Chromaflow™ 400-1000 columns

Operating Instructions

Original instructions

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Table of Contents

1 Introduction

About this chapter

This chapter contains important user information, safety notices, regulatory information, the intended use of Chromaflow 400-1000 columns, information about additional equipment and documentation.

In this chapter

This chapter contains the following sections:

1.1 About this manual

Purpose of the Operating Instructions

The *Operating Instructions* provide you with the instructions needed to install, operate and maintain Chromaflow 400-1000 columns in a safe way.

Scope of this document

This document describes the basic components and general principles of operation of Chromaflow 400-1000 columns and associated equipment. The instructions provided assume that the column is equipped with automatic nozzles and that it is operated with a Chromaflow packing station. Other configurations are possible.

For detailed information about individual products refer to the relevant information in the documentation package provided with each product. Contact your local GE representative for more information, if needed.

Images in this document

The Chromaflow column family encompasses a range of different column sizes and materials. The images depicted in this manual are included for general illustrative purposes and may not be representative of all column sizes and types. For detailed information about individual columns always refer to the relevant information in the documentation package provided with your column.

Typographical conventions

Hardware items are identified in the text by bold text (for example, **Power**).

1.2 Important user information

Read this before operating the product

All users must read the entire *Operating Instructions* before installing, operating or maintaining the product.

Always keep the *Operating Instructions* at hand when operating the product.

Do not operate the product in any other way than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

Intended use of Chromaflow columns

Chromaflow 400-1000 columns are designed for use in laboratory and industrial chromatography. Chromaflow 400-1000 columns are intended for purification only and should not be used for diagnostic purposes or in any clinical or *in vitro* procedures.

WARNING

Do not operate the product in any other way than described in the Chromaflow user documentation.

Prerequisites

In order to operate Chromaflow 400-1000 columns safely and according to the intended purpose the following prerequisites must be met:

- All users must read and understand the *Safety Instructions* chapter in these *Operating Instructions*.
- All equipment shall be installed according to the instructions in *Chapter 4 Installation, on page 50*.

- All users shall be acquainted with the use of process equipment and the handling of biological materials.
- All operations shall be performed by qualified personnel who are adequately trained.

Safety notices

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.

WARNING: EXPLOSION HAZARD

WARNING: EXPLOSION HAZARD highlights instructions that must be followed to avoid death or serious injury when working in a potentially explosive environment. It is important not to proceed until all stated conditions are met and clearly understood.

WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Notes and tips

Note: *A note is used to indicate information that is important for trouble-free and optimal use of the product.*

Tip: *A tip contains useful information that can improve or optimize your procedures.*

1.3 Regulatory information

Introduction

This section lists the directives and standards that are fulfilled by Chromaflow 400-1000 columns.

Manufacturing information

The table below summarizes the required manufacturing information. For further information, see the EU Declaration of Conformity (DoC) document.

Requirement	Content
Name and address of manufacturer	GE Healthcare Bio-Sciences AB, Björkgatan 30, SE 751 84 Uppsala, Sweden

International standards

Harmonized standard requirements fulfilled by this product are summarized in the table below.

Standard	Description	Notes
EN ISO 12100	Safety of machinery. General principles for design. Risk assessment and risk reduction.	EN ISO standard is harmonized with EU directive 2006/42/EC
ASME BPVC VIII, div 1	Boiler and Pressure Vessel Code (BPVC)	Applies only to stainless steel columns
ASME BPE	Bioprocessing equipment	
EMEA/410/01, CPMP Note	All wetted polymer and elastomeric parts are animal origin-free or comply with the conditions in the standard, and are also classified according to USP Class VI, 21 CFR Part 177	
EN 1127-1:2011	Explosive atmospheres - Explosion prevention and protection - Part 1: Basic concepts and methodology	Harmonized with 94/9/EC
EN 13463-1:2009	Non-electrical equipment for use in potentially explosive atmospheres - Part 1: Basic method and requirements	Harmonized with 94/9/EC

Conformity with EU Directives

This product complies with the European directives listed in the table, by fulfilling the corresponding harmonized standards.

