraction vessel 1 inlet valve port (MV2–V1), press `F5` on the handheld terminal.

    Result: The CO_2 pump is now primed.

19. Quickly power-off the CO_2 pump by pressing the switch located at the rear of the unit.

9.7.2 Cleaning the flow path

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

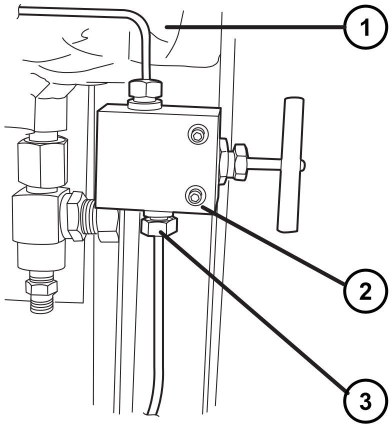
Required tools and materials:

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 3/8-inch open-end wrench

To clean the flow path:

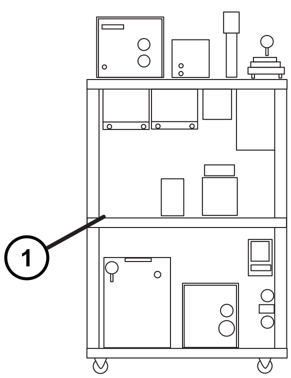
1. Use the 3/8-inch open-end wrench to disconnect the outlet fitting from the CO_2 recycler outlet (isolation) valve and connect it to the MV2 vessel 1 inlet port (MV2-V1).

Figure 9–75: CO_2 recycler outlet (isolation) valve fitting



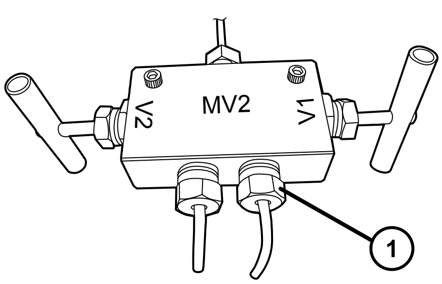
1. Bottom of CO_2 recycler
2. CO_2 recycler outlet (isolation) valve from the recycle tank to the 5 µM filter, before the mass flow controller
3. Outlet fitting

Figure 9–76: Location of MV2 valve on module cart



1. Location of MV2 valve

Figure 9–77: Fitting connected to MV2-V1 inlet port

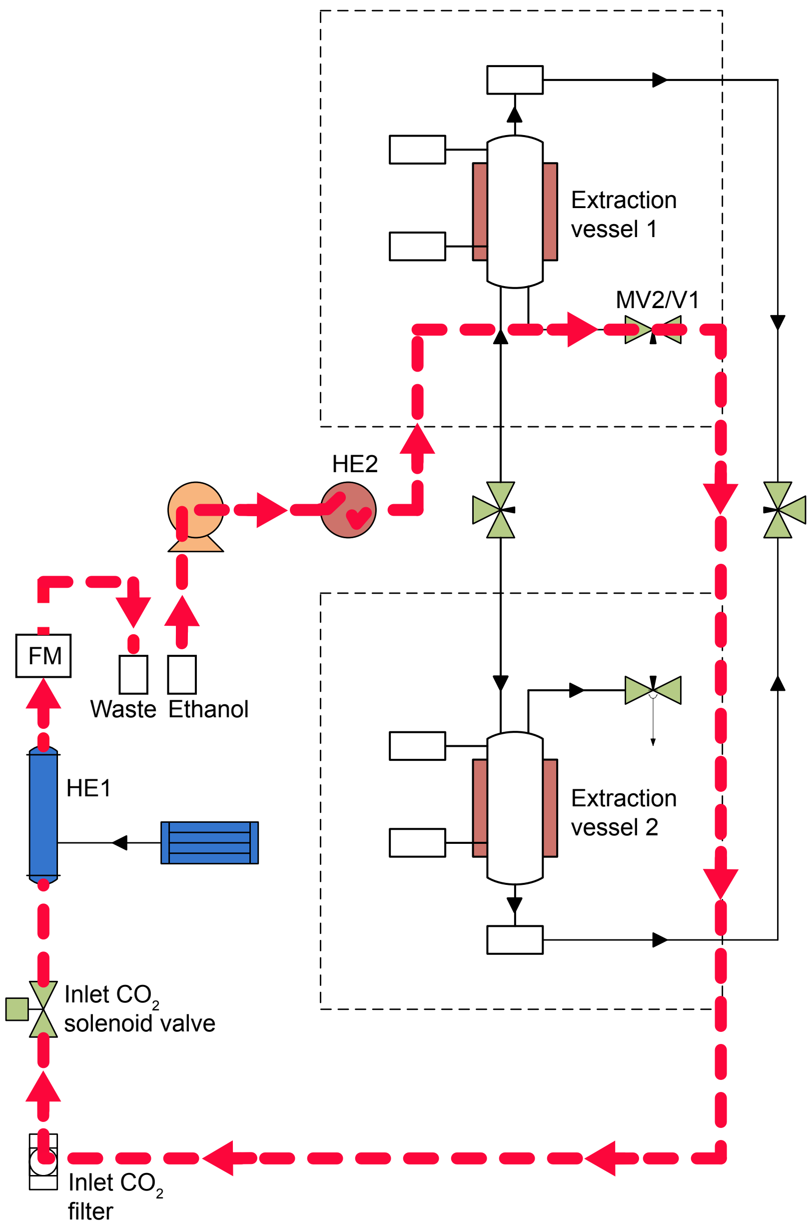


1 Fitting connected to MV2-V1 inlet port

2. On the handheld terminal, press `F2`, then press `E`, enter a flow rate of `5`, and then press `Enter`.

Note: The ethanol flows through the CO_2 pump (P-200 pump), extracting solvent heat exchanger (HE2), needle valve (MV2) on extraction vessel 1, inlet line, inlet filter, union, CO_2 cooling heat exchanger (HE1), and flow meter (FM).

Figure 9–78: Level 3 cleaning flow path

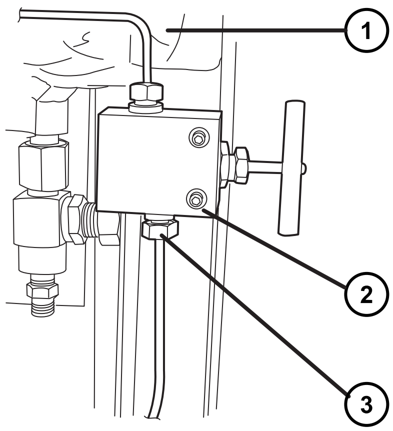


Legend:

Cleaning flow path

3. Periodically swap waste containers to determine when the ethanol begins to clear, indicating a clear flow path.
4. When the effluent is clear for 5 minutes, press `F5` to stop the CO_2 pump.
5. Use the 3/8-inch open-end wrench to disconnect the fitting from the MV2-V1 inlet port, and reconnect it to the CO_2 recycler outlet (isolation) valve.

Figure 9–79: CO_2 recycler outlet (isolation) valve fitting



1 Bottom of CO_2 recycler

2 CO_2 recycler outlet (isolation) valve from the recycle tank to the 5 µM filter, before the mass flow controller

3 Outlet fitting

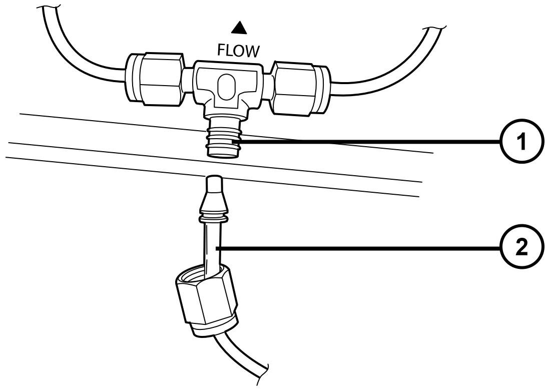
6. Set the chiller temperature to 3 °C.

Requirement: Do not proceed to the next step until the chiller is at the set temperature of 3 °C.

7. Remove the 1/8-inch tube from the pump inlet tee and reconnect the inlet tube to the tee.

Tip: Loosen the left nut on the tee before the pump.

Figure 9–80: Reconnecting inlet tube to CO_2 pump inlet tee

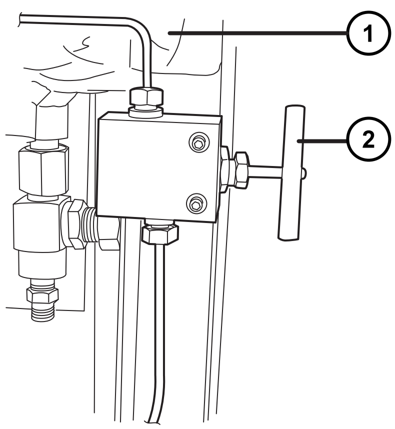


1 CO_2 pump inlet tee

2 Inlet tube

8. Open the outlet valve on the CO_2 recycler.

Figure 9–81: CO_2 recycler outlet (isolation) valve



1 Bottom of CO_2 recycler

2 CO_2 recycler outlet (isolation) valve handle

9. On the handheld terminal, press `F2`, then press `E`, enter a flow rate of `5`, and then press `Enter`.
10. When the CO_2 begins to exit from the MV2-V1 inlet port, press `F5` to stop the CO_2 pump.
11. Power-off the CO_2 pump by pressing the switch located at the rear of the unit.
12. Disconnect the handheld terminal from the port on the right-side, rear of the CO_2 pump, and then reconnect the RS-232 cable.

9.7.3 Verifying the system

To verify the system:

1. Power on the CO_2 pump and wait for the diagnostic routine to complete.

   Note: The diagnostic routine takes 20 to 30 seconds to complete.

2. Reconnect the inlet line from extraction vessel 1 to the MV2 vessel 1 inlet port (MV2/V1).
3. Run the system for 2 minutes at a flow rate of 50 g/min to ensure that the system is working correctly and that all modules are recognized.

9.8 Performing the Level 3 cleaning procedure on a system without an automation module

This procedure is intended for systems that do not include an automation module or a co-solvent pump.

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid electrical shock, power-off and unplug each module before performing any maintenance operation on it.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Perform the Level 3 cleaning procedure:

- After every six extractions of heavy-wax material
- After every ten extractions of low-wax material
- Before running a material that differs substantially from the last material run
- After running any material suspected of being contaminated with pesticides or mold
- When the CO_2 inlet density is too low (less than 0.87 g/cm^3) but the CO_2 recycler pressure is within an acceptable range 4654 to 5171 kPa (47 to 52 bar, 675 to 750 psi)

Tip: Ensure that you have all of the required tools and materials in stock before performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 3/8-inch open-end wrench
- 7/16-inch open-end wrench
- 1/2-inch open-end wrench
- 1/8-inch Stainless steel tubing cutter
- Handheld terminal (included in system startup kit)
- RS-232 adapter (included in system startup kit)
- 1/8-inch compression fitting nut
- 1/8-inch compression fitting ferrule set
- 1/8-inch tubing, Teflon inlet with filter, and 1/8-inch Valco type fitting
- 1/8-inch union
- Food-grade ethanol or 50:50 Food-grade ethanol/Water (4 L)
- 4-L bottle (2)

Performing the Level 3 cleaning procedure involves these steps:

1. Preparing the system for cleaning.
2. Cleaning the flow path.
3. Verifying the system.

9.8.1 Preparing a system without an automation module for cleaning

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 7/16-inch open-end wrench
- 1/2-inch open-end wrench
- Food-grade ethanol
- Handheld terminal (included in system startup kit)
- RS-232 adapter (included in system startup kit)

To prepare the system for cleaning:

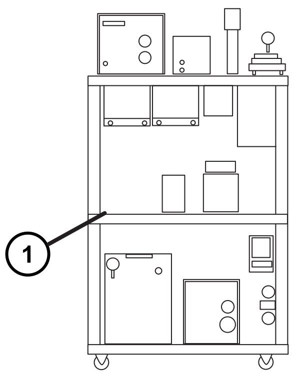
1. Set the chiller temperature to 38 °C.

   Note: The chiller is used with the CO_2 pump and CO_2 cooling heat exchanger 1 (HE1).

   Tip: Allow a minimum of 20 minutes for the chiller to reach the set temperature. To set the temperature, see the manufacturer instructions that were shipped with the chiller.

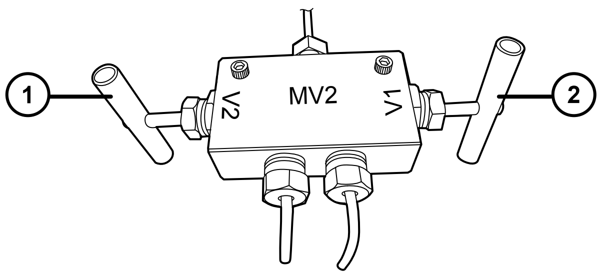
2. Close the inlet and outlet valves to extraction vessel 2 (MV2–V1 and MV2–V2) to isolate that extraction vessel from the system.

Figure 9–82: Location of MV2 valve on module cart



1 Location of MV2 valve

Figure 9–83: MV2–V1 and MV2–V2 valve handles

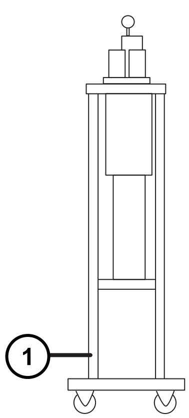


1 MV2–V2 valve handle

2 MV2–V1 valve handle

3. Ensure the extraction vessel 1 (EV1) pressure gauge displays 0 kPa, (0 bar, 0 psi). If necessary, carefully drain any residual pressure from the extraction vessel by slowly opening its exhaust valve (MV6).

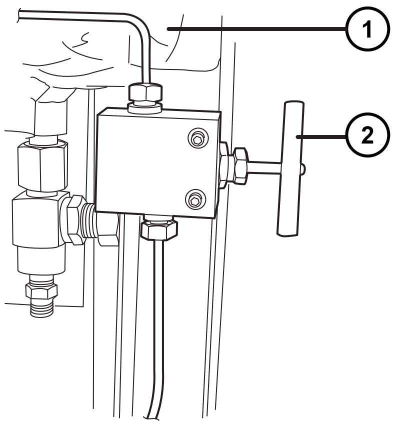
Figure 9–84: Location of MV6 valve on back side of extraction vessel



① Location of MV6 valve

4. Close the CO_2 recycler's supply (isolation) valve to isolate the recycler from the system.

Figure 9–85: CO_2 supply (isolation) valve

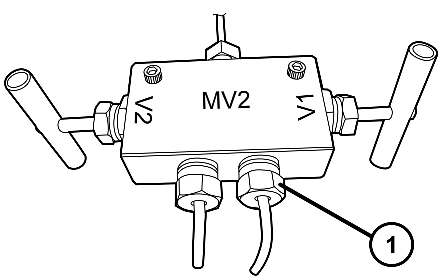


① Bottom of CO_2 recycler

② CO_2 supply (isolation) from the recycle tank to the 5 μM filter, before the mass flow controller

5. Exit ChromScope.
6. Use the 1/2-inch open-end wrench to remove the fitting from the MV2 vessel 1 inlet port.

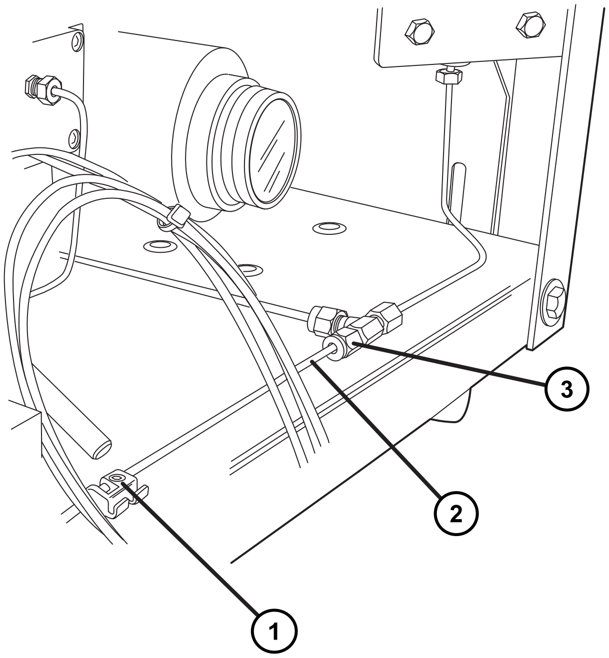
Figure 9–86: Fitting connected to MV2–V1 inlet port



① Fitting connected to MV2–V1 inlet port

7. Open the inlet valve to extraction vessel 1 (MV2–V1).
8. At the CO_2 pump inlet tee, use the 7/16-inch open-end wrench to loosen the nut on the left side of the tee, and then remove the tubing clamp to allow the pump inlet tubing to rotate.

Figure 9–87: Location of tubing clamp and nut on left side of CO_2 pump inlet tee



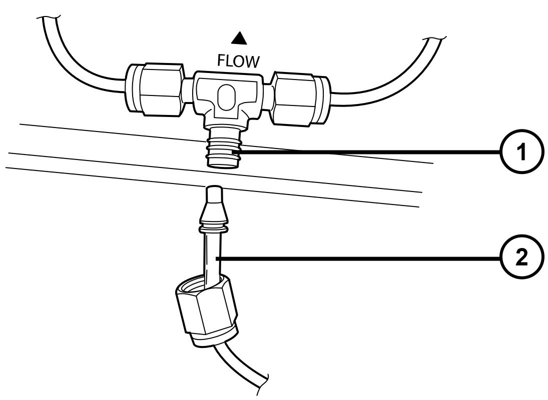
1 Tubing clamp

2 CO_2 pump inlet tubing

3 Left-side nut

9. At the CO_2 pump inlet tee, remove the inlet tube, then rotate the line and place it in an empty beaker to collect the solvent.

Figure 9–88: Removing inlet tube from CO_2 pump inlet tee



1 CO_2 pump inlet tee

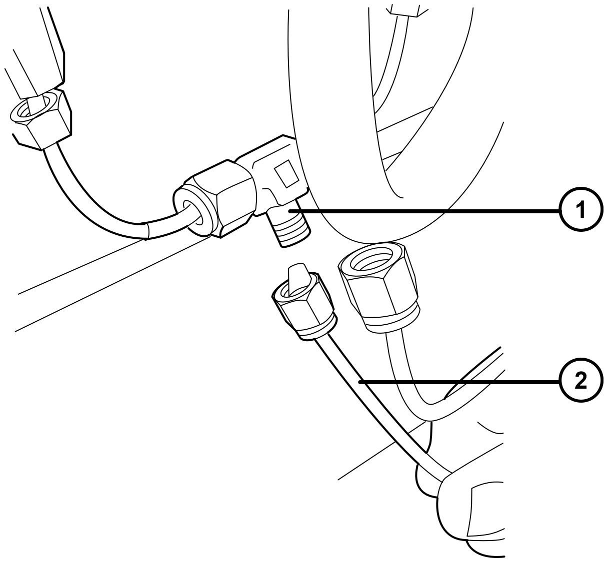
2 Inlet tube

10. Re-tighten the nut on the left side of the CO_2 pump inlet tee.

Warning: To avoid injuries arising from contact with spilled solvent, ensure that the solvent bottle is stable.

11. Connect a small, 1/8-inch tube to the CO_2 pump inlet tee, and then insert the other end of the tube into a 4-L bottle of 95% food-grade ethanol.

Figure 9–89: Removing inlet tube from CO_2 pump inlet tee

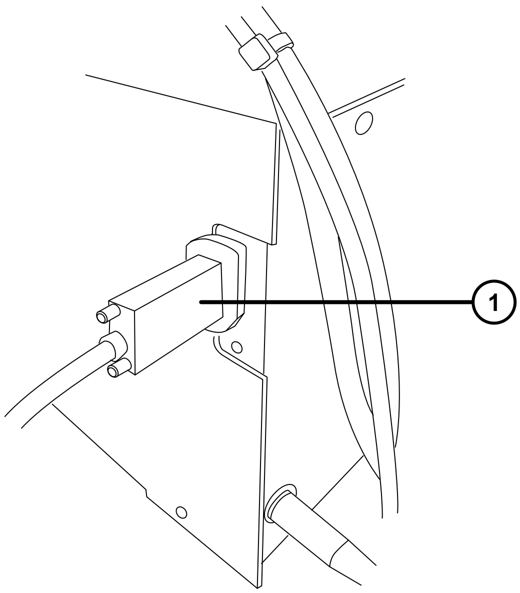


1. CO_2 pump inlet tee
2. Small, 1/8-inch tube

12. Disconnect the RS-232 cable (marked P0) from the port on the right-side, rear of the CO_2 pump, and then connect the handheld terminal cable to this port.

Requirement: If this is the first time using the handheld terminal, you must connect the RS-232 adapter (included in the system startup kit).

Figure 9–90: Handheld terminal cable in RS-232 port



1 Handheld terminal cable

13. Power-on the CO_2 pump by pressing the switch at the rear of the pump.

    Result: The pump emits a beep, verifying that it recognizes the handheld terminal.

14. Verify that the chiller is at the set temperature of 38 °C.

    Requirement: Do not proceed to the next step until the chiller temperature is 38 °C.

15. On the handheld terminal, press `F1`, and then enter an alarm value of `200`.

16. Move the handheld terminal to a location near the extraction vessel inlet valve (MV2), and then prime the pump:

    a. On the handheld terminal, press `F2`, and then press `E`.

    b. Enter a flow rate of `25` ml, and then press `ENTER`.

    Warning: To avoid injuries arising from contact with spilled solvent, use a cloth to collect the solvent as it begins to exit the MV2 port that was formerly attached to extraction vessel 1.

17. When ethanol begins to exit the extraction vessel 1 inlet valve port (MV2–V1), press `F5` on the handheld terminal.

    Result: The CO_2 pump is now primed.

18. Quickly power-off the CO_2 pump by pressing the switch located at the rear of the unit.

9.8.2 Cleaning the flow path

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

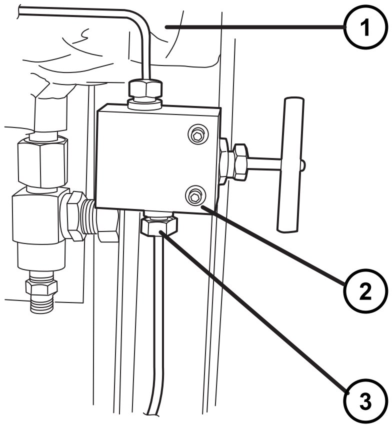
Required tools and materials:

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 3/8-inch open-end wrench

To clean the flow path:

1. Use the 3/8-inch open-end wrench to disconnect the outlet fitting from the CO_2 recycler outlet (isolation) valve and connect it to the MV2 vessel 1 inlet port (MV2-V1).

Figure 9–91: CO_2 recycler outlet (isolation) valve fitting

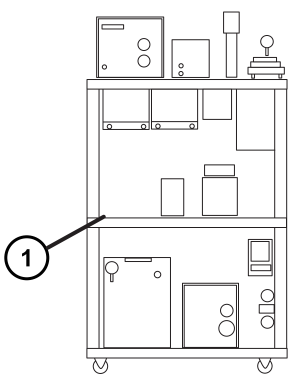


1. Bottom of CO_2 recycler

2. CO_2 recycler outlet (isolation) valve from the recycle tank to the 5 µM filter, before the mass flow controller

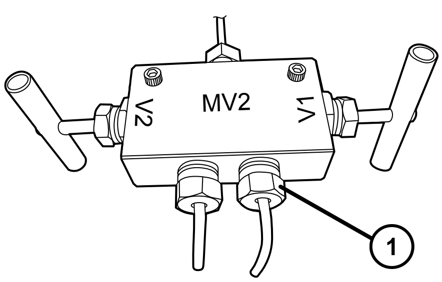
3. Outlet fitting

Figure 9–92: Location of MV2 valve on module cart



1. Location of MV2 valve

Figure 9–93: Fitting connected to MV2-V1 inlet port

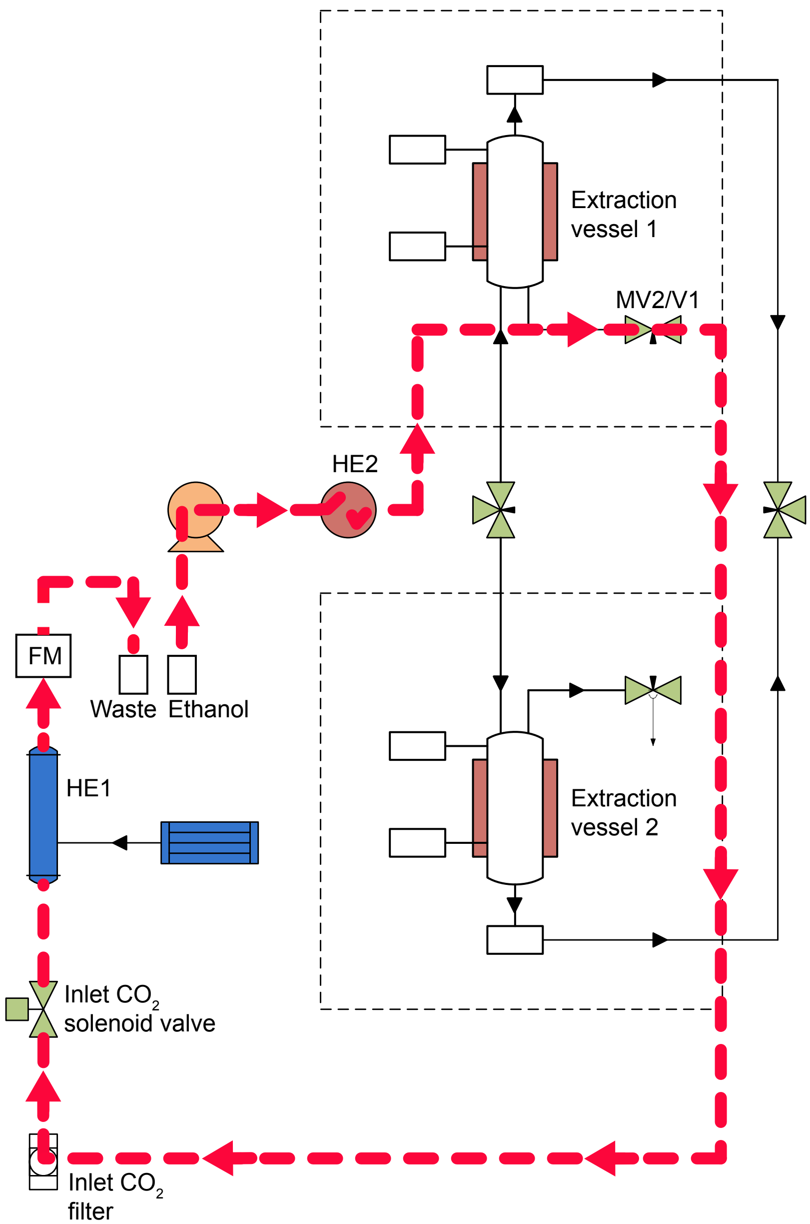


1 Fitting connected to MV2-V1 inlet port

2. On the handheld terminal, press `F2`, then press `E`, enter a flow rate of `5`, and then press `Enter`.

Note: The ethanol flows through the CO_2 pump (P-200 pump), extracting solvent heat exchanger (HE2), needle valve (MV2) on extraction vessel 1, inlet line, inlet filter, union, CO_2 cooling heat exchanger (HE1), and flow meter (FM).

Figure 9–94: Level 3 cleaning flow path

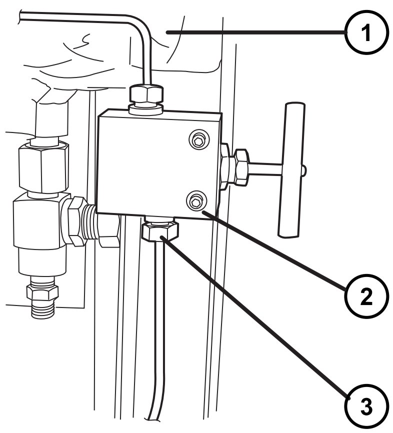


Legend:

Cleaning flow path

3. Periodically swap waste containers to determine when the ethanol begins to clear, indicating a clear flow path.
4. When the effluent is clear for 5 minutes, press `F5` to stop the CO_2 pump.
5. Use the 3/8-inch open-end wrench to disconnect the fitting from the MV2-V1 inlet port, and reconnect it to the CO_2 recycler outlet (isolation) valve.

Figure 9–95: CO_2 recycler outlet (isolation) valve fitting



1. Bottom of CO_2 recycler
2. CO_2 recycler outlet (isolation) valve from the recycle tank to the 5 µM filter, before the mass flow controller
3. Outlet fitting

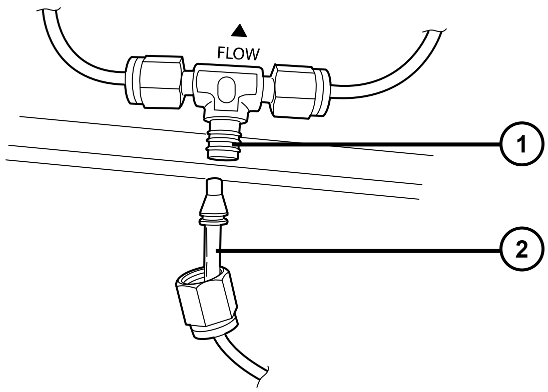
6. Set the chiller temperature to 3 °C.

   Requirement: Do not proceed to the next step until the chiller is at the set temperature of 3 °C.

7. Remove the 1/8-inch tube from the pump inlet tee and reconnect the inlet tube to the tee.

   Tip: Loosen the left nut on the tee before the pump.

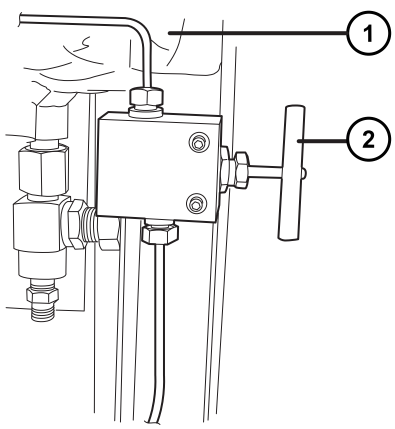
Figure 9–96: Reconnecting inlet tube to CO_2 pump inlet tee



1. CO_2 pump inlet tee
2. Inlet tube

8. Open the outlet valve on the CO_2 recycler.

Figure 9–97: CO_2 recycler outlet (isolation) valve



1. Bottom of CO_2 recycler
2. CO_2 recycler outlet (isolation) valve handle

9. On the handheld terminal, press `F2`, then press `E`, enter a flow rate of `5`, and then press `Enter`.
10. When the CO_2 begins to exit from the MV2-V1 inlet port, press `F5` to stop the CO_2 pump.
11. Power-off the CO_2 pump by pressing the switch located at the rear of the unit.
12. Disconnect the handheld terminal from the port on the right-side, rear of the CO_2 pump, and then reconnect the RS-232 cable.

9.8.3 Verifying the system

To verify the system:

1. Power on the CO_2 pump and wait for the diagnostic routine to complete.

   Note: The diagnostic routine takes 20 to 30 seconds to complete.

2. Reconnect the inlet line from extraction vessel 1 to the MV2 vessel 1 inlet port (MV2/V1).
3. Run the system for 2 minutes at a flow rate of 50 g/min to ensure that the system is working correctly and that all modules are recognized.

9.9 Performing the Level 4 cleaning procedure

This procedure, which describes how to clean the CO_2 recycler in the Bio-Botanical Extraction System and the SFE 5-liter and 10-liter systems, is intended for systems that include an automated or manual recycler.

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid electrical shock, power-off and unplug each module before performing any maintenance operation on it.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Clean the CO_2 recycler when you cannot control the recycler pressure to 5171 kPa (52 bar 750 psi).

Tip: Ensure that you have all of the required tools and materials in stock before performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 3/8-inch open-end wrench
- 7/16-inch open-end wrench
- 1/2-inch open-end wrench
- 1-1/2-inch open-end wrench
- PTFE tape
- 1/4-inch compression cap fitting
- Level sensor O-ring
- Food-grade ethanol or 50:50 Food-grade ethanol/Water (4 L)
- 48-inch, stainless steel, drain-cleaning brush
- Large beaker (polypropylene or metal, 1 L)
- Wash bottle

Cleaning the CO_2 recycler involves these steps:

1. Draining the CO_2 recycler.
2. Cleaning the stainless steel flexible tubing.
3. Cleaning the heat exchanger.
4. Cleaning the CO_2 recycler.
5. Filling the CO_2 recycler.

9.9.1 Draining the CO_2 recycler

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye or facial injury, wear a face shield when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

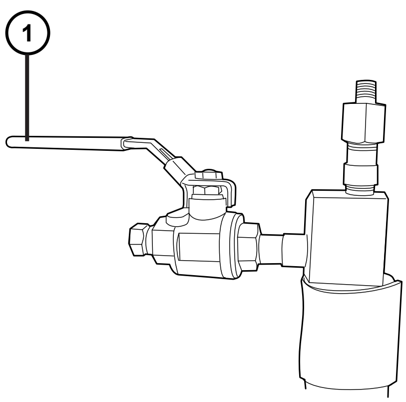
Required tools and materials

- Chemical-resistant, powder-free gloves
- Face shield
- 3/8-inch open-end wrench

To drain the CO_2 recycler:

1. Close the manual CO_2 supply valve at the top of the recycler leading from the CO_2 source.

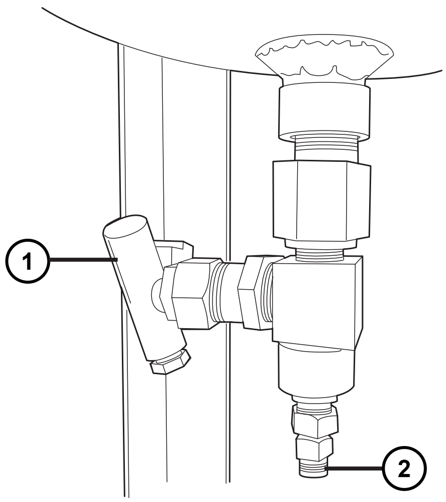
   Figure 9–98: Manual CO_2 supply valve

   1 Manual CO_2 supply valve handle

2. Close the drain valve on the bottom of the recycler.

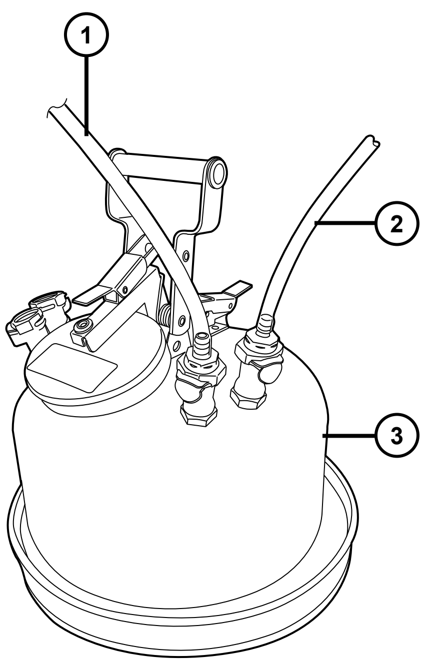
Figure 9–99: Drain valve outlet and handle on bottom of CO_2 recycling vessel



1. Drain valve handle
2. Drain outlet

3. Ensure that the CO_2 recycler drain outlet is routed to a suitable waste container.

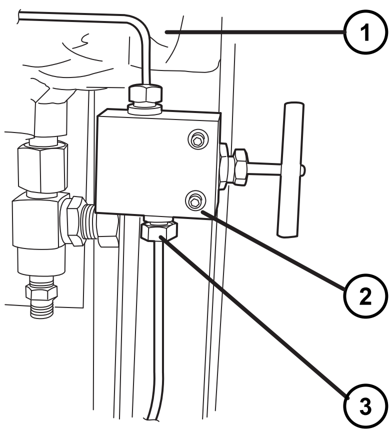
Figure 9–100: Example of a suitable waste container



1. Waste tubing (from CO_2 recycler drain outlet)
2. Waste-container vent tubing (to fume hood)
3. Waste container

4. Use the 3/8-inch open-end wrench to disconnect the outlet fitting from the CO_2 supply valve.

Figure 9–101: CO_2 supply valve outlet fitting



1. Bottom of CO_2 recycler
2. CO_2 supply isolation valve
3. Outlet fitting

5. Slowly open the CO_2 recycler manual drain valve.

Warning: To avoid freeze burns when closing the CO_2 recycler manual drain valve, wear thermally insulated gloves. During draining, the drain valve can freeze due to Joule-Thompson cooling, and its temperature can fall as low as -78 °C.

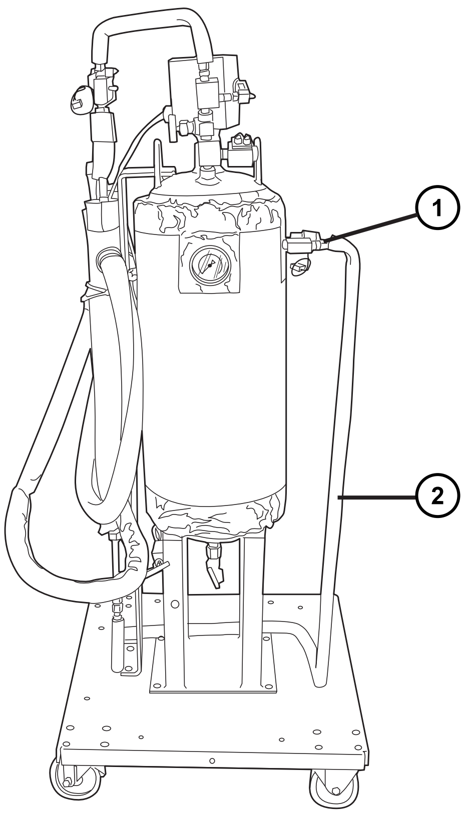
Warning: To avoid eye or facial injury, wear a face shield when closing the CO_2 recycler manual drain valve. As the drain valve begins to warm, frozen CO_2 blocking the outlet could be explosively expelled.

6. Once the CO_2 recycler pressure reaches 0 psi and CO_2 is no longer coming out of the drain outlet, close the CO_2 recycler manual drain valve, and remove the recycler tubing.

Tip: The CO_2 recycler tank can take several hours to drain, depending on the amount of CO_2 in tank. When draining, the drain valve may freeze due to Joule-Thompson cooling and clog the drain outlet with frozen CO_2. As the drain outlet begins to warm, the frozen CO_2 may be explosively expelled causing a loud sound and an explosive release of CO_2.

Eye protection (preferably a face shield) and thermally insulated gloves to protect from freeze burns are required. The drain valve on the recycle tank will go to as low as -78 °C during draining.

Figure 9–102: CO_2 recycler tubing



1 Recycler inlet

2 Recycler tubing

9.9.2 Cleaning the stainless steel flex tubing

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 9/16-inch open-end wrench
- Food-grade ethanol or 50:50 Food-grade ethanol/Water (4 L)
- Wash bottle

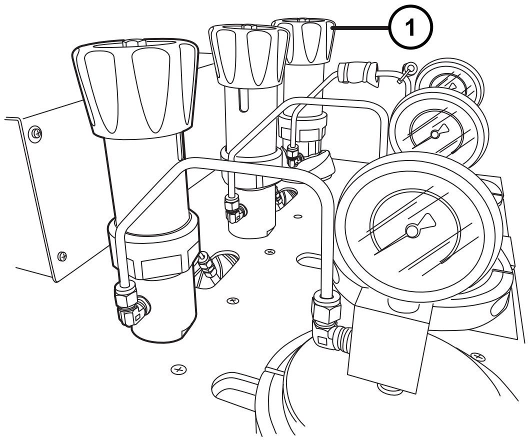
To clean the stainless steel flex tubing:

1. While the CO_2 recycler is draining, set the recycler chiller to 50 °C.

   Tip: To set the temperature, see the manufacturer instructions that were shipped with the chiller.

2. Turn the manual back-pressure regulator (MBPR) on cyclone separator 3 (CS3) two full turns clockwise.

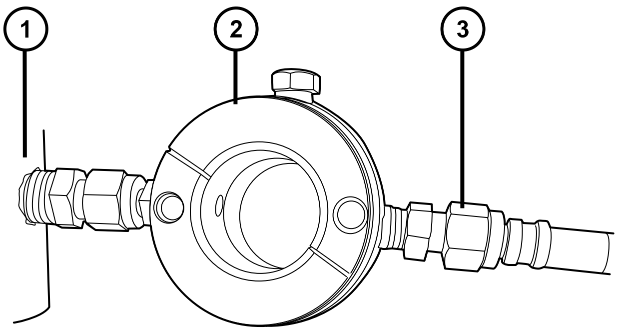
   Figure 9–103: Location of CS3 MBPR

① CS3 MBPR

3. Use the 9/16-inch open-end wrench to remove the stainless steel (SS) flex tubing connecting the outlet of the view cell on CS3 MBPR to the CO_2 recycler.