Directive	Title
2006/42/EC	Machinery Directive (MD)
1994/9/EC	ATEX Directive

CE marking

The CE marking and the corresponding EU Declaration of Conformity is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE, except for alterations described in the user documentation.

Regulatory compliance of connected equipment

Any equipment connected to Chromaflow 400-1000 columns should meet the safety requirements of EN/IEC 61010-1, or relevant harmonized standards. Within EU, connected equipment must be CE marked.

1.4 Additional documentation

Introduction

This section describes the additional documentation associated with Chromaflow columns.

Column-specific documentation

In addition to these *Operating Instructions*, the documentation package supplied with Chromaflow 400-1000 columns also includes documentation binders containing detailed specifications and traceability documents.

The table below presents the most important documents in the document package with regard to technical aspects of Chromaflow 400-1000 columns.

Document	Abbreviation	Purpose/Contents
*General Specification*or *Equipment Data Sheet*	GS or EDS	Technical data for the column.
Piping and Instrumentation Diagram	PID	Schematic overview of the entire process flow, components and instruments and the control system.
Assembly Drawing	AD	Physical layout. Provides dimensional data.
Equipment List	EQL	List of individual components

Third-party component documentation

Documentation for components produced by a third-party is included where applicable.

2 Safety instructions

About this chapter

This chapter contains safety precautions, descriptions of safety labels, emergency procedures and decommissioning information for Chromaflow columns.

Important

WARNING

Before installing, operating or maintaining the product, all users must read and understand the entire contents of this chapter to become aware of the hazards involved.

In this chapter

This chapter contains the following sections:

2.1 Safety precautions

Introduction

The safety precautions in this section are grouped into the following categories:

- General precautions
- Flammable liquids and explosive environments
- Personal protection
- Installing and moving the equipment
- Operation
- Maintenance

Note: *Some of the safety precautions in this chapter may concern components or situations described in other Chromaflow product documents.*

General precautions

WARNING

Only properly trained personnel may perform operation and user maintenance of the product.

WARNING

Do not operate the product in any other way than described in the Chromaflow user documentation.

WARNING

Observe extreme caution when in the vicinity of the column. The force generated by the movement of the column could crush hands and limbs.

WARNING

Max. column pressure. The working pressure of the column should never exceed 3 bar, otherwise there is a risk of personal injury and damage to the column. Always use appropriate pressure alarms, pressure vents or rupture discs, and safety equipment.

WARNING

High pressure. The flow rate may under no circumstances exceed the specified column maximum flow rate. High flows might affect the packed medium, causing the pressure to exceed the specified column maximum pressure.

WARNING

The working temperature range of the column should never fall outside the design temperature range.

WARNING

Power failure of connected systems. During a power failure of a connected system, or if the **EMERGENCY STOP** button is pressed on connected equipment, the equipment may remain pressurized. Opening a line or vessel at this point could result in the release of potentially hazardous process or cleaning fluid, and cause bodily harm.

When recovering from a power failure or emergency shutdown of a connected system, make sure all lines and vessels are depressurized before opening.

CAUTION

Make sure to protect adapter sealing surfaces. Sealing surfaces must always be clean and free from scratch, blemishes, wear or any other type of damage.

CAUTION

Do not expose hard piping to mechanical stress. Placing nozzle connections under strain may result in damage to column ports. Use flexible couplings and/or supports to avoid strain stress.

CAUTION

Make sure that any water used with the column is particulate-free (down to 1 µm). Particles can block and damage the bed supports.

NOTICE

Only use chemicals listed in the Chemical Resistance information.

The wetted parts of the product may be damaged by chemicals not listed in the Chemical Resistance information. Contact your GE representative before using chemicals that are not listed.

Flammable liquids and explosive environments

WARNING

Flammable liquids. This product is **not approved** to handle flammable liquids.

Personal protection

WARNING

Hazardous substances and biological agents. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of Chromaflow 400-1000 columns.

WARNING

For personal safety during the transportation, installation, operation, maintenance and service of columns, use protective glasses and other personal protective equipment appropriate for the current application at all times. The following personal protective equipment should always be available:

- Protective glasses
- Working gloves to protect against sharp edges
- Protective footwear, preferably with steel toe-cap
- Disposable gloves

Always use clean disposable gloves when manually handling parts.