Figure 9–104: Stainless steel flex tubing connected to view cell



① CS3 MBPR

② View cell assembly

③ Stainless steel flex tubing connection

4. Use a wash bottle to force 95% food-grade ethanol through the stainless steel flex line and let it soak for 15 minutes. Repeat this step until the ethanol runs clear.

9.9.3 Cleaning the heat exchanger

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Required tools and materials

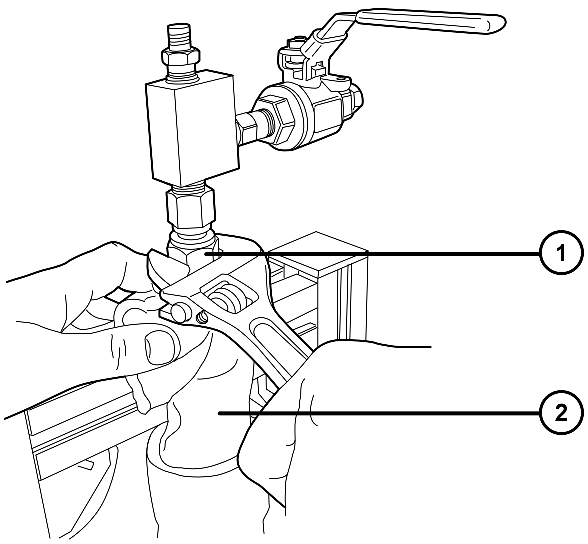
- Chemical-resistant, powder-free gloves
- Protective eyewear
- 3/4-inch open-end wrench
- 1/4-inch compression cap fitting
- Food-grade ethanol or 50:50 Food-grade ethanol/Water (4 L)
- Large beaker (polypropylene or metal, 1 L)
- Wash bottle

To clean the heat exchanger:

1. Use the 3/4-inch open-end wrench to remove the 1/2-inch Swagelok fitting on top of the CO_2 recycler heat exchanger.

   Tip: To access the fitting, you may need to remove the insulation and U-bracket.

   Figure 9–105: Opening the heat exchanger on $_2$ recycler

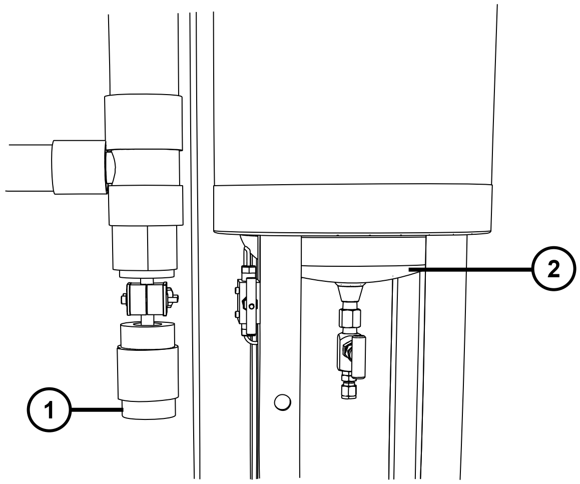
   

   1 Swagelok fitting

   2 Heat exchanger

2. Place a beaker under the heat-exchanger outlet.

Figure 9–106: Heat-exchanger outlet



1 Heat-exchanger outlet

2 Bottom of CO_2 recycler

3. Remove the tubing from the heat exchanger outlet.
4. Place the 1/4-inch compression cap fitting on the heat exchanger outlet, and then fill the heat exchanger with 95% food-grade ethanol.
5. Allow the heat exchanger to soak for 15 minutes, and then drain it.
6. Repeat the previous step until the ethanol is clear. Empty the beaker as necessary.

9.9.4 Cleaning the recycler

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

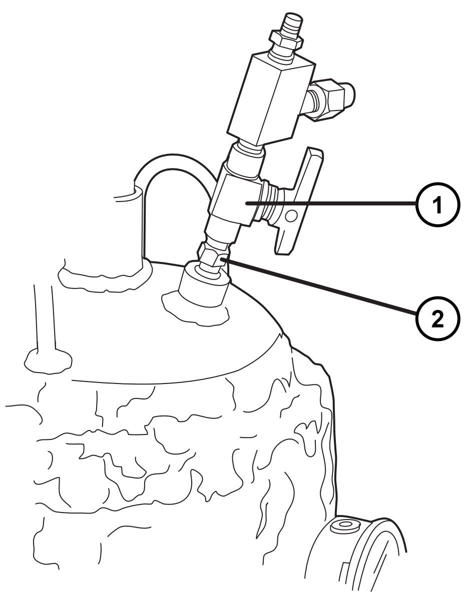
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 1/2-inch open-end wrench
- 1-1/2-inch open-end wrench
- PTFE tape
- Level sensor O-ring
- Food-grade ethanol or 50:50 Food-grade ethanol/Water (4 L)
- 48-inch stainless steel drain-cleaning brush
- Large beaker (polypropylene or metal, 1 L)
- Wash bottle

To clean the recycler:

1. While the heat exchanger soaks, disconnect all cables connected to the CO_2 recycler.
2. Use the 1/2-inch open-end wrench to loosen the recirculation valve fitting, and then remove the recirculation valve.

Figure 9–107: Recirculation valve and fitting

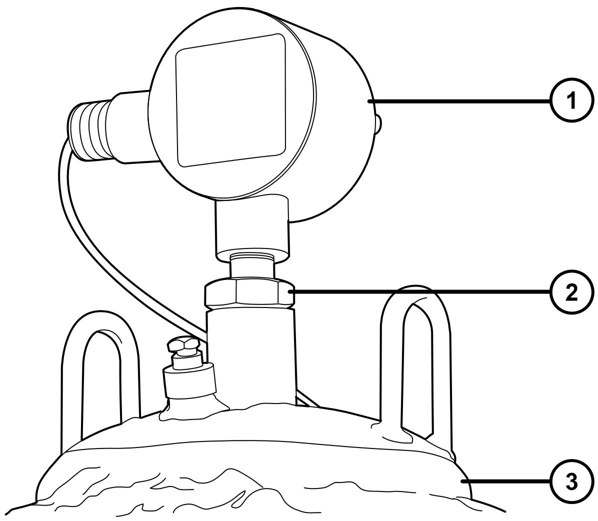


(1) Recirculation valve

(2) Fitting

3. Use the 1-1/2-inch open-end wrench to unscrew the large nut at the base of the level sensor, and then remove the sensor.

Figure 9–108: Large nut on level sensor base



(1) Level sensor

(2) Large nut

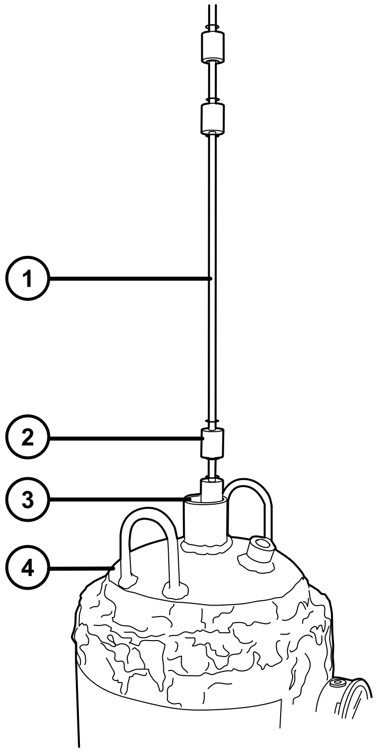
(3) CO_2 recycler tank

Warning: Retrieving a float from the bottom of the CO_2 recycler tank requires tipping the tank upside down. To avoid spinal and muscular injury, do not attempt to tip the tank upside down without assistance.

Notice: If you feel resistance when pulling upward on the dipstick, this indicates that dry ice has formed around its float. To avoid damaging the dipstick, wait two hours before attempting to remove it again.

4. Carefully remove the dipstick, ensuring that you do not contact the floats on the side of the orifice during removal.

Figure 9–109: Removing dipstick from CO_2 recycler tank



1. Dipstick
2. Float
3. Orifice
4. CO_2 recycler tank

5. Ensure that all valves are closed on the CO_2 recycler, and then place a beaker under the recycler drain outlet.
6. Use the wash bottle to squirt 95% food-grade ethanol around the inside of the recycler vessel, and then use the 48-inch stainless steel drain-cleaning brush to scrub the sides of the recycler.
7. Allow the recycler to soak for 15 minutes, and then drain it.
8. Continue scrubbing, soaking, and draining until the ethanol is clear. Empty the beaker as necessary.
9. Spray 95% food-grade ethanol to carefully clean out the springs and level sensor floats before reinstalling the level sensor.
10. Reinstall the level sensor, ensuring that you do not to contact the floats on the side of the orifice during installation.

11. Remove the old level-sensor O-ring and replace it with a new one.
12. Carefully reinstall the level sensor O-ring.

    Requirement: Replace the level sensor O-ring whenever you open the CO_2 recycler tank. If you wait until the O-ring fails, it will take two to three hours to replace.
13. Wrap the recirculation valve threads with PTFE tape, and then reinstall the valve on the CO_2 recycler tank, using the 1/2-inch open-end wrench to tighten the recirculation valve fitting.
14. Reinstall the stainless steel flex tubing, recycler tubing, and CO_2 supply tubing.

9.9.5 Filling the CO_2 recycler

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Warning: To avoid serious injury or death from CO_2 vapors, ensure that the room containing the system is well ventilated. The ventilation system must provide an air exchange rate of four rooms per hour.

Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Required tools and materials

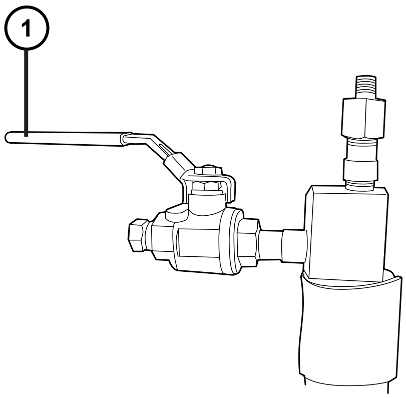
- Chemical-resistant, powder-free gloves
- Protective eyewear

To fill the CO_2 recycler:

1. Ensure the valves are in the following positions:

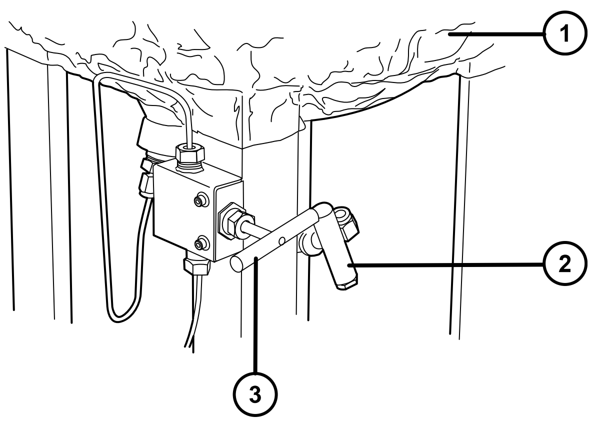
- Manual CO_2 supply valve: closed
- System supply valve: closed
- Recycler drain outlet: closed

Figure 9–110: Manual CO_2 supply valve



(1) Manual CO_2 supply valve handle

Figure 9–111: CO_2 recycler system supply and drain valves



(1) Bottom of CO_2 recycler tank

(2) Drain valve handle

(3) System supply valve handle

Notice: Opening the CO_2 supply valve too quickly will cause dry ice to form at the bottom of the CO_2 recycler. If this occurs, allow the ice to melt overnight or use a heat gun to melt it.

2. Slightly open the manual CO_2 supply valve.

Tips:

- Filling the CO_2 recycler takes approximately 20 minutes. To monitor the fill rate, check the recycler chiller temperature. If the recycler chiller temperature drops more than 2 °C, the recycler is filling too quickly.
- If you are using automated refilling (solenoid control), ensure that the manual valve remains only slightly open for the initial filling. After the initial filling is finished, open the valve completely.

3. Wait until the low light on the level sensor is illuminated. Keep the manual CO_2 supply valve open for 1 minute, and then close it.
4. Turn on the pump, and then slightly open the manual CO_2 supply valve again. If the low light on the level sensor is not illuminated after 2 minutes, check the CO_2 supply, and refill it if necessary.

9.10 Preparing for system shutdown

You must prepare a system for shutdown before actually shutting it down. To do so, you can set the conditions via the ChromScope software for cleaning and flushing the system. You can then run these conditions to perform those functions before the system-shutdown conditions apply.

Always observe Good Laboratory Practice (GLP) guidelines and your laboratory's standard operating procedures.

9.10.1 Shutting down the system for short periods

Short-term shutdown is appropriate for shutting down the system for a few days, such as a weekend.

To perform a short-term system shutdown:

1. Use an acceptable cleaning solvent to empty and clean all extraction and collection vessels of residual product.
2. Set conditions suitable for cleaning the solvent and fraction-collection flow lines for your application.

   Tip: Minimally set the flow for CO_2 and, optionally, co-solvent, to flush to the co-solvent and fraction-collection flow lines for as long as 15 minutes.
3. When the flushing ends, shut off the CO_2 inlet supply.
4. If the pumps and ABPR remain in operation, in the ChromScope Main window, click **Stop** to switch off the system pumps, ABPR, and heaters.
5. Leave the system pressurized.

6. Optionally, shut down the CO_2 recycler chiller.

   Tip: If you shut down the chiller, you must refill the CO_2 recycler with CO_2 before starting up the system. Depending on the CO_2 tank temperature and its distance from the recycler, refilling can take up to two hours.

7. If the system is stopped for more than 1 hour, open the recirculation valve on the CO_2 recycler.

9.10.2 Shutting down the system for long periods

Long-term shutdown is appropriate for shutting down the system for several days or longer.

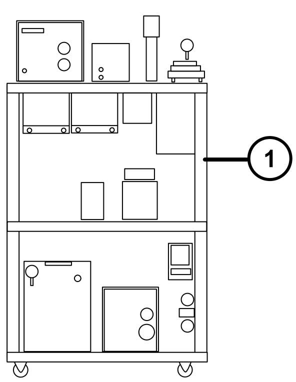
To perform a long-term system shutdown:

1. Perform the shutdown procedure described in Shutting down the system for short periods.
2. Exit ChromScope.
3. Power-off the PC workstation.
4. Power-off each component (including the CO_2 recycler chiller).
5. Disconnect all electrical connections to the system.
6. If shutting down the system for an extended period, or before moving the system to a new location, fully depressurize the system as follows:

   a. If the system is equipped with a CO_2 recycler, shut down and depressurize the recycler by opening its drain valve.

   b. For each cyclone separator, release any pressure remaining in the system's back end by fully opening the separator's associated manual back-pressure regulator (MBPR) valve (MBPR1, MBPR2, or MBPR3) and drain valve (MV8, MV9, or MV10).

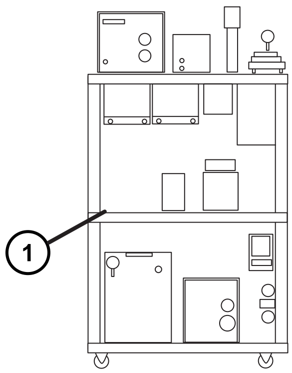
   Figure 9–112: Location of MV8, MV9, and MV10 valve on side of module cart



Location of MV8, MV9, and MV10 valve

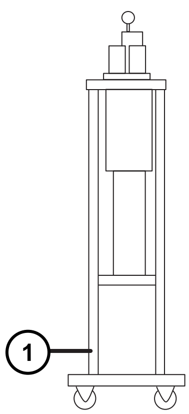
c. For each extraction vessel, release any pressure remaining in the system's front end by opening the MV2 valve stem that directs flow to the vessel, and then opening the vessel's associated drain valve (MV6 or MV7).

Figure 9–113: Location of MV2 valve on module cart



1 Location of MV2 valve

Figure 9–114: Location of MV6 or MV7 valve on back side of extraction vessel



1 Location of MV6 or MV7 valve

9.11 Servicing the CO_2 inlet filter

An in-line filter in the CO_2 inlet line helps prevent particles from entering the system and damaging components such as check valves. A clogged filter restricts flow to the pump, diminishing its performance. Clean or replace the CO_2 inlet filter as part of a scheduled maintenance routine or whenever a clogged filter restricts CO_2 flow.

Warning: To avoid serious injury, including asphyxiation, that can result from disconnecting a pressurized CO_2 line, before disconnecting the line from the CO_2 source, stop the flow of CO_2 from the system, and shut the valve at the CO_2 supply.

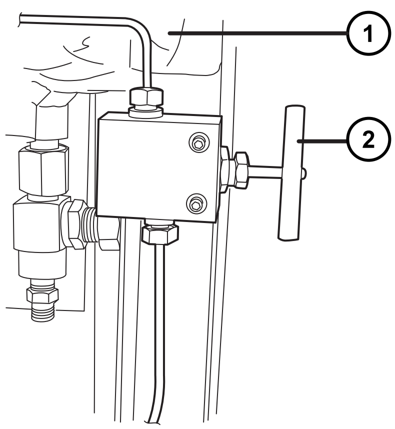
Required tools and materials

- 3/4-inch open-end wrench
- Food-grade ethanol (if cleaning)
- Ultrasonic bath
- CO_2 inlet filter (if replacing)
- CO_2 inlet filter seal (if replacing)

To service the inlet filter:

1. Close the CO_2 recycler outlet (isolation) valve.

Figure 9–115: CO_2 recycler outlet (isolation) valve



1 Bottom of CO_2 recycler

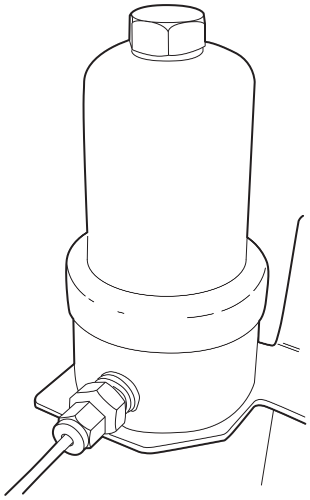
2 CO_2 recycler outlet (isolation) valve handle

2. On the ChromScope main page, set the CO_2 flow to `30 g/min` until it shuts down.

   Result: This will drain the CO_2 from the filter tubing and inlet.

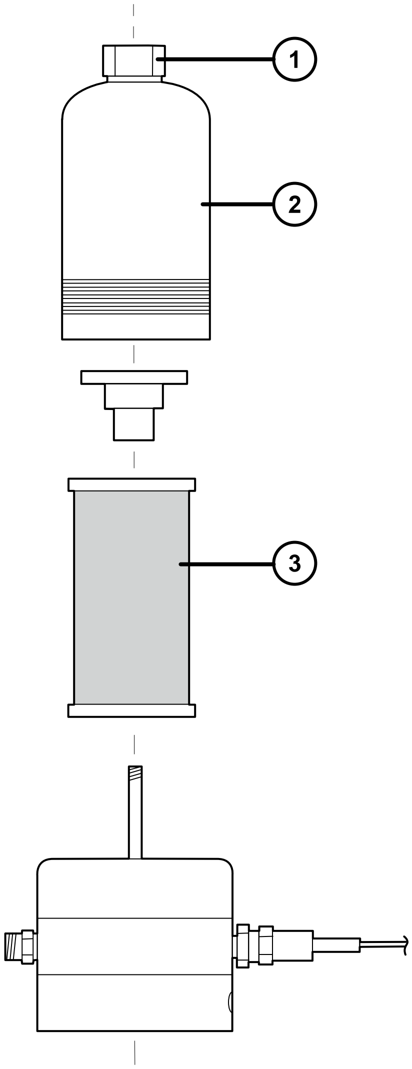
3. Locate the CO_2 inlet filter assembly where the CO_2 supply connects to the system.

Figure 9–116: CO_2 inlet filter assembly



4. Use a 3/4-inch open-end wrench to loosen and remove the cover of the inlet filter.

Figure 9–117: CO_2 inlet filter assembly exploded view



1. Place the 3/4-inch open-end wrench here
2. Inlet filter cover
3. Inlet filter

5. Remove the inlet filter from the inlet filter housing, and then do one of the following:
   - If the filter is partially blocked, sonicate the filter in 95% food-grade ethanol for 20 minutes, and then reinstall it.
   - If the filter is completely blocked, replace the filter, and perform the level 3 cleaning procedure.

   Tip: A partially or completely blocked filter indicates that the CO_2 source might be contaminated with particulate matter.

6. Replace and tighten the inlet filter cover.
7. Open the valve to the CO_2 supply source, and inspect the inlet filter assembly for leaks.

9.12 Maintaining the P-Series high-pressure pump

The SFE systems use P-Series high-pressure pumps to deliver precise quantities of liquid CO_2 and co-solvent, or modifier, to the system. The pump models, which are shipped fully assembled and ready to use, vary in size to provide sufficient flow capacity to accommodate extractions of different sample volumes. The pump models share a design that incorporates a low-dead-volume head and check valves, which provide efficient pumping of liquid CO_2 or co-solvent. As the pump's piston retracts, the lower check valve opens to allow fluid into the chamber. Concurrently, the upper check valve closes, to prevent the system fluid from entering the chamber. When the piston completes its fill cycle, it begins to move forward, causing the lower check valve to close. When the pressure in the piston chamber exceeds the system pressure, the upper check valve opens and allows the fluid to flow into the system. The pump consists of pistons that provide pulse-free solvent delivery.

Warning: To avoid electric shock, do not remove protective panels from system modules. The components within are not user-serviceable.

Requirements:

- Before servicing a P-Series pump used to pump liquid CO_2, shut the valve to the CO_2 supply source, and open the bleeder valve, to completely depressurize the pump. Then power-off the pump, disconnect it from its power source, and remove and plug the cooling lines attached to the front of the pump heads.
- Before servicing a P-Series pump used to pump co-solvent, power-off the pump, and disconnect it from its power source. Then depressurize the system, and remove the solvent inlet lines from the solvent reservoirs, or move the solvent reservoirs to a location below the pump.

Warning: To avoid injuries arising from contact with solvent spilled as a result of unintentional siphoning, before servicing a pump, remove the solvent inlet lines from the solvent reservoirs or move the solvent reservoirs to a location below the pump.

The topics in this section convey instructions for maintaining the system's high-pressure pumps.

9.12.1 Maintaining the P-200 high-pressure pump

Depending on the SFE system configuration, the system uses the P-200 high-pressure pump to deliver precise quantities of liquid CO_2 to the system.

The topics in this section convey instructions for maintaining the P-200 high-pressure pump.

9.12.1.1 Replacing the P-200 or P-350 pump's inlet and outlet check valves

The inlet check valves on the bottom of the pump heads allow flow into the heads as the piston retracts. As the piston advances and pressure in the chamber begins to build, the inlet check valves prevent retrograde flow.

The outlet check valves atop the pump heads allow flow out of the heads as the piston advances. As the piston retracts, the outlet check valves prevent system pressure from entering the heads.

The P-200 pump has a mass flow rate of approximately 1.125 to 1.0 times its RPM. Proportionately, the typical P-200 pump RPM is approximately .875 to 1.0 times its mass flow rate. For example, the estimated mass flow rate for a P-200 pump running at 117 RPM is 124 g/min (117 × 1.0625 = 124 g/min).

Notes:

- The example assumes that the pump is running well.
- The correction factor varies, based on check valve and seal wear.
- Because the thermophysical relationships present are highly nonlinear, the correction factor is only an estimate.

When a check valve fails, the pump's outflow decreases, causing the pump to run faster in order to maintain the specified flow rate. If the flow rate from the pump is lower than the stated RPM, one or more check valves can be defective. Other possible problems include cooling restriction on the inlet, leaking piston seals, and a low CO_2 supply.

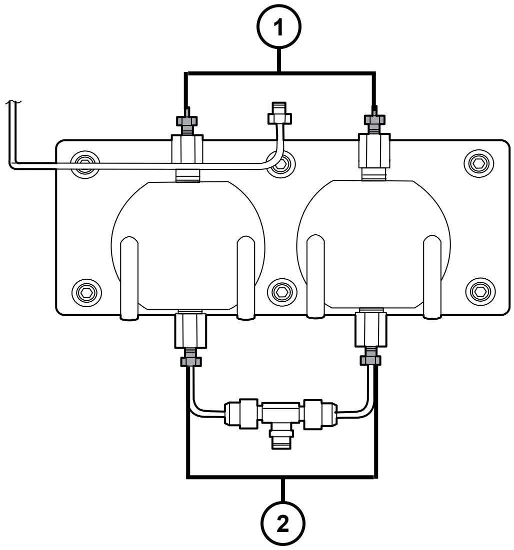
Required tools and materials

- 3/8-inch open-end wrench
- 1/2-inch open-end wrench
- Needle-nose pliers
- Inlet and outlet check valves designated for the P-Series model pump
- PTFE spray

To replace the check valve:

1. Use a 3/8-inch open-end wrench and a 1/2-inch open-end wrench as backup to disconnect the inlet line or outlet line from the pump check valve nut and the manifold.

Figure 9–118: Outlet and inlet connections on pump head



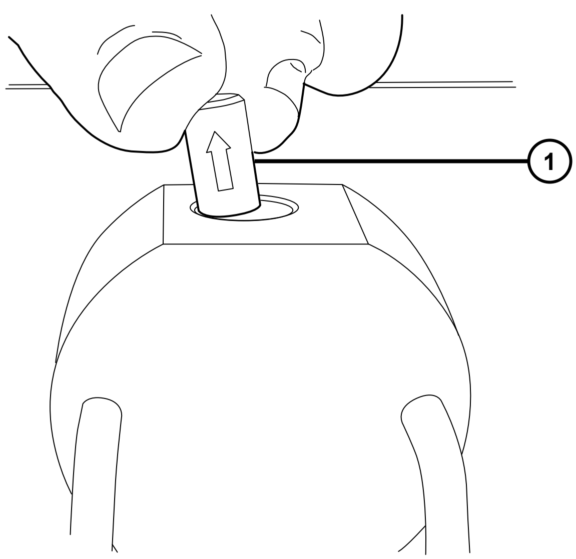
1 Outlet line

2 Inlet line

2. Remove the tubing.
3. Remove the check-valve nut.

Tip: If the check valve is an outlet valve, use needle-nose pliers to remove the valve from the head.

Figure 9–119: Outlet check valve - with arrow up



1 Outlet check valve

4. Clean the retainer-nut threads and spray them with a PTFE lubricant.
5. Install the new check valve with the arrow pointing up.
6. Finger-tighten the nut into the head until the nut contacts the check valve.
7. Use a 3/8-inch wrench to tighten the check-valve nut approximately 120°.
8. Reinstall the tubing.
9. After servicing the CO_2 pump, open the valve to the CO_2 supply source and inspect for leaks.

9.12.1.2 Replacing the P-200 or P-350 pump's piston seals

The P-Series pump incorporates low-friction sapphire for the pistons. The sapphire passes through a spring-loaded seal. After some time, the seal begins to wear and requires replacement. Typical symptoms of a leaking seal are decreased pump efficiency, excessive bubbles exuding from behind the head, and a buildup of frost on the CO_2 pump, from leaking CO_2.

Requirement: Always replace the backup ring when replacing the piston seal.

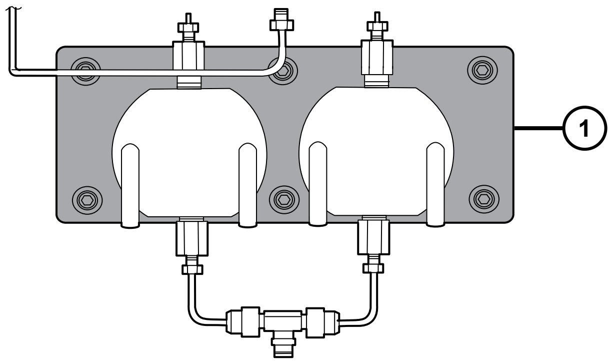
Required tools and materials

- 3/8-inch open-end wrench
- 1/2-inch open-end wrench
- 3/16-inch hex wrench
- 30W, non-detergent, motor oil
- Acetone
- Lint-free cloth
- Pump piston seal service kit designated for the P-Series model pump

To replace the piston seals:

1. Disconnect the inlet and outlet lines from the pump's check-valve nuts and the manifold using a 3/8-inch open-end wrench and a 1/2-inch open-end wrench as backup.

Figure 9–120: Pump heads showing tubing and six securing screws



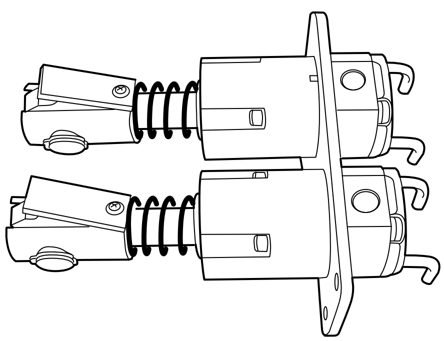
1 Pump head spacer plate

2. Remove the 6 screws securing the head-spacer plate to the front of the pump using a 3/16-inch hex wrench.

3. Withdraw the plate head assembly from the front of the pump.

   Tip: Mark the top of the plate, the left and right heads, pistons, and bearing blocks for future reference.

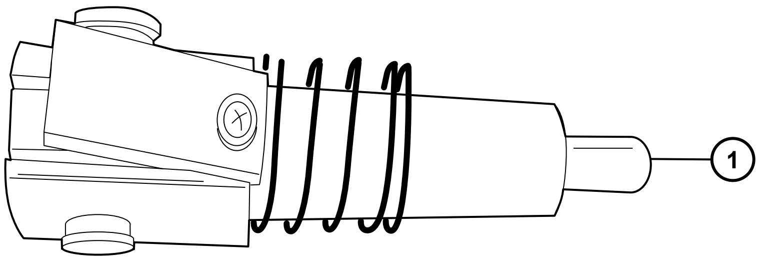
Figure 9–121: Pump head assembly removed



Warning: To avoid finger or hand lacerations from sharp-edged surfaces, use care when installing or removing head-assembly components. Bending or twisting the sapphire piston shaft can cause it to fracture or splinter.

4. Withdraw the piston from the first head and bearing-block assembly.

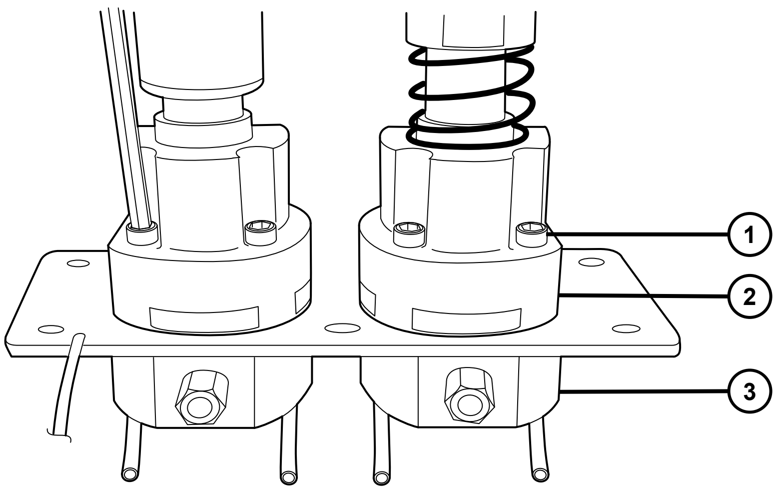
Figure 9–122: Piston assembly



(1) Piston sapphire

5. Clean the piston sapphire using acetone and a lint-free cloth.
6. Remove the 4 socket-head screws that secure the bearing block to the head-spacer plate and pump head using the hex wrench.

Figure 9–123: Pump head bearing block



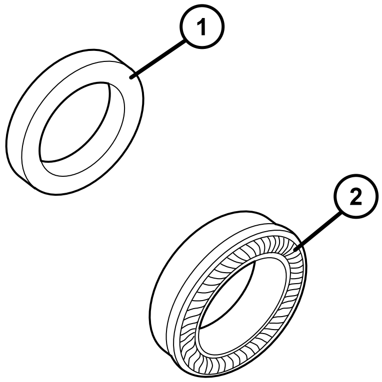
(1) Socket head screws

(2) Bearing block

(3) Pump head

7. Remove the backup ring and spring seal from the pump head using the plastic, hooked tool from the service kit.

Figure 9–124: Backup ring and piston spring seal



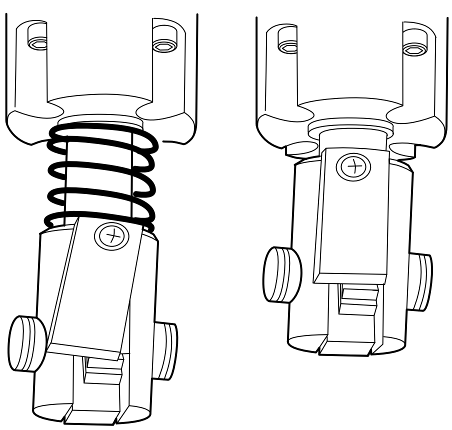
1 Backup ring

2 Piston spring seal

8. Clean the seal cavity using acetone using a cotton swab.
9. Slide the new spring seal into the head with the spring facing into the head.

   Requirement: The spring must not be visible when installed.
10. Slide the backup ring into the head until it seats flush.
11. Loosely install the 4 socket-head screws securing the head to the spacer plate and bearing block.
12. With the piston's return spring removed, insert the piston using a rotating motion into the bearing block, spacer plate, and head until it contacts the head seals.

    Figure 9–125: Installed piston with spring removed to seat the new seals

    
13. Tighten the 4 socket head screws into the head. Use the partially installed piston as a guide to center the bearing.
14. Repeat the preceding steps to replace the seals in the second pump head.

15. Remove each piston assembly, and then reinstall it with its piston-return spring mounted on the piston shaft.

16. Turn the pistons so the oiler wicks face upward, and slide the plate assembly back, into the front of the pump.

    Note: The P-Series pump uses sealed bearings. Under normal operating conditions, such bearings require no additional lubrication, except for the roller bearing at the rear of each piston assembly. To lubricate the piston's roller bearings, monthly apply 3-4 drops of 30W, non-detergent, motor oil to the oiler wicks.

17. Loosely install the 6 socket-head screws joining the spacer plate and pump chassis.

18. Begin tightening the screws, approximately 1 or 2 turns each and in a clockwise pattern until all are tight.

19. Reinstall the tubing to the check-valve nuts and the manifold.

20. Reattach the cooling lines to the front of the pump heads.

21. Confirm pump operation as follows, according to the type of the pump serviced:

    - For the P-Series pump that functions as the CO_2 pump, open the valve to the CO_2 supply source, and inspect the pump for leaks. In ChromScope, verify that the CO_2 flow rate is set to zero and the pump and ABPR pressure readings are within +/- 1.5 bar. If the readings are outside this tolerance, contact your Waters service representative, to request pump calibration service.

    - For the P-Series pump that functions as the co-solvent pump, reinstall the solvent reservoir lines, and use the appropriate MV valve to prime the pump. Start a solvent flow, and inspect the pump for leaks.

9.12.1.3 Replacing the P-200, P200X, or P-350 pump's rupture disc

The P-Series pump is equipped with a rupture-disc safety device. This device is designed to burst if the pressure exceeds the disc's limit. The rupture discs are of a special, nonfragmenting type and must not be replaced with any other type.

Recommendation: Before replacing a ruptured pressure disc, identify and correct the problem that caused the disc to burst. A burst event can result from any of these causes:

- Improperly calibrated pressure transducer
- Operating the pump with the outlet valve closed
- Running the pump with incorrect control parameters
- Using an incorrect rupture disc

Warning: To avoid injury from a bursting rupture disc, do not replace a rupture disc with one that is not prescribed for use with the pump, particularly one whose burst rating is higher than that specified for the pump. You cannot determine a disc's burst rating by examining the disc itself, for many discs appear to be identical though their burst ratings differ. You can verify a disc's rating only by inspecting its tag.

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Notice: To avoid damaging pump components, replace a rupture disc only with one of the prescribed burst rating, which appears on the disc's tag.

Notice: To avoid contaminating system components, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Warning: To avoid eye injury, use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Requirement: Use eye protection when performing this procedure.

Required tools and materials

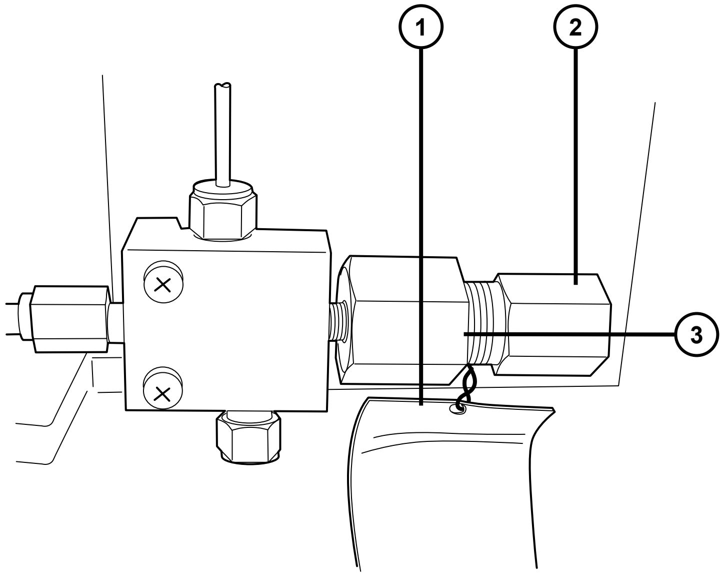
- Chemical-resistant, powder-free gloves
- Protective eyewear
- 13/16-inch open-end wrench
- 1-inch open-end wrench
- Calibrated torque wrench capable of measuring torque of 10 to 150 foot/pounds
- PTFE spray
- Rupture-disc replacement kit designated for the P-Series model pump

To replace a burst rupture disc:

Warning: To avoid injury from a burst outlet valve, do not completely close the valve before performing this procedure.

1. Use the 13/16-inch open-end wrench and the 1-inch open-end wrench as backup to loosen and remove the disc-retainer nut from the housing.

Figure 9–126: Rupture disc rating tag and retainer nut



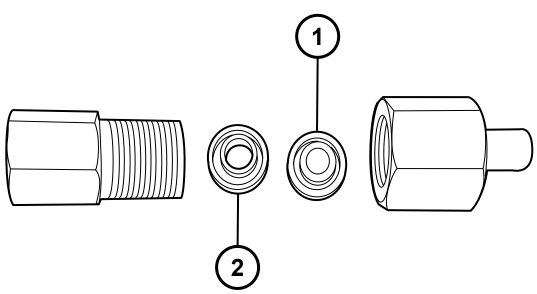
1 Rupture-disc rating tag

2 Disc-retainer nut

3 Housing

2. Remove the burst rupture disc.
3. Clean the retainer-nut threads and spray them with PTFE lubricant.
4. Place the replacement disc onto the disc retainer.

Figure 9–127: Rupture disc components



1 Replacement disc

2 Disc retainer

5. Use your finger to slide the holder and disc into the housing, disc-first with the dome side facing inward (downstream or towards the outdoor vent), and carefully thread the disc-retainer nut into the housing.

6. Use the calibrated torque wrench and the 1-inch open-end wrench to tighten the disc-retainer nut and housing to 15 ft/lbs.
7. Install the tag supplied with the rupture disc around the holder.

9.12.2 Maintaining the P-50 high-pressure pump

Depending on the system configuration, the P-50 high-pressure pump module is provided with the system. The pump delivers precise quantities of co-solvent to the system flow.

The topics in this section convey instructions for maintaining the P-50 high-pressure pump.

9.12.2.1 Replacing the P-50 pump's inlet and outlet check valves

The inlet check valves, on the bottom of the pump heads, allow flow into the pump heads as the piston retracts. As the piston advances, and pressure in the chamber begins to build, the inlet check valves close, preventing retrograde flow.

The outlet check valves, atop the pump heads, allow flow out of the pump heads as the piston advances. As the piston retracts, the outlet check valves prevent system pressure from entering the pump heads.

When a check valve fails, the pump's outflow decreases, causing the pump to run faster, to maintain the specified flow rate. A typical pump's flow rate slightly exceeds the pump's RPM multiplied by a factor of four. If the flow rate from the pump is lower than the stated RPM, one or more check valves can be defective. Other possible causes for diminished flow rate include cooling restriction on the inlet, leaking piston seals, and low supply source.