Installing and moving the equipment

WARNING

Heavy object. Use suitable lifting equipment when moving the unit. All lifting and moving must be performed in accordance with local regulations.

WARNING

Packing crates and columns shall only be moved by personnel with appropriate training, and in adherence with local regulations.

Even if the instructions in the Chromaflow *Operating Instructions* are followed, it is the responsibility of the customer to guarantee the safety of personnel while working with the column.

WARNING

Move transport crates. Make sure that the forklift has capacity to safely lift the crate weight. Make sure that the crate is properly balanced so that it will not accidentally tip when moved.

WARNING

Move column. Make sure that the center of gravity of the column is well balanced over the lifting arms of the forklift. Otherwise the column may tip off the forklift.

WARNING

Move column. When using a pallet truck or fork lift, do not lift the column higher than required for clearance. There is an increased risk that the column might accidentally tip when moved if the column is lifted more than 10 cm from the floor.

WARNING

The lifting eyebolts of Chromaflow 400-1000 columns are intended for lifting specific column components only. The lifting eyebolts are not intended to be used for lifting the entire column. Non-ferritic lifting slings with sufficient lifting capacity to safely lift the weight of the column may be wrapped around the column flange to lift the entire column with a crane or hoist. Use sling protectors to protect lifting slings from sharp edges.

WARNING

Heavy object. Because of the significant weight of Chromaflow 400-1000 columns, great care must be taken not to cause squeezing or crushing injuries during movement.

WARNING

Pay extra attention when moving the adapter as there is a risk of crushing your hands.

WARNING

Safety valves must be fitted in accordance with local pressure vessel regulations.

CAUTION

Do not expose hard piping to mechanical stress. Placing nozzle connections under strain may result in damage to column ports. Use flexible couplings and/or supports to avoid strain stress.

CAUTION

To prevent bacterial growth, the product may be filled with denatured alcohol (18% C_2H_5OH (ethanol), 2% C_3H_7OH (isopropanol) and 80% H_2O (water)) at delivery.

The denatured alcohol mixture can be hazardous to humans if consumed.

Flush out the denatured alcohol before assembling, testing or integrating the product into the intended process context.

CAUTION

Do not turn on the air supply before all connections have been made and checked.

NOTICE

When moving a column with an acrylic tube from a warm environment to a cold room it is advisable to control bolt tensions. Adjust to recommended bolt torque settings if necessary.

Operation

WARNING

There should **NEVER** be air or gas under pressure in the column.

WARNING

If the column is equipped with caster wheels, the wheel brakes must be activated at all times during operation or storage.

WARNING

Do not adjust the feet more than 160 mm out from the base of the column stand to the floor. There is a risk of the column tipping over if the feet are screwed out too far.

WARNING

Never use compressed air to raise the adapter.

WARNING

When reactivation is performed, pumps that were in use when the **EMERGENCY STOP** on the connected equipment button was pressed may start at the same setting.

If a pump setting was previously on maximum, reduce the pump setting prior to reactivation to avoid an immediate high pressure increase.

WARNING

Pressure may remain in the column and tubing for an extended period after shutdown. Make sure all lines and chambers are de-pressurized before opening or disconnecting.

WARNING

Secure connections so that tubing cannot suddenly whip around during use and cause personal injury.

WARNING

Some of the chemicals used with Chromaflow 400-1000 columns may be flammable under certain conditions. Make sure to use chemicals only under conditions where they are not flammable. Refer to local and/or national classifications of flammable liquids.

WARNING

Do not reset after an emergency shutdown if the column or piping is suspected to be damaged or otherwise defective. Damaged or defective columns or piping might leak or rupture.

WARNING

Do not exceed maximum packing pressure for the column.

CAUTION

Make sure there is adequate over-pressure relief for the Chromaflow column.

CAUTION

Do not use chemicals at temperatures above the specified limits.

CAUTION

Make sure that all tubing, hoses and cables are placed so that the risk of tripping accidents is minimized.

CAUTION

Do not strain nozzle connections. Connect tubing carefully. Failure to do so may damage nozzle components and cause leakage.

CAUTION

Make sure that safety valve connecting tubing has an unrestricted flow path.