Required tools and materials

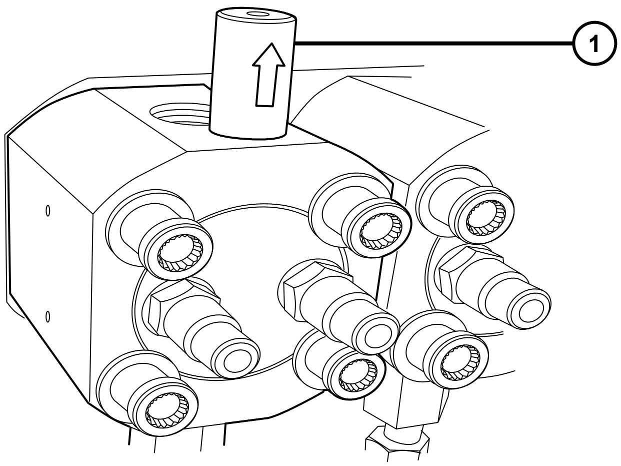
- 3/8-inch open-end wrench
- 1/2-inch open-end wrench
- Needle-nose pliers
- Inlet and outlet check valves designated for the P-50 model pump
- PTFE spray

To replace the check valve:

1. If using the P-50 to pump liquid CO_2 to the system, shut the valve to the CO_2 supply source, and then open the bleeder valve, to completely depressurize the pump.
2. For the check valve that you are replacing, use the 3/8-inch open-end wrench and the 1/2-inch open-end wrench, as backup, to disconnect the inlet or outlet line from the pump check-valve nut and the manifold.
3. Remove the tubing.
4. Remove the check-valve nut.

Tip: If the check valve is an outlet valve, use needle-nose pliers to remove the valve from the head.

Figure 9–128: Outlet check valve - with arrow up



1. Check valve with arrow pointing up

5. Clean the retainer-nut threads, and spray them with a PTFE lubricant.
6. Install the new check valve with the arrow pointing up.
7. Finger tighten the nut into the head until the nut contacts the check valve.
8. Using the 1/2-inch open-end wrench, tighten the check-valve nut approximately 120°.
9. Reinstall the tubing.
10. If using the P-50 to pump liquid CO_2 to the system, open the valve to the CO_2 supply source, and inspect for leaks.

9.12.2.2 Replacing the P-50 pump's piston seals

The P-50 high-pressure pump's seals incorporate low-friction sapphire. The sapphire passes through a spring-loaded seal, which eventually wears, and requires replacement.

Requirement: Always replace the backup ring when replacing the piston seal.

Required tools and materials

- 3/8-inch open-end wrench
- 1/2-inch open-end wrench
- 3/16-inch hex wrench
- Acetone
- Cotton swabs

- Lint-free cloth
- Pump piston seal service kit designated for the P-50 model pump

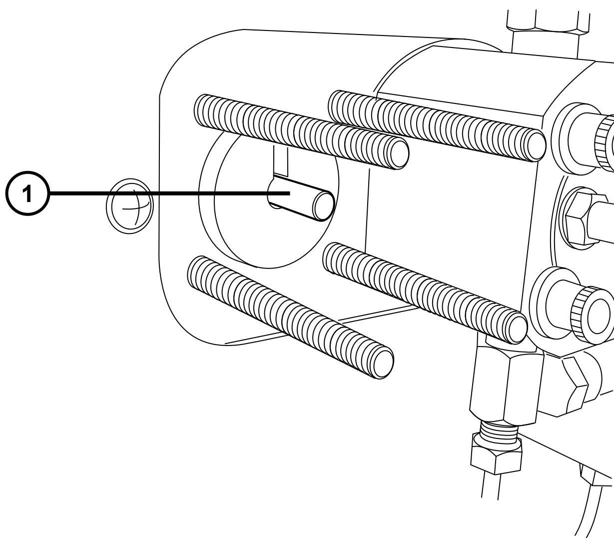
To replace the piston seals:

1. Use the 3/8-inch open-end wrench and the ½-inch open-end wrench as backup to disconnect the inlet and outlet lines from the pump's check-valve nuts and the manifold.
2. Use the 3/16-inch hex wrench to remove the 4 head nuts and washers securing the head to the front of the pump.

   Warning: To avoid finger or hand lacerations from sharp-edged surfaces, use care when installing or removing head-assembly components. Bending or twisting the sapphire piston shaft can cause it to fracture or splinter.

3. Withdraw the head assembly from the front of the pump.

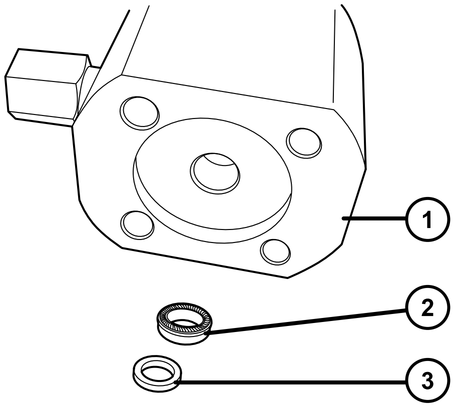
   Figure 9–129: Pump head assembly removed

   1 Sapphire piston

4. Clean the sapphire piston using acetone and a lint-free cloth.
5. Remove the backup bearing and piston seal from the pump head using the plastic, hooked tool from the service kit.

Figure 9–130: Piston spring seal and backup seal

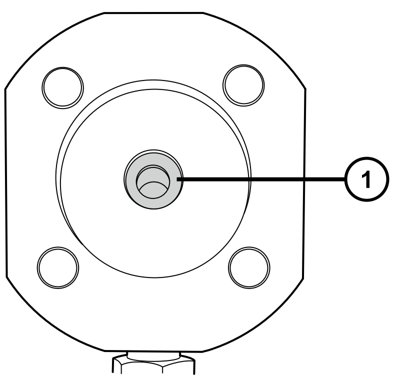


1. Pump head
2. Piston spring seal
3. Backup seal

6. Clean the seal cavity using acetone using a cotton swab.
7. Slide the new seal into the pump head with the spring facing toward the front of the head.

   Requirement: When installed, the spring must not be visible.

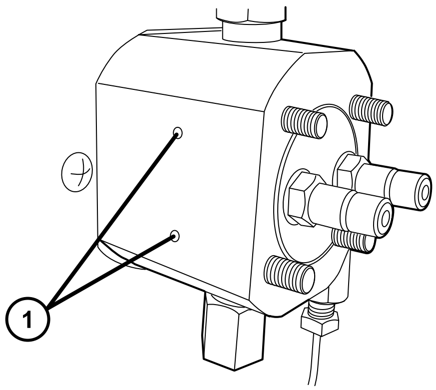
Figure 9–131: Pump head with installed spring seal



1. Spring seal

8. Slide the backup bearing into the head until it is seats flush.
9. Orient the head so that the two bleed holes on its side are visible when both heads are installed.

Figure 9–132: Bleed holes on side of pump head



1 Bleed holes

10. With the head properly oriented, align it with the head bolts, and slide it into place.
11. Loosely install the 4 head nuts between the head and the pump chassis.
12. Use the hex wrench to tighten the nuts with approximately 1 or 2 turns each and in a clockwise pattern until all are tight.
13. Reinstall the tubing to the check-valve nuts and the manifold.
14. If using the P-50 pump to deliver liquid CO_2 to the system, reinstall the cooling lines attached to the front of the pump heads. Open the valve to the CO_2 supply source, and inspect for leaks.

9.12.2.3 Replacing the P-50 pump's rupture disc

The P-50 high-pressure pump is equipped with a rupture disc safety device. This device is designed to burst if the pressure exceeds the disc limit. The rupture discs are a special nonfragmenting type and must not be replaced with any other type.

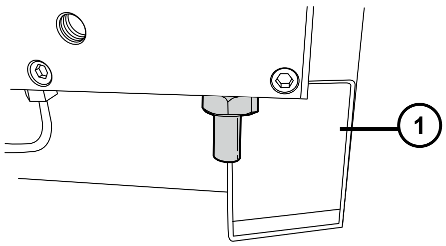
Recommendation: Before replacing a rupture disc, identify and resolve the problem that caused the disc to burst. A burst event can result from any of these causes:

- Improperly calibrated pressure transducer
- Operating the pump with outlet valve closed
- Running the pump with incorrect control parameters
- Using an incorrect rupture disc

Warning: To avoid injury from a bursting rupture disc, do not replace a rupture disc with one that is not prescribed for use with the pump, particularly one whose burst rating is higher than that specified for the pump. You cannot determine a disc's burst rating by examining the disc itself, for many discs appear to be identical though their burst ratings differ. You can verify a disc's rating only by inspecting its tag.

Notice: To avoid damaging pump components, replace a rupture disc only with one of the prescribed burst rating, which appears on the disc's tag.

Figure 9–133: Rupture disc rating tag



(1) Rupture disc rating tag

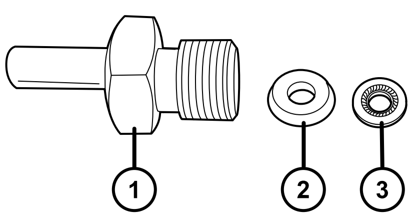
Required tools and materials

- 3/4-inch open-end wrench
- PTFE spray
- Rupture disc replacement kit designated for the P-50 model pump

To replace a burst rupture disc:

1. Use the 3/4-inch open-end wrench to loosen and remove the disc retainer nut from the manifold.
2. Remove the burst rupture disc.
3. Clean the retainer nut threads and spray with a PTFE spray lubricant.
4. Install the replacement disc into the disc holder with the dome side facing inward.

   Figure 9–134: Rupture disc components

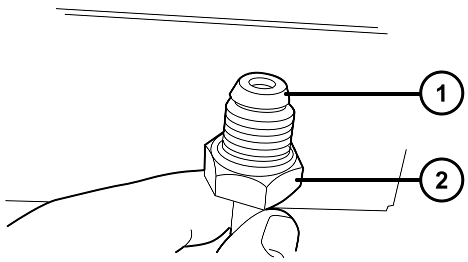
   (1) Retainer nut

   (2) Disc holder

   (3) Rupture disc

5. Place the disc holder with the disc on top of the lower nut, and carefully thread the disc retainer nut into the manifold.

Figure 9–135: Rupture disc and holder installed in retainer nut



(1) Disc holder and rupture disc

(2) Retainer nut

6. Finger tighten the nut until the rupture disc contacts the base of the manifold.
7. Use the 3/4-inch open-end wrench to tighten the nut to 60°.
8. Around the holder, install the tag supplied with the rupture disc.

9.13 Maintaining the automated back-pressure regulator

For information on maintaining the automated back-pressure regulator (ABPR), see the *Automated Back-Pressure Regulator Overview and Maintenance Guide*.

9.14 Maintaining the heat-exchanger module

The heat exchanger heats a fluid circulating through it, in preparation for the next phase of a system's operation. A temperature controller monitors the temperature of the heat exchanger, compensating for variations produced by different flow rates.

The heat exchanger contains no user-serviceable components, so it does not require routine maintenance. For models of the heat exchanger fitted with fuses, the two fuses are installed in an external power-entry module on its rear panel.

9.15 Replacing the vessel's rupture disc

The extraction and cyclone separator vessels are equipped with a rupture disc safety device. This device is designed to burst if the pressure exceeds the disc limit. The rupture discs are a special nonfragmenting type and must not be replaced with any other type.

Recommendation: Before replacing a rupture disc, identify and resolve the problem that caused the disc to burst. A burst event can result from any of these causes:

- Improperly calibrated pressure transducer
- Running the system with incorrect control parameters
- Using an incorrect rupture disc

Warning: To avoid injury from a bursting rupture disc, do not replace a disc with one that is not prescribed for use with the vessel, particularly one whose burst rating is higher than that specified for the vessel. Note that you cannot determine a disc's burst rating by examining the disc itself, for many discs appear to be identical, though their burst ratings differ. You can verify a disc's rating only by inspecting its tag.

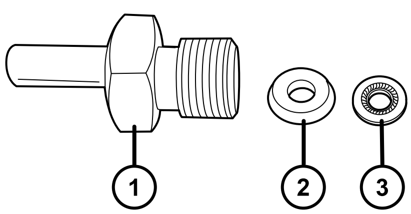
Required tools and materials

- 3/4-inch open-end wrench
- PTFE spray
- Rupture disc replacement kit designated for the vessel

To replace a burst rupture disc:

1. Use a 3/4-inch open-end wrench to loosen and remove the disc retainer nut from the manifold.
2. Remove the burst rupture disc.
3. Clean the retainer nut threads and spray with a PTFE lubricant.
4. Install the replacement disc into the disc holder with the dome side facing inward.

Figure 9–136: Rupture disc components



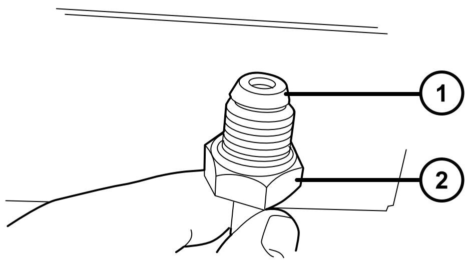
1 Retainer nut

2 Disc holder

3 Rupture disc

5. Place the disc holder with the disc on top of the lower nut, and carefully thread the disc retainer nut into the manifold.

Figure 9–137: Rupture disc and holder installed in retainer nut



1 Disc holder and rupture disc

2 Retainer nut

6. Finger tighten the nut until the rupture disc contacts the base of the manifold.
7. Use a 3/4-inch open-end wrench to tighten the nut to 60°.
8. Around the holder, install the tag supplied with the rupture disc.

9.16 Replacing the component fuses

The system components are equipped with fuses that interrupt the electric current when something within the component fails. They protect both the operator and the equipment. Fuse failure is, under normal conditions, an extremely rare occurrence. Fuses that regularly fail indicate a probable fault in the component and must be investigated. Contact Waters Customer Support for assistance.

Exception: The fuses inside heat exchanger models equipped with an internal power-entry module are not customer-serviceable. Their replacement requires contacting a Waters service representative.

The fuse holder is located above the power switch on the rear of the module. Always replace a fuse with one of equal rating. The fuse-replacement information is printed on the rear of the module and described in the tables below.

Important: For modules that support dual-voltage settings, always refer to the fuse-replacement information printed on the rear of the module for fuse-replacement instructions that accord with the module's voltage setting.

Table 9–4: System and component fuses

System module	Power rating	Fuse rating
Six-zone temperature controller module (CN6)	100-240 Vac, 50-60Hz	1A-250V
ABPR	100-240 Vac, 50-60Hz	3A-250V

Table 9–4: System and component fuses (continued)

System module	Power rating	Fuse rating
Heat exchanger (for models with external power-entry module on rear panel only)	500/1000 watt - 120/240 Vac, 50/60 Hz 2000 watt - 240 Vac, 50/60 Hz	500 watt - 6.3A-250V 1000/2000 watt - 10A-250V
P-50 high-pressure pump	100-240 Vac, 50-60Hz	10A-250V
P-200/P-350 high-pressure pump	100-240 Vac, 50-60Hz	10A-250V
Mass flow meter	115-130 Vac, 50/60 Hz	0.4A-250V
Chiller unit	100-240 Vac, 50/60 Hz	10 A, 5 × 20 mm, IEC 127, fast acting

Notice: To avoid damaging system modules, before replacing an opened fuse, determine the cause of the fuse's failure. It is possible that a serious underlying problem requires attention. Contact Waters Technical Service for assistance.

Warning: To avoid electrical fire, ensure that replacement fuses comply with the ratings affixed to the rear panel of the module.

Warning: To avoid electrical shock, before removing a fuse, power-off the module, and then remove the power cord from the receptacle on the back of the module.

Required tools and materials

- Appropriate replacement fuses

To replace the fuses in a module:

1. Move the power switch to the "off" (0) position, and remove the power cord from the back of the unit.
2. Open the fuse-holder door located above the power switch.
3. Remove the fuse, and replace it with one of the same type and rating.
4. Close the fuse-holder door, reconnect the power cord, and move the power switch to the "on" (1) position.

9.17 Cleaning the exterior of the equipment

Warning: To avoid electric shock,

- ensure that the electrical power to the equipment is interrupted;
- when cleaning the surface of the equipment, apply water to a cloth, and then wipe the instrument or device. Do not spray or otherwise apply water directly onto any equipment surface.

Warning: To avoid personal injury, use eye and hand protection during the cleaning process.

Requirement: Use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

To clean the exterior of the equipment:

Clean surfaces of the equipment using only a clean, soft, lint-free paper or clean cloth dampened with water.

10 Diagnostic tests and troubleshooting

10.1 System alarms

10.1.1 Software alarm is triggered, then resets

Problem	Solution
A software alarm occurred and then reset. A system overpressure condition, caused by a pressure spike, is a typical example of such a alarm.	1. Open and view the System Status panel or the current history file to determine the cause of the problem. 2. From the **Tools** menu, click **Reset System Alarm(s)** > **OK**. 3. If the problem persists, exit the ChromScope software, and cycle power to the failed module. 4. If the alarm persists, report its occurrence, along with any supplemental information, to your Waters service representative.

10.1.2 Communication failure

Problem	Solution
Communication with a module fails, triggering a software alarm.	1. Open and view the System Status or Status Control panel, or the current history file to determine the cause of the problem. 2. Inspect the failed component's cables and connections, to ensure they are tight. 3. Exit the ChromScope software, and cycle power to the failed module. 4. Restart the ChromScope software. 5. If the alarm persists, report its occurrence, along with any supplemental information, to your Waters service representative.

10.1.3 Internal hardware or software alarm shutting-down system

Problem	Solution
An internal hardware or software alarm is triggered causing a module or the system to shut-down.	1. Open and view the System Status or Status Control panel, or the current history file to determine the cause of the problem. 2. Exit the ChromScope software and cycle power to the failed module. 3. Restart the ChromScope software. 4. If the alarm persists, report its occurrence, along with any supplemental information, to your Waters service representative.

10.2 Reviewing the data-log files

ChromScope software automatically logs data about each extraction run performed by the system and stores it in data-log files (with the extension ".tta"). Data-log files are organized under the Project's Data Files folder, by date, and labeled with a time stamp indicating when the run ended.

Whenever you suspect a problem occurred with the system, open and view the most recent data-log file to try and diagnose the problem and determine its corrective action. You can adopt this approach to solve most problems that occur during a run.

To review data-log files:

1. In the Folder View panel for the project, in the `Data Files` folder, click the folder name that represents the date on which the files were generated.

   Result: The data-log files generated on that date are displayed in the File View panel.

2. In the File View panel, click the file name.

   Result: The data-log file opens in the ChromScope main window. Click the tabs on the open data-log file to view the run's temperature, flow, and pressure plots. You can also click the **General Info** and **Method Info** tabs to view a record of the run's general and method information.

10.3 Troubleshooting system problems

Follow these basic steps to perform system troubleshooting:

Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Material Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

- Examine the system and consider the likely causes of the problem. For example, unresponsive instruments can mean that power or signal cables are disconnected or connected improperly.
- Compare the system's performance with its performance before the problem arose. When a system is installed, document parameters and operating conditions, establishing a performance baseline. Likewise, in a daily log, document system performance. You must know whether the parameters specified when tuning a system approximate those specified for a previous test sample. If they do not, and the settings needed to achieve optimum performance and a good result differ significantly from those previously specified, some part of the system requires attention.
- Using known samples, measure the performance of individual instruments. To identify atypical parameters, methodically investigate and eliminate possible causes of a problem:
  - System pressure (high, low, erratic)
  - Baseline (flow path or electronics problem)
  - Incorrect qualitative or quantitative results
- Consult the other troubleshooting sections in the system documentation to identify potential causes of a problem and find corrective actions. If you isolate a problem to a system module that has additional documentation, consult the documentation for that module.

10.4 Troubleshooting the high-pressure CO_2 pump

10.4.1 Unable to achieve CO_2 pump pressure or flow rate

Problem	Solution
Low CO_2 supply	Replace CO_2 supply tank, or confirm the bulk supply of CO_2. A cylinder or in-house source is adequate. If using an optional flow meter, confirm that the density is above 0.87 g/cm^3. If the density is lower, the CO_2 supply is low, or the CO_2 flow path leaks.
Inadequate cooling	At the chiller unit, ensure that the bath temperature is between 0 and +5 °C, inspect cooling lines to the pump heads and heat exchangers, and determine coolant fluid level.

Problem	Solution
CO_2 supply does not contain a dip tube	Contact CO_2 supplier; switch tank.
Defective check valve	Replace the CO_2 pump's check valve.
Leaking piston seal	Replace the CO_2 pump's piston seal.
Defective flow meter	Contact your Waters technical service representative.

10.4.2 Pressure reading reported on the display fails to match that of gauge

Problem	Solution
Defective gauge	Replace gauge.
Defective pressure transducer	Contact your Waters technical service representative.
Miscalibrated pressure transducer	Contact your Waters technical service representative.

10.4.3 Pressure reading reported on display fails to match that of other system components

Problem	Solution
Restriction in lines: closed valves	Ensure valves are in proper positions, inspect for proper tubing sizes and properly cut tubing, and confirm valve alignments.
Defective pressure transducer	Contact your Waters technical service representative.
Miscalibrated pressure transducer	Contact your Waters technical service representative.

10.4.4 CO_2 pump stops because of high RPMs

Problem	Solution
Problem with CO_2 source	• Confirm that the CO_2 supply is adequate (flow meter shows density greater than 0.85 g/mL). • Add a new supply tank, or confirm that the CO_2 recycler is operating properly.

Problem	Solution
Tubing connector leaking	Replace tubing connector fitting.
Extraction vessel frozen	• Verify that the temperature in the extraction chamber, as reported in the software's Heater Controller panel, reflects or approximates the temperature set point. • Inspect the extraction vessel's lower exterior area for evidence of ice buildup. • Confirm that the heater controller and heater are functioning properly.

10.4.5 Repeated failure of pump check valves

Problem	Solution
Contaminated solvent	Replace solvent supply.
PTFE particles in lines	Inspect for proper application of PTFE tape on fittings and for the integrity of the seal between tank and supply-line fitting.
Dirt particles in lines	Clean lines after cutting; add an in-line filter to the supply line.

10.4.6 Burst rupture disc

Problem	Solution
Outlet valve closed	Open outlet valve.
Defective pressure transducer	Replace pressure transducer. Request Waters technical service representative to recalibrate unit.
Defective or incorrect rupture disc	Replace rupture disc.

10.5 Troubleshooting the extraction and cyclone separator vessels

10.5.1 Frost forming on a cyclone separator

A cyclone separator can form frost when the following conditions exist:

- The vessel's heater controller settings are switched off or set too low.
- The temperature controller module configured for that vessel is not operating, or its cables are improperly connected.
- During depressurization when collecting a sample, even when the vessel's heater and temperature control module are operating correctly.

See also: Unable to achieve temperature set point for heat exchanger or vessel

10.5.2 Unable to achieve or maintain system pressure

Problem	Solution
No power to the ABPR module	• Confirm connectivity to the ac power source. • Replace the module's fuse. • The model of ABPR module is equipped with an internal temperature safety switch that was activated because of a problem with the module. Call a Waters service representative.
Leaks in the system or problems with pump output	Inspect the system for leaks or problems associated with pump output.
ABPR needle out of alignment	ABPR needle may need alignment, due to either wear on the needle and seat or a defective valve component. Call a Waters service representative.

10.5.3 Unable to achieve temperature set point for heat exchanger or vessel

Problem	Solution
No power to temperature controller module, heat exchanger, or vessel's electrical heater.	• Confirm that the temperature controller module for the heat exchanger or vessel heater is connected to the AC power source. • Confirm that the heat exchanger or vessel heater is connected to the AC power source. • Replace the fuse in the temperature controller module. • The internal temperature safety switch of the temperature controller, the heat exchanger, or the vessel's electrical heater was activated because of a problem with the controller or heater element. Contact a Waters service representative.

Problem	Solution
Zone is not enabled on temperature controller.	In the software's Heater Controller panel, verify that the correct zone is enabled.
Control cable or thermocouple cable is disconnected or connected to different zones on the temperature controller.	Verify that the control cable and thermocouple cable are connected to the same zone on the temperature controller, and that the zone is the correct one specified for the heater or heat exchanger.
Insufficient time allowed to achieve temperature set point.	Allow up to one hour to achieve a thermal steady-state. **Note:** The tolerance for the system temperature set point is +/-5 °C.

10.5.4 Burst rupture disc

Problem	Solution
Outlet valve closed	Open outlet valve.
Defective pressure transducer	Replace pressure transducer. Request Waters technical service representative to recalibrate unit.
Defective or incorrect rupture disc	Replace rupture disc.

10.6 Troubleshooting the optional high-pressure co-solvent pump

10.6.1 Unable to achieve pump flow rate

Problem	Solution
Low solvent supply	Refill solvent reservoir.
Clogged solvent filter	Replace solvent filter.
Defective pump check valve	Clean or replace check valves.
Leaking piston seal	Replace piston seal.

10.6.2 Pressure reading reported on the display fails to match that of gauge

Problem	Solution
Defective gauge	Replace gauge.
Defective pressure transducer	Contact your Waters technical service representative.
Miscalibrated pressure transducer	Contact your Waters technical service representative.

10.6.3 Pressure reading reported on display fails to match that of other system components

Problem	Solution
Restriction in lines: closed valves	Ensure valves are in proper positions, inspect for proper tubing sizes and properly cut tubing, and confirm valve alignments.
Defective pressure transducer	Contact your Waters technical service representative.
Miscalibrated pressure transducer	Contact your Waters technical service representative.

10.6.4 Repeated failures of pump check valves

Problem	Solution
Contaminated CO_2	Contact CO_2 supplier.
PTFE particles in lines	• Ascertain the proper application of PTFE tape on fittings. • Ascertain the integrity of the seal between the tank and fitting of the supply-supply line.
Dirt particles in solvent lines	• Clean lines after cutting them. • Insert a filter in the solvent-supply line.

10.6.5 Burst rupture disc

Problem	Solution
Outlet valve closed	Open outlet valve.

Problem	Solution
Defective pressure transducer	Replace pressure transducer. Request Waters technical service representative to recalibrate unit.
Defective or incorrect rupture disc	Replace rupture disc.

10.7 Troubleshooting the CO_2 recycler

Problem	Solution
CO_2 recycler will not fill	• Confirm that electrical power to the level indicator is uninterrupted. • For the manual CO_2 recycler only, confirm that the manual supply valve is open. • Confirm the sufficiency of the CO_2 supply. • Confirm that the chiller is powered-on and operational.
SFE system CO_2 pump stops due to high rpm alarm, but the recycler indicates adequate supply	• Confirm that the recycler is not isolated from the SFE system. • Verify that the recycler pressure is sufficient. If pressure is too low, increase the chiller temperature (up from 13.5 °C).
Recycler relief valve opens	• Confirm that both manual valves are open. • Inspect the chiller temperature setting. If it is above or near 14 °C, decrease it by 1 °C, and repeat this decrease until the recycler pressure stabilizes at 5500 to 6000 kPa (55 to 60 bar, 798 to 870 psi).

Problem	Solution
Sample carryover to CO_2 recycler	**Possible cause:** Large pressure drops between cyclone separators 2 (CS2) and 3 (CS3) cause significant cooling in CS3, which condenses the exhaust from CS3 into a liquid, resulting in carryover to the CO_2 recycler. **Solution:** Install an inline collection heater between CS2 and CS3 to reduce the manual back- pressure on CS2, which maintains the exhaust from CS3 as a gas and eliminates carryover.
	Possible cause: The temperature in the room where the system is located is too low. Because room temperature affects the CO_2 recycler pressure, too-low temperatures cause the recycler to have more liquid CO_2 than gas, which drives the recycler pressure lower. The large pressure drop has a waterfall effect, pulling the extractables from the cyclone separators into the recycler. Low-polarity extractables, such as terpenes and alkanes, are especially vulnerable to such pressure drops and polarity changes. **Solution:** Maintain a room temperature of between 18 and 24 °C.

Problem	Solution
Sample carryover to CO_2 recycler (continued)	**Possible cause:** The temperature in the room where the system is located is too high. A high room temperature can cause the CO_2 recycler pressure to rise to too high of a level. High recycler pressure can cause terpenes and waxes to remain in the CO_2 as it exits CS3, and then carryover to the recycler. As the recycler heat exchanger gets coated with extract, it becomes less efficient at cooling the liquid CO_2 entering the recycler, which causes the recycler temperature to rise. As this continues, the waxes and terpenes continue to carry over to the Bio-Botanical Extraction System inlet filter and CO_2 pump. **Solution:** Maintain a room temperature of between 18 and 24 °C.
	Possible cause: High CO_2 recycler pressure caused by clogs in the recycler's cooling heat exchanger. **Solution:** Perform the Level 4 cleaning procedure. For more information, see Performing the Level 4 cleaning procedure.
	Possible cause: High CO_2 recycler pressure caused by an unexpected system shutdown from a power outage or other system failure. **Solution:** Reduce the CO_2 recycler pressure. For more information, see Reducing the CO2 recycler pressure.

A Safety advisories

Waters products display safety symbols that identify hazards associated with the product's operation and maintenance. The symbols also appear in product manuals with statements that describe the hazards and advise how to avoid them. This appendix presents all safety symbols and statements that apply to Waters' product offerings. The symbols and statements can apply to a specific product, or apply to other products within the same system.

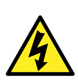
A.1 Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with the misuse of an instrument or device. Heed all warnings when you install, repair, or operate any Waters instrument or device. Waters accepts no liability in cases of injury or property damage resulting from the failure of individuals to comply with any safety precaution when installing, repairing, or operating any of its instruments or devices.

The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device or component of an instrument or device. When one of these symbols appears in a manual's narrative sections or procedures, an accompanying statement identifies the applicable risk and explains how to avoid it.

Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)

Warning: (Risk of burn injury from contacting hot surfaces.)



Warning: (Risk of electric shock.)

Warning: (Risk of fire.)

Warning: (Risk of sharp-point puncture injury.)

Warning: (Risk of hand crush injury.)

Warning: (Risk of injury caused by moving machinery.)

Warning: (Risk of exposure to ultraviolet radiation.)

Warning: (Risk of contacting corrosive substances.)

Warning: (Risk of exposure to a toxic substance.)

Warning: (Risk of personal exposure to laser radiation.)

Warning: (Risk of exposure to biological agents that can pose a serious health threat.)

Warning: (Risk of tipping.)

Warning: (Risk of explosion.)

Warning: (Risk of high-pressure gas release.)

A.1.1 Specific warnings

A.1.1.1 Burst warning

This warning applies to Waters instruments and devices fitted with nonmetallic tubing.

Warning: To avoid injury from bursting, nonmetallic tubing, heed these precautions when working in the vicinity of such tubing when it is pressurized:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to compounds with which it is chemically incompatible: tetrahydrofuran, nitric acid, and sulfuric acid, for example.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, significantly reducing the pressure at which the tubing can rupture.

A.1.1.2 Biohazard warning

The following warning applies to Waters instruments and devices that can process material containing biohazards, which are substances that contain biological agents capable of producing harmful effects in humans.

Warning: To avoid infection with potentially infectious, human-sourced products, inactivated microorganisms, and other biological materials, assume that all biological fluids that you handle are infectious.
Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).
Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the biohazard safety representative for your organization regarding the proper use and handling of infectious substances.

A.1.1.3 Biohazard and chemical hazard warning

This warning applies to Waters instruments and devices that can process biohazards, corrosive materials, or toxic materials.

Warning: To avoid personal contamination with biologically hazardous, toxic, or corrosive materials, you must understand the hazards associated with their handling.
Guidelines prescribing the proper use and handling of such materials appear in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards*.
Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the safety representative for your organization regarding its protocols for handling such materials.

A.2 Notices

Notice advisories appear where an instrument, device, or component can be subject to use or misuse that can damage it or compromise a sample’s integrity. The exclamation point symbol and its associated statement alert you to such risk.

Notice: To avoid damaging the case of the instrument or device, do not clean it with abrasives or solvents.

A.3 Bottles Prohibited symbol

The Bottles Prohibited symbol alerts you to the risk of equipment damage caused by solvent spills.

Prohibited: To avoid equipment damage caused by spilled solvent, do not place reservoir bottles directly atop an instrument or device or on its front ledge. Instead, place the bottles in the bottle tray, which serves as secondary containment in the event of spills.

A.4 Required protection

The Use Eye Protection and Wear Protective Gloves symbols alert you to the requirement for personal protective equipment. Select appropriate protective equipment according to your organization’s standard operating procedures.

Requirement: Use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

A.5 Warnings that apply to all Waters instruments and devices

When operating this device, follow standard quality-control procedures and the equipment guidelines in this section.

Warning: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user’s authority to operate the equipment.

Avertissement : Toute modification sur cette unité n’ayant pas été expressément approuvée par l’autorité responsable de la conformité à la réglementation peut annuler le droit de l’utilisateur à exploiter l’équipement.

Warnung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Advertencia: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

警告： 未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

警告： 未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

경고: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

警告: 規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Avertissement : Manipulez les tubes en polymère sous pression avec precaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l’instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Warnung: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.

Avvertenza: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告： 當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

警告： 当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

This warning applies to Waters instruments fitted with nonmetallic tubing. This warning applies to instruments operated with flammable solvents.

Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Avertissement : L’utilisateur doit être informé que si le matériel est utilisé d’une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d’être défectueuses.

Warnung: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Avvertenza: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告： 使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。

警告： 使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。

경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告: ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

A.6 Warnings that address the replacing of fuses

The following warnings pertain to instruments and devices equipped with user-replaceable fuses. Information describing fuse types and ratings sometimes, but not always, appears on the instrument or device.

Finding fuse types and ratings when that information appears on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.

Avertissement : pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boite à fusible de l'instrument.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.

Avvertenza: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.

Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.

警告： 為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。

警告： 为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。

경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告: 火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Finding fuse types and ratings when that information does not appear on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating indicated in the “Replacing fuses” section of the Maintenance Procedures chapter.

Avertissement : pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués dans la rubrique "Remplacement des fusibles" du chapitre traitant des procédures de maintenance.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert im Abschnitt "Sicherungen ersetzen" des Kapitels "Wartungsverfahren" angegeben sind.

Avvertenza: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate nel paragrafo "Sostituzione dei fusibili" del capitolo "Procedure di manutenzione".

Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características indicados en la sección "Sustituir fusibles".

警告： 為了避免火災，更換保險絲時，應使用「維護步驟」章節中「更換保險絲」所指定之相同類型與規格的保險絲。

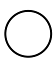
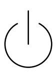
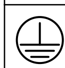
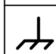
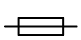
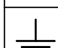
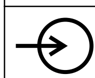
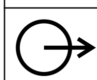
警告： 为了避免火灾，应更换“维护步骤”一章的“更换保险丝”一节中介绍的相同类型和规格的保险丝。

경고: 화재의 위험을 막으려면 유지관리 절차 단원의 “퓨즈 교체” 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告: 火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

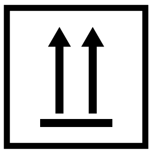
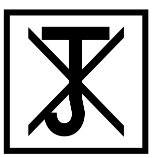
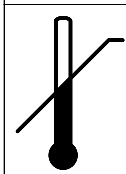
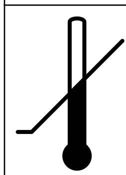
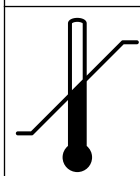
A.7 Electrical symbols

The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument’s front or rear panels.

Symbol	Description
	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
3 	Alternating current (3 phase)
	Safety ground
	Frame, or chassis, terminal
	Fuse
	Functional ground
	Input
	Output

A.8 Handling symbols

The following handling symbols and their associated statements can appear on labels affixed to the packaging in which instruments, devices, and component parts are shipped.

Symbol	Description
	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!
	Upper limit of temperature
	Lower limit of temperature
	Temperature limitation

B Specifications

B.1 System specifications

The Waters Bio-Botanical Extraction System is a bulk-scale, preparative system designed to serve as a high-pressure separator for collecting extracted compounds, to support various applications, including quantitative recovery of botanicals and natural products.

Table B–1: System specifications

Item	Specification
Operating pressure range	10,000 to 60,000 kPa (100 to 600 bar, 1450 to 8702 psi) ±200 kPa (±2 bar, ±29 psi)
Liquid CO_2 inlet pressure	5500 to 10,000 kPa (55 to 100 bar, 798 to 1,450 psi)
Liquid CO_2 inlet temperature	18 to 24 °C **Note:** CO_2 Supply tanks should be at ambient temperature a minimum of 8 hours before use.
Cooling of CO_2 pump	Circulating coolant
System flow rate	3 to 200 g/min
CO_2 pump flow rate	3 to 200 g/min ±2 g/min
Optional co-solvent pump flow rate	1 to 50 mL/min ±1 mL/min
Temperature control	Ambient +5 °C to 60 °C ±1.5 °C
High-pressure extraction	One or two, 5-L or 10-L extraction vessels
High-pressure fraction collection	Three, 2-L cyclone separators
Process control	Automated operation via computer
Software	ChromScope software

It is possible to operate individual components outside the range of system defined parameters. The system is designed and tested only for the ranges defined in the "System Specifications" table.

B.1.1 Optional mass flow meter specifications

Item	Specification
Maximum pressure	10,000 kPa (100 bar, 1,450 psi)
Note: The mass flow meter on the inlet of the CO_2 pump is set at the factory to provide precise flow control.	

B.1.2 High-pressure extraction vessel specifications

Item	Specification
Nominal capacity	5 L or 10 L
Maximum operating pressure	60,000 kPa (600 bar, 8,702 psi)
Maximum operating temperature	150 °C
Body material	SST, 17-4 PH H-1150
Cap material	SST, 17-4 PH H-1150
Seal material	Polyimide
Outside diameter, without fittings	15.24 cm
Inside diameter	10.16 cm
Cap port	1/4 NPT (2)

B.1.3 High-pressure cyclone separator specifications

Item	Specification
Nominal capacity	2 L
Maximum operating pressure	37,200 kPa (372 bar, 5,395 psi)
Maximum operating temperature	150 °C
Body material	SST, 17-4 PH H-1150
Cap material	SST, 17-4 PH H-1150
Seal material	Polyimide
Diameter, without fittings	11.4 cm
Length, without fittings	32.8 cm

B.1.4 Optional CO_2 recycler system specifications

Item	Specification
Storage capacity	15 L
Operating temperature	5 to 15 °C
Operating pressure	6,000 to 6,500 kPa (60 to 65 bar, 870 to 943 psi)
Bleed valve	1
Level sensor	Low, low-low, high, high-high

B.1.5 Heat exchanger specifications

Item	Specification
Process connection	Tubing: 1/8-inch SST, high-pressure
Maximum temperature	100 °C

B.1.6 Six-zone temperature controller module specification

Item	Specification
Inputs and outputs	6
Operating temperature	5 to 40 °C
Temperature sensor input	J-type thermocouple
Control output	24 Vdc, non-inductive (50 mA max load per channel)

B.1.7 Automated back-pressure regulator (ABPR 200) specifications

Item	Specification
Flow rate	up to 200 g/min
Fluid temperature	up to 80 °C
Maximum pressure	32,000 kPa (320 bar, 4641 psi)

B.2 System and component dimensions and weights

Table B–2: System and component dimensions

System component	Width	Depth	Height
System cart with extraction vessel and CO_2 recycler carts and chiller	208.2 cm	162.6 cm	127.0 cm for system with 5-L vessel cart 172.7 cm for system with 10-L vessel cart
Cooling heat exchanger	6.4 cm	25.4 cm	6.4 cm
P-200 high-pressure CO_2 pump	35.6 cm	61.0 cm	31.2 cm
Optional P-50 high-pressure co-solvent pump	27.9 cm	66.0 cm	43.2 cm
Relay	17.8cm	17.8 cm	11.4 cm
Automated back-pressure regulator (ABPR)	17.8 cm	43.2 cm	15.9 cm
Manual back-pressure regulator (MBPR)	7.6 cm	7.6 cm	10.4 cm
Heat exchanger	11.2 cm	46.4 cm	10.7 cm
Six-zone temperature controller module	21.1 cm	27.6 cm	20.3 cm
Mass flow meter	15.2 cm	20.3 cm	14.0 cm
System chiller	23.1 cm	48.8 cm	60.0 cm
PC workstation (computer/monitor)	23.0 cm/45 cm	45 cm/25 cm	45 cm/42 cm
	Requirement: Specifications for the PC workstation are representative and can change depending on the model. In all cases, the PC workstation must be located adjacent to the system.		