CAUTION

Make sure to protect adapter sealing surfaces. Sealing surfaces should always be clean and free from scratches, blemishes, wear or any other type of damage.

Spray the column tube wall above the top piston seal with a 20% ethanol solution. Failure to do so may cause damage to the column wall and seals.

CAUTION

Before using the column, make sure that the adapter and adapter O-rings are clean and absolutely free of all dust and particulate matter. Such matter can scratch and damage the tube.

CAUTION

Do not use chemicals harmful to the column.

Maintenance

WARNING

When unpacking the chromatography media from the column, check the integrity of all connections and make sure that the collection vessels can accommodate the volumes involved.

WARNING

LOCK OUT / TAG OUT (LOTO)! Before any maintenance or decommissioning work is performed on the system, make sure that:

- the system is empty and depressurized.
- the system is disconnected from process feed, electrical power and pneumatic supply.
- the system is prevented from accidentally becoming re-energized during maintenance.
- the system is clearly tagged as taken out of operation.
- all process wetted areas are clean and decontaminated.

WARNING

Some of the procedures outlined in this document involve the lifting of heavy parts. Two people as well as a suitable hoist should always be at hand, when performing column service and maintenance.

WARNING

Do not perform any type of maintenance work on the column while the adapter is in motion. Do not reach inside the column while moving the adapter.

WARNING

During removal of the adapter, or any other movement performed through applying hydrostatic pressure, a pressure gauge must be connected to the system so that the internal column pressure can be monitored.

CAUTION

Do not leave O-rings or seals in hot water for longer than the time period recommended in GE product documentation.

CAUTION

Twisted or deformed O-rings will cause leakage.

CAUTION

Handle stainless steel parts with care and use only stainless steel tools. Using inappropriate tools will cause damage to surfaces and may lead to leakage and corrosion.

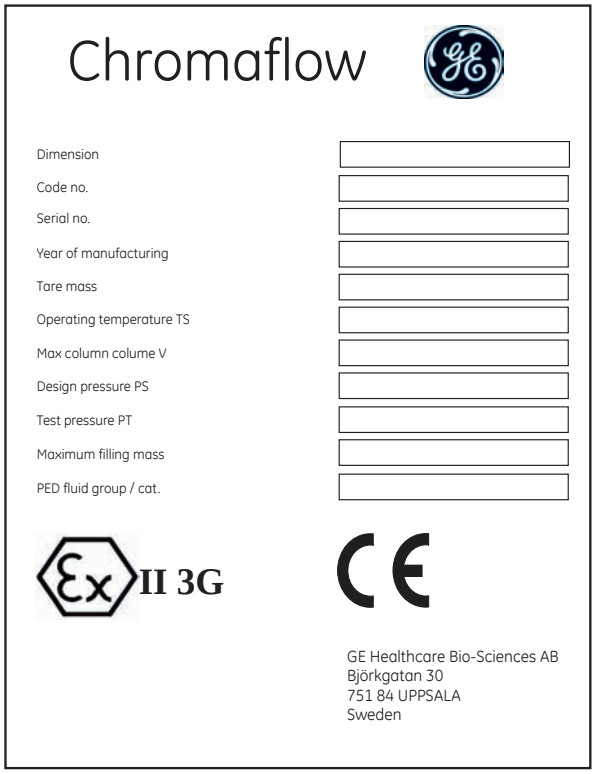
2.2 Labels

Introduction

This section describes the various labels found on Chromaflow 400-1000 columns and associated equipment and explains their meaning.

Column labels

The illustration below shows an example of the identification label found on Chromaflow 400-1000 columns. The label on a column shows the specific manufacturing and performance data for the individual column and therefore exact information details may vary from the example shown.



The Chromaflow label information is explained in the following table.