Table B–3: System and component weights

System component	Weight
Base system minimum configuration	100.0 kg
Base system with P-50 high-pressure co-solvent pump and mass flow meter	122.0 kg
5-L extraction vessel cart (containing two vessels)	91.0 kg
10-L extraction vessel cart (containing two vessels)	190.0 kg
Recycler system	102.0 kg
System chiller	30.0 kg

Table B–3: System and component weights (continued)

System component	Weight
Recycler chiller	66.0 kg
PC workstation (computer/monitor)	14 kg (5 kg)

B.2.1 Additional space requirements

Use these guidelines in concert with the information from the "System and component dimensions and weights" section for planning your system's laboratory space requirements:

- Maintain a minimum clearance of 30.5 cm at the front and rear of the system, for adequate ventilation and access to system cables.
- Other than the exhaust tubing, keep the area above the system clear of overhanging obstructions, to allow access to the top of the system.
- If installing the system inside a fume hood, contact your local Waters service representative for additional requirements.
- Locate the chillers on the floor as close to the system as possible while maintaining a minimum clearance of 30.5 cm at the front and rear of the chiller, for adequate ventilation.
- Locate the data system (computer CPU, monitor, keyboard, and mouse) on a laboratory bench next to the system and within reach of the data system's cables.
- Locate the optional recycle cart next to extraction-vessel cart.

B.3 System and component power requirements

System component	Input voltage/frequency	Rated power draw	Fuse rating
ABPR	100-240 Vac, 50/60 Hz	220 VA	3A-250 V
Six-zone temperature controller module	100-240 Vac, 50/60 Hz	22 VA	1A-250 V
Heat exchanger	100-240 Vac, 50/60 Hz	1000 VA	10A-250 V
P-200 high-pressure CO_2 pump	100-240 Vac, 50/60 Hz	880 VA	10A-250 V
Optional P-50 high-pressure co-solvent pump	110/220 Vac, 50/60 Hz	352 VA	10A-250 V
Relay module-12A	200-240 Vac, 50/60 Hz	12A-2640 VA	(2x) 12A-250 V
Relay module-20A	200-240 Vac, 50/60 Hz	20A-2640 VA	(4x) 12A-250 V

System component	Input voltage/frequency	Rated power draw	Fuse rating
Optional mass flow meter	115/230 Vac, 50/60 Hz	20 VA	0.4A-250V
2-L cyclone separator vessel heater	200-240 Vac, 50/60 Hz	1000W	N/A
5-L or 10-L extraction vessel heater	200-240 Vac, 50/60 Hz	2000W	N/A
Optional recycler	100-240 Vac, 50/60 Hz	18W	N/A

B.4 Electrical specifications

Attribute	Specification
Protection class[a]	Class I, (Grounded ac)
Overvoltage category[b]	II
Pollution degree[c]	2
Moisture protection[d]	Normal (IPXO)
Line voltages, nominal	200 - 240 Vac
Frequency	50 - 60 Hz
Maximum power draw	11.5 kVA (split between two circuits)

a. Protection Class I – The insulating scheme used in the instrument to protect from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.

b. Overvoltage Category II – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.

c. Pollution Degree 2 – A measure of pollution on electrical circuits that can produce a reduction of dielectric strength or surface resistivity. Degree 2 refers only to normally nonconductive pollution. Occasionally, however, expect a temporary conductivity caused by condensation.

d. Moisture Protection – Normal (IPX0) – IPX0 means that no Ingress Protection against any type of dripping or sprayed water exists. The “X” is a placeholder that identifies protection against dust, if applicable.

B.5 Environmental specifications

Attribute	Specification
Operating temperature	15 °C to 40 °C
Operating humidity	20% to 80%, noncondensing
Non-operating temperature	-30 °C to 60 °C
Non-operating humidity	10% to 85%, noncondensing
Transportation and storage temperature	-30 °C to 60 °C
Transportation and storage humidity	20% to 85%, noncondensing

B.6 Wetted materials of construction

Item	Specification
Wetted materials	316, 17-4, and 304 stainless steel, gold, GFPM, polyimide, Nitronic 60, fused silica, fluoropolymer, fluoropolymer blend, fluoroelastomer, PPS, natural PPS, platinum, PEEK, sapphire, ruby, UHMWPE, and silicone elastomer (for cooling bath tubing)

C Best practices

C.1 Supplies

Waters recommends using the following lab and safety supplies with the Bio-botanical Extraction System. For information about ordering supplies, visit the Waters website (www.waters.com), or consult your Waters sales representative.

C.1.1 Lab supplies

Waters recommends the following lab supplies for the Bio-Botanical Extraction System:

System

- Computer table with room for notebooks and manuals
- Computer protectors or dust covers
- Uninterrupted power supply (1 KVa recommended), for computer, monitor, and CO_2 level indicator
- Plexiglas or some other type of floor protection (2 by 2-ft), to be placed on the floor under the collection containers (beakers)
- Tubing for venting CO_2 waste gas, to be connected to a 6.0-cm (1/4-inch) compression fitting
- 6 portable temperature monitors to verify +/- 5 °C room temperature (placed in different areas to determine where the air flow is needed after the instrument is installed)

Scales

- Balance or balances (0.1 g to 2 kg, upper limit), for weighing trim and extracts
- Tank scale for weighing CO_2 tank

Gases and liquids

- Minimum of six 23-kg (50-lb) CO_2 cylinders with dip tube (siphon) for liquid delivery (food grade)

  Notice: To avoid damaging the chiller, do not use automotive antifreeze as the cooling agent.

- 8-L supply of 50:50 ethylene glycol/water mix or propylene glycol/water mix (see www.dynalene.com)

Extraction preparation

- Suitable container for sample material

  Tip: Oven bags work well.

- Stainless steel funnel with a 2 to 4-inch exit diameter, for adding trim to the extraction vessel. The extraction vessel interior diameter is approximately four inches. For more information, see Additional funnel information.
- Grinder with the ability to grind the plant material to a sand or coffee-ground consistency. For more information, see Additional grinder information.
- Wooden dowel or plastic rod, 2-inch by 3-ft, for packing trim in the extraction vessel.

  Tip: Wooden dowels used for closet clothes hangers work well.

- Step stool (for use when packing 10 L vessel)

Sample collection

Warning: To avoid injuries caused by shattering glass fragments, do not use laboratory glassware to collect fractions or waste components. When opening drain valves, a high-pressure release of solvent or extracted compounds can knock a glass container out of your hand and cause it to shatter.

- At least 24 1-L storage containers for extracted product. Stainless steel is recommended (stainless steel 1.5 qt Bain-marie vessels from a restaurant supply store work well)

  Recommendation: Use stainless steel containers, which do not shatter if dropped or subjected to extreme temperatures. 1.5 quart Bain-marie vessels form a restaurant supply store are suitable.

- Heat gun (low wattage), used for thinning extract when pulling from the sample vessels and for melting clogs in the system tubing

Cleaning

- 10 L of 95% food-grade ethanol

  Note: Store the ethanol appropriately and follow all state laws.

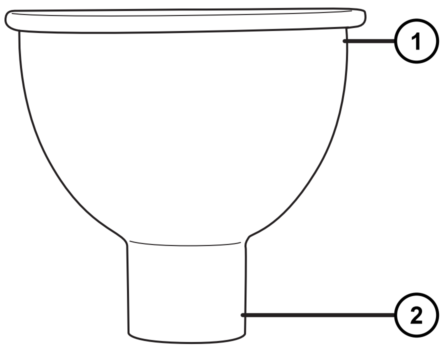
- 4 squirt bottles (for cleanup solvent and checking for CO_2 leaks)

- Cotton swabs of varying lengths
- Paper towels or shop rags
- Paint brush, 2 to 3-inches wide, for cleaning trim from the threads in the extraction vessel
- Wet/dry vacuum with a 3-inch hose diameter (not necessary if you use vessel baskets)

C.1.1.1 Additional funnel information

A funnel with a 3.8-L capacity, 8-inch or larger mouth, and 4-inch neck opening is available for purchase online. With this type of funnel, you can pack the extraction vessel without removing the funnel.

Figure C–1: Funnel example



1. 8-inch or larger mouth
2. 4-inch neck opening

Local hardware stores sell galvanized steel HVAC duct diameter reducers that you can use as funnels. A 6-inch to 4-inch reducer works well as a funnel, but ensure that you cover the sharp edges with tape before use.

C.1.1.2 Additional grinder information

Common food blenders are inexpensive, but tend to unevenly grind the botanical material and generate excessive heat that can degrade the product. You must open the cover and push the intact material to the bottom of the blender jar several times during the run to ensure that the material is evenly ground.

For large amounts of botanical material, a large commercial grade grinder works best. These grinders are more expensive (about $1,000 to $1,500) than common food blenders, but are manufactured for continuous use in a commercial kitchen and are more durable than common food blenders. Their larger bowls (3 to 6 L), enable them to process more botanical material with each run. The design of the grinder bowl ensures that all of the material can be ground without opening the cover.

C.1.2 Safety equipment and supplies

Waters recommends the following safety equipment and supplies for the Bio-Botanical Extraction System:

- Disposable lab coats
- Disposable, lint-free gloves
- Protective eyewear
- Tank safety rack and tank cart, for restraining CO_2 tanks
- CO_2 monitor, 0 to 5000 ppm (see www.co2meter.com)
  - Install the monitor 18 inches above the floor, away from the collection vessels
  - CO_2 must be vented externally
- Positive pressure air exchange system, to exchange the air in the room

Requirement: The ventilation system must provide an air exchange rate of four rooms per hour.

Note: Check local regulations for installation guidelines.

C.1.3 Tools

Waters recommends the following tools for the Bio-Botanical Extraction System:

- 7/16-inch open-end wrench, for extraction vessel fittings
- 3/8-inch open-end wrench, for pump fittings
- 1/2-inch open-end wrench, for black CO_2 fittings on pump, vessel, and vessel selection valves
- 9/16-inch open-end wrench, for collection vessel fittings
- Large, adjustable wrench, 1-1/4-inch minimum, for CO_2 cylinder
- 3/16-inch hex wrench, for pump seal replacement
- Slip-joint pliers, 4-inch minimum opening, for servicing extraction vessel frits and replacing extraction vessel seals

C.2 Preparing botanical material

Observe these guidelines when preparing botanical material for an extraction:

- Dry the botanical material to a moisture content of 5% to 8% wt/wt. Botanical material that is too wet (greater than 8%) adversely impacts extraction efficiency.
- Choose a suitable grinder. For more information, see Additional grinder information.

- Grind the botanical material to at least to a coffee ground consistency, and preferably to a sand consistency. Grinding the material to a sand consistency increases extraction efficiency.
- Grind the material in a separate room from where the Bio-botanical Extraction System is located. Ground botanical material can stick to the electronics and cause overheating.

C.3 Loading the sample

When loading botanical material into the extraction vessel, observe these guidelines:

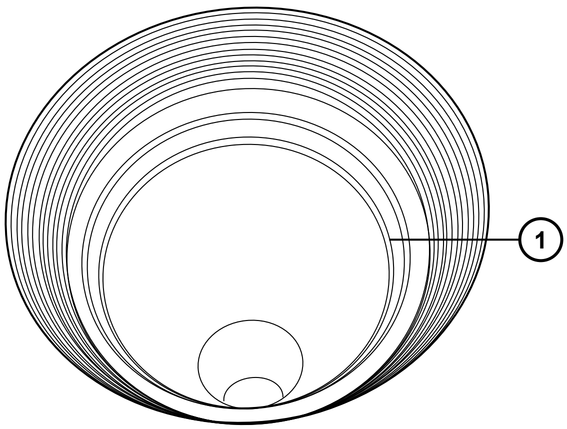
Warning: To avoid injury from flying debris, ensure that the extraction vessel is completely depressurized before loading botanical material. Failure to do so can cause the cap to forcefully pop off the extraction vessel.

- Turn on the extraction vessel heater and specify the temperature so that it can reach the set point while loading the sample.
- The extraction vessel holds a weight of botanical material (in pounds) that is approximately half the volume of the vessel (in liters). For example, a 5-L vessel holds at least 2.5 lbs (1.13 kg) of botanical material; a 10-L vessel holds at least 5 lbs (2.25 kg) of botanical material.
- When botanical material is finely ground (fine sand), a 5-L vessel holds from 3.5 to 5 lbs. (1.5 to - 2.25 kg)
- If there is not enough botanical material present, use clear, inert glass or stainless steel beads in a filter bag to account for the difference. For example, 3 lbs is 75% of 4 lbs, so you should use a filter bag that has a height that is 25% of the vessel. For more information, see www.filterbag.com.
- Place the beads against the inlet of the extraction vessel so the extract oil does not adhere to them. Ensure that the glass beads are in front of the botanical material, so the direction of the flow contacts the inert glass or metal before the botanical material.
- The inlet of the vessel is normally located at the bottom.
- Weigh a large plastic bucket, add the bio-botanical material, and then re-weigh the bucket to determine the weight of the empty bucket and the weight of the bucket and ground bio-botanical material.
- Use the large-mouth funnel to add the bio-botanical material to the 5 or 10-L extraction vessel.
- A dust cloud may form around the extraction vessel while you add the finely ground material to it. Wear a face mask to avoid breathing in particles from the air surrounding you.
- Re-weigh the plastic bucket and any bio-botanical material that remains in it. The difference between this weight and the initial bucket weight is the amount of bio-botanical material that you added to the extraction vessel.
- Use the packing tool or dowel (a wooden baseball bat with the end cut off) to pack the material down into the extraction vessel. A well-packed vessel is important to extraction efficiency. If the botanical material plug is not well-packed, CO_2 creates channels in the

material, travels through the path of least resistance (the channel), and fails to interact with the material. Do not completely fill the extraction vessel before packing. Add a small amount (approximately 3 inches of material at a time), and then pack it down with the dowel or packing tool.

- When packing directly into the extraction vessel, fill the sample to the vessel seal line scored in the stainless steel, which allows enough room for the filter cap to fit.

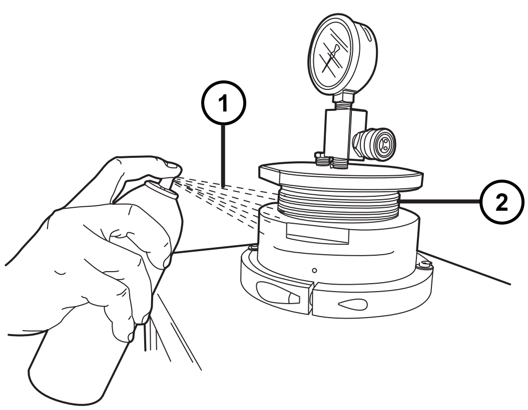
Figure C–2: Fill line on extraction vessel



1 Vessel's fill line

- Use the paint brush to clean off the material from the threads on the vessel.
- Spray the threads on the cap with Teflon lubricant prior to closing, being careful not to over-spray on the U-cup seals.

Figure C–3: Spraying extraction vessel cap threads with Teflon lubricant



1 Teflon lubricant

2 Threads on extraction vessel cap

- Ensure that the U-cup seal is placed on the cap prior to closing and that the spring faces the pressure gauge (visible to you).

C.3.1 Vessel basket

For 5 L extraction vessels, you can use a vessel basket to load botanical material into the vessel, which makes cleaning the vessel easier. When using a vessel basket, observe these guidelines:

- The vessel basket reduces the loading capacity by 8%, so it is best for indoor grow customers who process lower volumes.
- When using the vessel basket to load botanical material, you must remove the bottom filter frit of the main extraction vessel, so that you cannot alternate between using the basket and using the vessel.

  Note: The extraction vessel uses a standard 5-µm frit. An optional, 100-µm frit is also available.

C.4 CO_2 recycler

The CO_2 recycler receives the spent CO_2 from the last collection vessel, chills it, and sends it to the inlet of the Bio-botanical Extraction System. When using the recycler, observe these guidelines:

- The extraction system requires liquid CO_2. Use only dip (siphon) tube CO_2 supply cylinders.

  Note: Do not use CO_2 gas in the extraction system. Doing so will cause the pump to shut down.

  - You can easily identify dip tube cylinders by the red ring around the neck of the tank.
  - Prior to use, store the dip tube cylinders in the laboratory at room temperature for 24 hours.
- The CO_2 recycler tank has a floating ball inside of it to measure the liquid CO_2 level. Older display panels indicate the level as low-low, low, high, or high-high. Newer display panels indicate the level as low, medium, or high. The recommended level of the recycler is low, to allow the liquid CO_2 enough space to expand.
  - Before the first run on a newly installed system, you must fill the chiller and system with CO_2, which will most likely consume an entire CO_2 cylinder. Additional CO_2 cylinders are required only to top off the system, and will last much longer. Once the system is full, a cylinder typically lasts for five extractions.
- The pressure in the recycler must be 4895 to 5102 kPa (49 to 51 bar, 710 to 740 psi). Pressures below 4826 kPa (48 bar, 700 psi) cause CO_2 liquid to flash to a gas in the pump head. Pressures above 5102 kPa (51 bar, 740 psi) can cause carryover of extracted matter from cyclone separator 3 (CS3) to the recycler tank.
- The pressure is controlled by the amount and temperature of CO_2 in the recycler. The valve between the dip tube cylinder and the recycler controls the flow of CO_2 into the recycler, and the temperature of the recycler's chiller can be used to control the pressure of the recycler.

Adjust temperatures 0.5 degree every 15 minutes with the recycler flow rate. As the temperature decreases, the recycler pressure also decreases.

Recommendation: Use portable thermometers to monitor the room temperature.

- The recycler chiller temperature can range from 5 to 15 °C, which determines the recycler tank pressure. Assuming the room temperature is 66 to 74 °F, start at 11 °C, and adjust the chiller temperature as necessary.

C.4.1 CO_2 care and use

Observe these guidelines when preparing and storing CO_2:

- Initially run the system without recycling (two cylinders), if permitted by local law.
- Store tanks overnight in a temperature-controlled environment (18 to 24 °C), preferably in the extraction room itself, prior to the service engineer's arrival.
- Secure tanks to the bench or wall.
- If necessary, wrap a gas cylinder heater (purchased at a local gas supply dealer) around the tank to generate tank pressure.

Requirements:

- CO_2 tanks must have dip tubes (liquid CO_2).
- CO_2 tanks must be equilibrated at room temperature for approximately three hours.

Tips:

- Warm CO_2 tanks stored outside in cool weather using a heating blanket to raise the pressure to higher than 5861 kPa (59 bar, 850 psi).
- CO_2 tanks stored in sunlight will have higher pressure and may not be able to deliver any liquid CO_2 to the CO_2 recycler.

C.5 Operating the system

Tip: New users should run the system overnight for the first few runs, which results in maximum recovery and a low chance of clogging the system. After the first few runs, run the system for four to six hours per day and compare the recovery against the overnight run to determine the optimum run time. A Waters Technical Service representative must be present for at least two full runs.

Important: Ensure that the manual outlet valve for extraction vessel 1 (MV1–V1), manual inlet valve for extraction vessel 1 (MV2–V1), and P-200 high-pressure pump outlet valve are open before powering-on the pump.

To operate the system:

1. Power-on the six-zone temperature controller modules [CN6(1) and CN6(2)], CO_2 pump, co-solvent pump, automated back-pressure regulator (ABPR), automation unit, and extracting solvent heat exchanger (HE2).
2. Ensure that the chillers are at these set points:
   - CO_2 chiller = 3.0 °C
   - Recycler chiller = 11.0 °C, or whatever temperature is required to achieve 4999 kPa (50 bar, 725 psi)
3. After the system components are powered on for at least 30 seconds, launch the ChromScope software.
4. Click **Tools** > **Clear alarms**.
5. Power-on the extraction vessel heater, if necessary.
6. Specify the appropriate flow rate, and then start the CO_2 pump.

   Note: Initially, the flow rate typically exceeds the limit for triggering the "free flow error", which causes the CO_2 pump to power-off. To resolve this, power-on the pump.
7. Set the extraction vessel temperature to 50 °C.
8. Set the main heat exchanger temperature to 60 °C (10 °C higher than the extraction vessel).
9. Set the ABPR setting to 22,477 kPa (225 bar, 3260 psi), at a temperature of 45 °C.

C.5.1 Recommended extraction parameters

The following tables list the recommended extraction parameters for plant material.