Label text	Description
CE	The equipment complies with applicable European directives. Refer to *Section 1.3 Regulatory information, on page 10*.
Ex II 3G	This symbol indicates that the product is capable of functioning in conformity with the operating parameters established by the manufacturer and ensuring a normal level of protection. Equipment with this category rating may be used in areas in which explosive atmospheres caused by gases, vapors, or mists are not likely to occur or, if they do occur, are likely to do so only infrequently and for a short period only.
Code No.	Number indicating that the instrument is a Chromaflow product.
Serial No.	Number of the specific instrument.
Model No.	Number indicating the specific type of instrument.
Year of Manufacture	Year the equipment was manufactured.
Operating Temperature, TS	Permitted operating temperature range.
Design Pressure, PS	Maximum pressure the instrument has been designed to operate at
Test Pressure, PT	Pressure the instrument has been tested to tolerate.
Tare mass	Weight of the instrument when empty.
Maximum filling mass	Maximum amount of packing media.
Maximum column volume, V	Maximum amount of chromatography media.

Safety labels

The table below describes various safety labels that may be found on Chromaflow columns and associated equipment.

Note: *The labels shown in the following table are examples only.*

Symbol/text	Description
	**Warning!** Read the user documentation before using the equipment. Do not open any covers or replace parts unless specifically stated in the user documentation.
	**WARNING!** Max operating pressure as stated on the label.
	**WARNING!** When using corrosive solutions containing e.g. chlorides, the column must be rinsed thoroughly with more than 5 column volumes of pure water. Failure to do this could result in corrosion of the stainless steel components.
	**EMERGENCY STOP** label, yellow with black text. (The emergency stop button is red). See *Section 2.3 Emergency procedures, on page 29* for further information regarding the emergency stop.
	**IMPORTANT!** Before service/maintenance or return to GE, clean the equipment and accompany it with a de-contamination statement, specifying substances with which it has been in contact during use and the method of cleaning.
	**CAUTION!** Do not use any solvent solution when clean-ing transparent plastic items.
	**CAUTION!** Label stating pressure control valve settings for instrument air supply.

2.3 Emergency procedures

Introduction

This section explains how to perform an emergency shutdown of Chromaflow 400-1000 columns and associated equipment. How to restart the equipment following an emergency shutdown or power failure is also described.

Precautions

WARNING

Pressure may remain in the column and tubing for an extended period after shutdown. Make sure all lines and chambers are depressurized before opening or disconnecting.

WARNING

Do not reset after an emergency shutdown if the column or piping is suspected to be damaged or otherwise defective. Damaged or defective columns or piping might leak or rupture.

EMERGENCY STOP buttons

As the operation of Chromaflow columns are controlled by the associated equipment, the column itself does not have an **EMERGENCY STOP** button. To stop any operation you must use the equivalent button on the connected equipment used.

If the column is connected to a packing station, pressing the **EMERGENCY STOP** button on the packing station will stop the packing station pumps and cut the supply of compressed air to the column. This will in turn stop adapter movement and will stop the packing or unpacking operation.

If the column is connected to a process system to perform a run, pressing the **EMERGENCY STOP** button on the process system will stop the run operation.

In an emergency situation

Press the **EMERGENCY STOP** button on the equipment being used with the column (packing station or process system). Call medical help or apply first aid if the emergency situation has caused injury.

For the rest of the procedures in an emergency situation, refer to the user documentation of the equipment being used with the column (packing station or process system).

Restart

Follow the instruction below to restart after an emergency shut down.

Step	Action
1	Make sure that the condition that was the root cause of the emergency stop has been corrected.
2	 Turn the **EMERGENCY STOP** button on the equipment that was used to perform the emergency stop clockwise. This will reset the **EMERGENCY STOP** button.

Step	Action
3	Restart the process by pressing the appropriate reset button. • If a Chromaflow column is used with a packing station, then press **START/RESET** to restart the process. • If a Chromaflow column is used with a process system, then refer to the user documentation for the system to restart the process.

WARNING

When reactivation is performed, pumps that were in use when the **EMERGENCY STOP** on the connected equipment button was pressed may start at the same setting.

If a pump setting was previously on maximum, reduce the pump setting prior to reactivation to avoid an immediate high pressure increase.

2.4 Recycling information

Introduction

This section contains information about the decommissioning of Chromaflow 400-1000 columns.

Decontamination

Chromaflow 400-1000 columns shall be decontaminated before decommissioning and all local regulations shall be followed with regard to scrapping of the equipment.

Disposal, general instructions

When taking Chromaflow 400-1000 columns out of service, the different materials must be separated and recycled according to national and local environmental regulations.

3 System description

About this chapter

This chapter provides a general description of Chromaflow columns and the basic operational principles of the nozzle. It also briefly describes packing stations and how these are used together with Chromaflow columns.

In this chapter

This chapter contains the following sections:

3.1 Overview of Chromaflow and associated equipment

Introduction

This section describes the main components of Chromaflow columns and the associated equipment required for operation.

Chromaflow column

Chromaflow columns from GE are made for convenient and cost-efficient large scale chromatography. The patented Chromaflow nozzle enables columns to be packed, run, cleaned-in-place, and unpacked without disassembling the column.

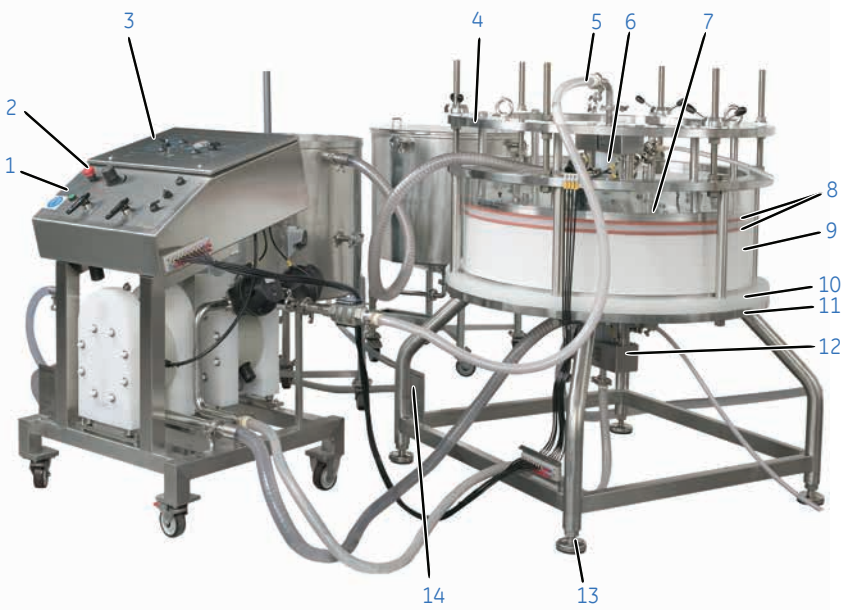
Chromaflow columns are variable bed height columns with a fixed bottom-end piece and a position variable top adapter assembly. The column tube is manufactured in cast acrylic or stainless steel. Both materials are precision-bored and polished. Chromaflow columns are equipped with manual or automatic nozzles, and with stainless steel or polyethylene bed supports.

Chromaflow setup

The normal setup includes the following:

- A Chromaflow column
- A Chromaflow packing station
- A slurry vessel
- A packing buffer / water vessel

An example of a setup with Chromaflow column and a Chromaflow packing station is shown below.



Part	Description
1	Packing station lower panel
2	Emergency stop
3	Packing station upper panel
4	Adapter flange
5	Slurry and buffer hose connected to top nozzle
6	Top nozzle
7	Adapter plate
8	Adapter O-ring(s)
9	Column tube
10	Bottom distributor
11	Bottom backing plate
12	Bottom nozzle
13	Adjustable foot
14	Column label

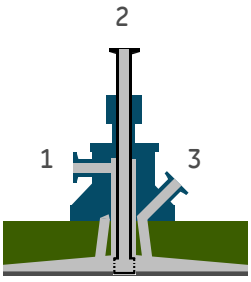
3.2 Chromaflow nozzles

Introduction

The Chromaflow column is equipped with one nozzle at the top of the column and one at the bottom. The top and bottom nozzles are identical.

The nozzles are multifunctional, allowing for packing, running, unpacking and cleaning, all without dismantling the column. Nozzles on a column can either be manually or automatically operated. In the automatic mode they are operated from the packing station.

A nozzle has three ports.