50 g/min: Overnight run of 14 hours (ChromScope v1.6)

Total duration: 840 min

Parameter	Step 1: Overnight run
Co-solvent flow (ml/min)	0
CO_2 flow (g/min)	50
Extraction vessel	Extraction vessel 1
Extraction vessel 1 temperature (°C)	50
Extraction vessel 2 temperature (°C)	0
Cyclone vessel 1 temperature (°C)	45
Cyclone vessel 2 temperature (°C)	35

Parameter	Step 1: Overnight run
Cyclone vessel 3 temperature (°C)	22
In-line heater temperature (°C)	60
In-line collection heater temperature (°C), if installed	30
Extraction pressure (bar)	225
Dynamic duration 1 (min)	0.00
Static duration (min)	0.00
Dynamic duration 2 (min)	840.00
Cycles to execute	1

100 g/min: Method for botanical material (ChromScope v1.6)

Total duration: 420 min

Parameter	Step 1: Collect terpenes	Step 2: Collect product
Co-solvent flow (ml/min)	0	0
CO_2 flow (g/min)	75	100
Extraction vessel	Extraction vessel 1	Extraction vessel 1
Extraction vessel 1 temperature (°C)	50	50
Extraction vessel 2 temperature (°C)	0	0
Cyclone vessel 1 temperature (°C)	45	45
Cyclone vessel 2 temperature (°C)	35	35
Cyclone vessel 3 temperature (°C)	22	22
In-line heater temperature (°C)	60	60
In-line collection heater temperature (°C), if installed	30	30
Extraction pressure (bar)	150	225
Dynamic duration 1 (min)	0.00	0.00
Static duration (min)	0.00	0.00
Dynamic duration 2 (min)	60.00	360.00
Cycles to execute	1	1

150 g/min: Method for botanical material (ChromScope v1.6)

Total duration: 360 min

Parameter	Step 1: Collect terpenes	Step 2: Collect product
Co-solvent flow (ml/min)	0	0
CO_2 flow (g/min)	75	150
Extraction vessel	Extraction vessel 1	Extraction vessel 1
Extraction vessel 1 temperature (°C)	50	50
Extraction vessel 2 temperature (°C)	0	0
Cyclone vessel 1 temperature (°C)	45	45
Cyclone vessel 2 temperature (°C)	35	35
Cyclone vessel 3 temperature (°C)	22	22
In-line heater temperature (°C)	60	60
In-line collection heater temperature (°C), if installed	30	30
Extraction pressure (bar)	150	225
Dynamic duration 1 (min)	0.00	0.00
Static duration (min)	0.00	0.00
Dynamic duration 2 (min)	60.00	300.00
Cycles to execute	1	1

100 g/min: Method for trim (ChromScope v1.5)

Total duration: 300 min

Parameter	Step 1: Collect terpenes	Step 2: Collect product
Co-solvent flow (ml/min)	0	0
CO_2 flow (g/min)	75	100
Extraction vessel	Extraction vessel 1	Extraction vessel 1
Extraction vessel 1 temperature (°C)	50	50
Extraction vessel 2 temperature (°C)	0	0
Cyclone vessel 1 temperature (°C)	45	45
Cyclone vessel 2 temperature (°C)	35	35
Cyclone vessel 3 temperature (°C)	22	22
In-line heater temperature (°C)	60	60
Extraction pressure (bar)	150	225
Dynamic duration 1 (min)	0.00	0.00

Parameter	Step 1: Collect terpenes	Step 2: Collect product
Static duration (min)	0.00	0.00
Dynamic duration 2 (min)	60.00	240.00
Cycles to execute	1	1

150 g/min: Method for trim (ChromScope v1.5)

Total duration: 240 min

Parameter	Step 1: Collect terpenes	Step 2: Collect product
Co-solvent flow (ml/min)	0	0
CO_2 flow (g/min)	75	150
Extraction vessel	Extraction vessel 1	Extraction vessel 1
Extraction vessel 1 temperature (°C)	50	50
Extraction vessel 2 temperature (°C)	0	0
Cyclone vessel 1 temperature (°C)	45	45
Cyclone vessel 2 temperature (°C)	35	35
Cyclone vessel 3 temperature (°C)	22	22
In-line heater temperature (°C)	60	60
Extraction pressure (bar)	150	225
Dynamic duration 1 (min)	0.00	0.00
Static duration (min)	0.00	0.00
Dynamic duration 2 (min)	60.00	180.00
Cycles to execute	1	1

C.5.2 Cyclone separator temperatures and settings

The system runs dynamically for the allotted time at the temperatures listed in the following table. Dynamic extractions are recommended to prevent material from accumulating in the tubing and potentially falling out of solution.

Table C–1: Six-zone temperature controller module temperatures

Zone	Temperature
1	Extraction vessel 1 heater: 50 °C, when in use
2	Extraction vessel 1 internal temperature (read only): 47 to 50 °C

Table C–1: Six-zone temperature controller module temperatures (continued)

Zone	Temperature
3	Extraction vessel 2 heater: 50 °C, when in use
4	Extraction vessel 2 internal temperature (read only): 47 to 50 °C
5	Main heat exchanger: 60 °C, 10 °C higher than extraction vessels 1 and 2 (V1/V2)

The following table lists the recommended settings for the cyclone separators.

Table C–2: Recommended cyclone separator settings

Cyclone separator	Material	Pressure during extraction	Temperature during extraction
CS1	Waxes, lipids, oil acids	13,790 kPa (138 bar, 2000 psi)	45 °C
CS2	Oil neutrals	• 6895 kPa (69 bar, 1000 psi), without the in-line collection heater. This setting reduces the pressure drop, which helps prevent carryover. • 8274 kPa (83 bar, 1200 psi), with the in-line collection heater installed	35 °C
CS3	Terpenes, water, 95% food-grade ethanol (if used), and small amounts of oils	4999 to 5171 kPa (50 to 52 bar, 725 to 750 psi)	25 °C (To help prevent carryover, increase to 35 °C)

Important: The CO_2 recycler must be at 4826 to 4999 kPa (48 to 50 bar, 700 to 725 psi) to ensure that the cyclone separator 3 (CS3) pressure during extraction is no more than 172 kPa (1.5 bar, 25 psi) higher.

Notes:

- To prevent a buildup of waxes, which can be caused by carryover from the other cyclone separators, increase the temperature during extraction. Always drain terpenes before increasing the temperature.
- Do not set CS3 above the recommended temperature and pressure, which can increase the CO_2 solvating power and carry oils, waxes, terpenes, and water into the recycler.
- The ratio of oils in CS1:CS2:CS3 is 1:4:1, so most oils are in CS2.
- Cyclone separators are increased in temperature 10 minutes before the pull to thin the extract.

C.5.3 Filling the CO_2 recycler

Observe these guidelines when filling the CO_2 recycler:

- Ensure that the dip tube cylinder is open fully, and that the recycler inlet valve is closed.
- Ensure that the drain valve on the bottom of the recycler is closed.
- Ensure that the system (isolation valve) supply is open.
- If you are using manual filling, slightly open the valve to fill the recycler. The filling process takes approximately 20 minutes to complete. Opening the valve too quickly causes dry ice to form at the bottom of the recycler. You must allow any dry ice build-up to melt overnight, or use a heat gun to melt it.
- You can monitor the fill rate by monitoring the recycler chiller temperature. If the chiller temperature decreases by more than 2 °C, the recycler is filling too quickly. When the low light illuminates, keep the valve open for 1 minute, and then close the valve. Repeat this process when you power-on the pump.
  - If the green low light does not illuminate after 2 minutes, replace the CO_2 supply.
- If you are using automated refilling (solenoid control), ensure that the manual valve is only slightly open for the initial fill. When the initial fill is complete, open the valve completely.
  - With automated refilling, the green low light should illuminate no longer than 2 minutes after it turns off. If the light takes longer than 2 minutes to illuminate, replace the CO_2 supply.
- When running the extraction system, ensure that the recirculation valve on top of the recycler is closed (horizontal).
- When the system is not in use, leave the chiller powered-on and the recirculation valve open (vertical), to chill (equilibrate) the CO_2 in the recycler.

Important: The pressure in the recycler must be between 4895 to 5102 kPa (49 to 51 bar, 710 to 740 psi). Before you connect the recycler, set the cyclone separator 3 (CS3) pressure to 2068 kPa (21 bar, 300 psi). After you connect the recycler, it maintains the CS3 pressure to 172 kPa (2 bar, 25 psi) higher than its (recycler) pressure through the inline check valve.

C.6 Preparing for sample collection

To prepare for sample collection:

1. After the extraction completes, reduce the pump flow to 25 to 40 g/min.
2. Drain the contents from cyclone separator 3 (CS3), most of which will be terpenes and water.

3. Increase the temperature of all three collection vessels to 55 °C.
4. Begin reducing the ABPR pressure from 22,477 kPa (225 bar, 3260 psi) to 13,996 kPa (140 bar, 2030 psi).
   - For a 5-L extraction vessel, reduce the pressure in 1999 kPa/min (20 bar/min, 290 psi/min) increments.
   - For a 10-L extraction vessel, reduce the pressure in 1000 kPa/min (10 bar/min, 145 psi/min) increments.

   Note: You can specify the ABPR depressurization ramp in the method using ChromScope.

   Important: Incremental depressurization is critical in preventing carryover from one collection vessel to another, and ultimately into the CO_2 recycler.

Example of incremental depressurization used to prevent carryover:

Total duration: 184 min

Parameter	Step 1	Step 2	Step 3	Step 4	Step 5
Co-solvent flow (ml/min)	0	0	0	0	0
CO_2 flow (g/min)	150	150	150	150	150
Extraction vessel	Extraction vessel 1	Extraction vessel 1	Extraction vessel 1	Extraction vessel 1	Extraction vessel 1
Extraction vessel 1 temperature (°C)	50	50	50	50	50
Extraction vessel 2 temperature (°C)	35	35	35	35	35
Cyclone separator 1 temperature (°C)	45	45	45	45	45
Cyclone separator 2 temperature (°C)	35	35	35	35	35
Cyclone separator 3 temperature (°C)	25	25	25	25	25
Inline heater temperature (°C)	60	60	60	60	60
Inline collection heater temperature (°C), if installed	35	35	35	35	35
Extraction pressure (bar)	225	205	185	165	145

Parameter	Step 1	Step 2	Step 3	Step 4	Step 5
Dynamic duration 1 (min)	0.00	0.00	0.00	0.00	0.00
Static duration (min)	0.00	0.00	0.00	0.00	0.00
Dynamic duration 2 (min)	180.00	1.00	1.00	1.00	1.00
Cycles to execute	1	1	1	1	1

C.7 Collecting the sample

To collect the sample:

1. Ensure that the collection rack is set to an appropriate height so that the collection tube is in the top quarter of the storage container.

   ! **Notice:** To avoid damage to the splash guard, including freezing and breakage, ensure that it is installed properly. Splash guards are designed to fit over a 1-L beaker.

2. Ensure that the splash guard is installed properly, so that it covers the opening on the storage container.
3. Slowly open the drain valve on cyclone separator 3 (CS3) until the plant material flows out.
4. When the material is exhausted and CO_2 is visible, close the drain valve.
5. Warm the cyclone separator 2 (CS2) and cyclone separator 1 (CS1) outlet lines, by moving the heat gun slowly up and down the entire tube.
6. When the outlet lines are warm, slowly open the drain valves on CS2 and CS1 until the plant material flows out.
7. When the material is exhausted and CO_2 is visible, close the drain valves.
8. If the flow stops and CO_2 is not visible, close the drain valves and use the heat gun to melt any clogs in the line.
9. Re-open the drain valves, continue the collection, and then close the valve when CO_2 is visible.

C.8 Transferring from extraction vessel 1 to extraction vessel 2

Transfers between extraction vessels conserves CO_2, pressurizes the new vessel faster, and depressurizes the old vessel faster.

Note: This procedure applies to manual mode only.

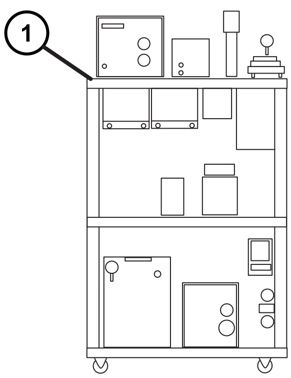
To transfer from extraction vessel 1 to extraction vessel 2:

1. Set the flow to `0` and ensure that the CO_2 and co-solvent pumps are powered off.

   Note: If the CO_2 and co-solvent pumps are on, the rupture discs will trigger and must then be replaced.

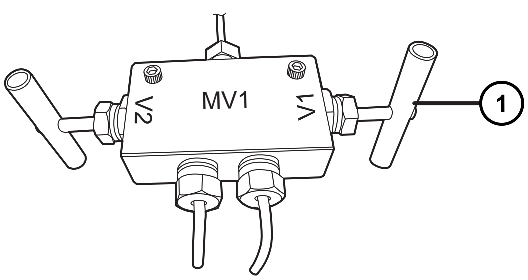
2. Ensure that the manual outlet valve for extraction vessel 1 (MV1–V1) is open.

   Figure C–4: Location of MV1 valve on module cart



   1 Location of MV1 valve

   Figure C–5: MV1–V1 outlet valve handle

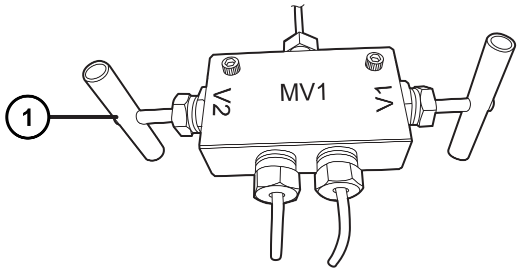


   1 MV1–V1 outlet valve handle

   Notice: To avoid damaging the filter frit, do not open the manual outlet valve for the extraction vessel too quickly.

3. Slowly open the manual outlet valve for extraction vessel 2 (MV1–V2) approximately one full turn over 1 minute.

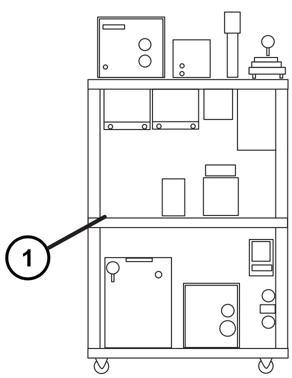
Figure C–6: MV1–V2 outlet valve handle



1 MV1–V2 outlet valve handle

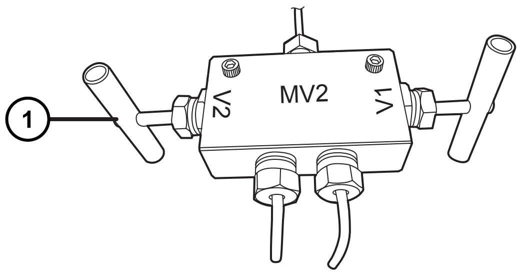
4. When the line that runs from MV1–V2 starts to feel cold, stop opening the manual outlet valve.
5. Maintain the position of the manual outlet valve for approximately 10 minutes or until the readings on both pressure gauges on the extraction vessels are equal.
6. Close MV1–V1 completely.
7. Close MV1–V2 completely.
8. Close the manual inlet valve for extraction vessel 2 (MV2–V2) completely.

Figure C–7: Location of MV2 valve on module cart



1 Location of MV2 valve

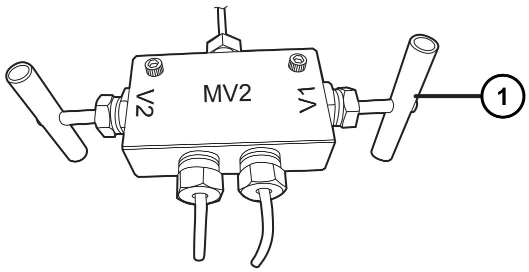
Figure C–8: MV2–V2 inlet valve handle



1 MV2–V2 inlet valve handle

9. Close manual inlet valve for extraction vessel 1 (MV2–V1) completely.

Figure C–9: MV2–V1 inlet valve handle



1 MV2–V1 inlet valve handle

C.9 Transferring from extraction vessel 2 to extraction vessel 1

Transfers between extraction vessels conserves CO_2, pressurizes the new vessel faster, and depressurizes the old vessel faster.

Note: This procedure applies to manual mode only.

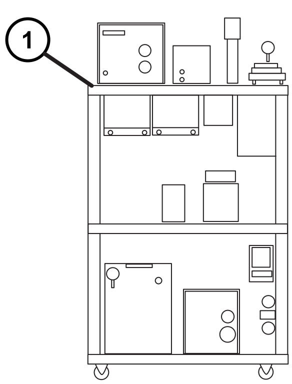
To transfer from extraction vessel 2 to extraction vessel 1:

1. Set the flow to `0` and ensure that the CO_2 and co-solvent pumps are off.

   Note: If the pumps are on, the rupture discs will trigger and must then be replaced.

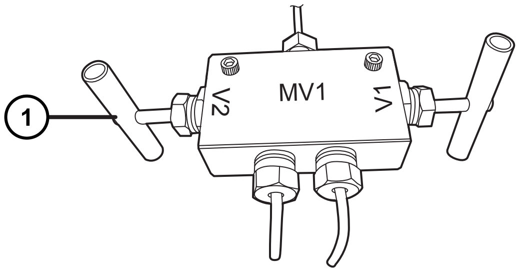
2. Ensure that the manual outlet valve for extraction vessel 2 (MV1–V2) is open.

   Figure C–10: Location of MV1 valve on module cart



1 Location of MV1 valve

Figure C–11: MV1–V2 outlet valve handle

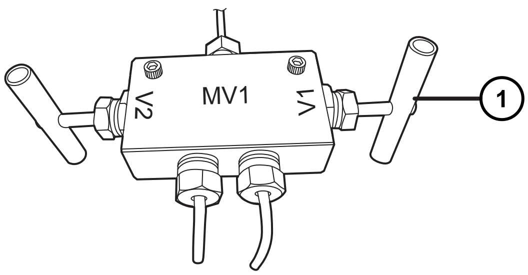


1 MV1–V2 outlet valve handle

Notice: To avoid damaging the filter frit, do not open the manual outlet valve for the extraction vessel too quickly.

3. Slowly open the manual outlet valve for extraction vessel 1 (MV1–V1) approximately one full turn over 1 minute.

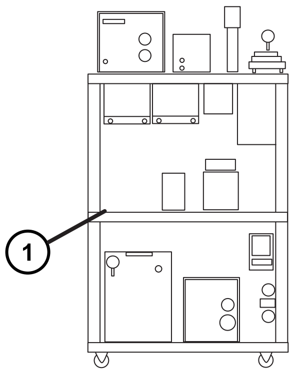
Figure C–12: MV1–V1 outlet valve handle



1 MV1–V1 outlet valve handle

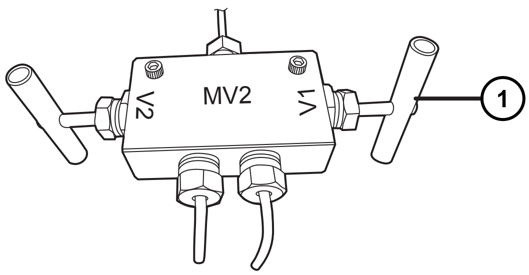
4. When the line that runs from MV1–V1 starts to feel cold, stop opening the manual outlet valve.
5. Maintain the position of the manual outlet valve for approximately 10 minutes or until the readings on both pressure gauges on the extraction vessels are equal.
6. Close MV1–V2 completely.
7. Close MV1–V1 completely.
8. Close the manual inlet valve for extraction vessel 1 (MV2–V1) completely.

Figure C–13: Location of MV2 valve on module cart



1 Location of MV2 valve

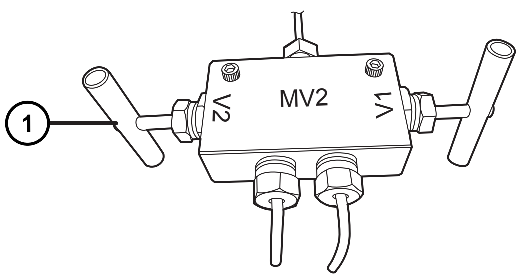
Figure C–14: MV2–V1 inlet valve handle



1 MV2–V1 inlet valve handle

9. Close the manual inlet valve for extraction vessel 2 (MV2–V2) completely.

Figure C–15: MV2–V2 inlet valve handle



1 MV2–V2 inlet valve handle

C.10 Maintaining the system

To maintain the system, observe these guidelines:

- Clean the system on a regular basis. For specific cleaning procedures and intervals, see Cleaning guidelines for all configurations.
- Replace the seals when a persistent leak (greater than five minutes) is observed around the P-200 high-pressure pump head.
- Replace the check valves when the P-200 high-pressure pump efficiency drops significantly (increased RPM/flow rate).
- Realign the needle in the automated back-pressure regulator (ABPR) valve if the ABPR fails to reach target pressure and there are no system leaks (see the Rebuilding the ABPR valve instructional video). A fully packed 5-L vessel at 150 g/min takes approximately ten minutes to reach the target pressure of 29,992 kPa (300 bar, 4350 psi).

  Note: After a second needle realignment, replace the needle and seat. The recommended routine replacement of the needle and seat is 6 months to 1 year.