Part	Function	Illustration
1	Slurry outlet	
2	Slurry inlet	
3	Mobile phase port	

Slurry outlets

The slurry outlets are ports used for emptying during unpacking, or for venting depending on the position of the nozzle and the procedure undertaken. The slurry outlet ports are labeled **SOT** (Slurry Outlet Top) and **SOB** (Slurry Outlet Bottom).

Slurry inlets

The slurry inlets ports are used to either introduce slurry during packing or water during priming or cleaning. The slurry inlet is completely isolated from the mobile phase.

The slurry inlet ports are labeled **SIT** (Slurry Inlet Top) and **SIB** (Slurry Inlet Bottom).

Mobile phase ports

The mobile phase ports, set at an angle, are the inlet or outlet ports for the mobile phase, allowing flow into and out of the column via the distributors and bed supports. The mobile phase pathway is isolated from the other nozzle pathways during normal chromatographic operations.

The mobile phase ports are labeled **MPT** (Mobile Phase Top) and **MPB** (Mobile Phase Bottom).

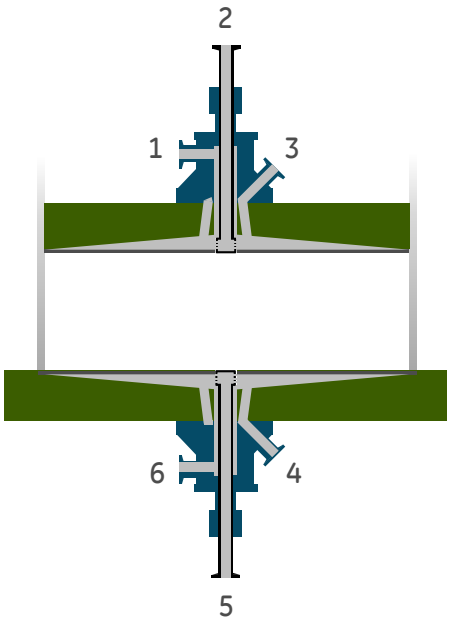
3.3 Basic principles of nozzle operation

Introduction

The nozzles, adapter and bed support are crucial parts of Chromaflow columns. This section provides a general overview of how the nozzles function.

Nozzle illustration

The following illustration shows an overview of the Chromaflow nozzle ports.

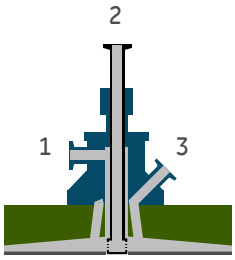
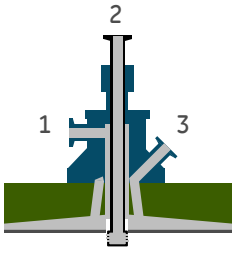


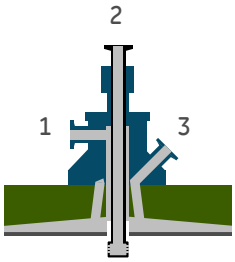
Port	Acronym	Function
1	**SOT**	Slurry Outlet Top on the top nozzle is the connection point for slurry or water exiting the column or for venting.
2	**SIT**	Slurry Inlet Top on the top nozzle is the connection point for slurry or water entering the column.
3	**MPT**	Mobile Phase Top on the top nozzle is the connection point for the mobile phase inlet.

Port	Acronym	Function
4	**MPB**	Mobile Phase Bottom on the bottom nozzle is the connection point for the mobile phase outlet.
5	**SIB**	Slurry Inlet Bottom on the bottom nozzle is the connection point for slurry or water entering the column.
6	**SOB**	Slurry Outlet Bottom on the bottom nozzle is the connection point for slurry or water exiting the column.

Nozzle positions

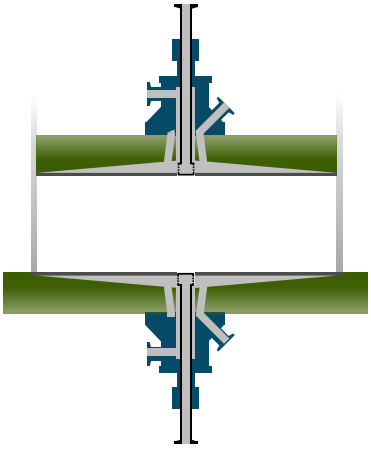
The Chromaflow nozzle positions are illustrated in the following table.

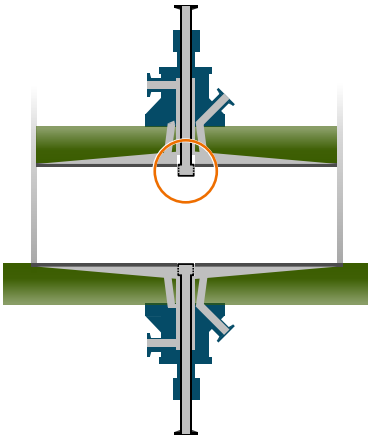
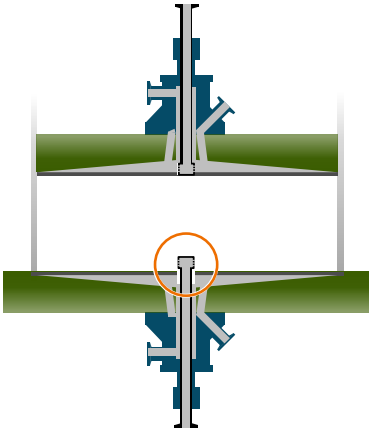
Nozzle position	Description	Illustration
RUN	The nozzle is fully retracted. • Liquids can only enter into the column and exit the column via the mobile phase ports **MPB** and **MPT** (3 in the illustration to the right). • Liquids can, without entering the column, pass via the slurry inlet ports **SIT** and **SIB** (2) and exit through the slurry outlet ports **SOT** and **SOB** (1).	
PACK	The nozzle is halfway inserted into the column. • Liquids can enter the column via the slurry inlet ports **SIT** and **SIB** (2). • Liquids can enter and exit the column via the mobile phase ports **MPB** and **MPT** (3).	

Nozzle position	Description	Illustration
UNPACK	The nozzle is fully inserted into the column. • Liquids can enter the column via the slurry inlet ports **SIT** and **SIB** (2). • Liquids can exit the column via the slurry outlet ports **SOT** and **SOB** (1). • Liquids can enter and exit the column via the mobile phase ports **MPT** and **MPB** (3).	

Nozzle function

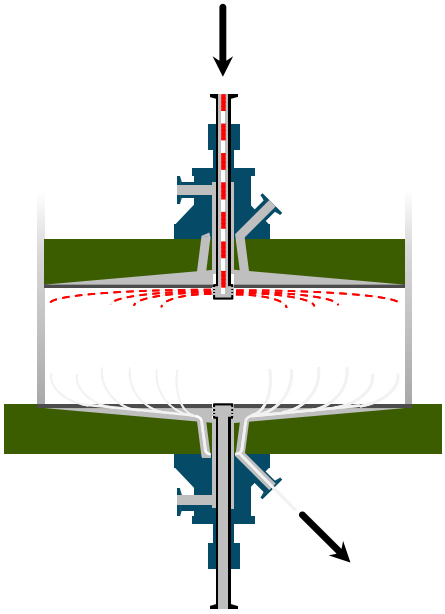
The top and bottom nozzles of Chromaflow columns are controlled separately and can be set in one of three different positions.

Nozzle position	Description	Illustration
RUN	When the nozzle tip is fully retracted and level with the bed support the nozzle is in the **RUN** position. There is no liquid pathway to the column open through the nozzle. The **RUN** nozzle position is illustrated by both nozzles in the image to the right. Buffers or other process fluids can enter and exit the column via the mobile phase ports. However you can use the **RUN** position to clean the slurry lines as the pathway, in this nozzle position, is closed off from the column.	

Nozzle position	Description	Illustration
PACK	When the nozzle tip is halfway inserted it is in the **PACK** position. With the nozzle in this position a passage to the slurry inlet is open, and it is possible to pack by pumping slurry into the column. Liquids can enter and exit the column via the mobile phase ports **MPB** and **MPT**. Positioning the top nozzle in the **PACK** position is used for packing the column using downflow. Positioning the bottom nozzle in the **PACK** position is used for packing the column using upflow.	
UNPACK	When the nozzle tip is fully inserted it is in the **UNPACK** position. This opens a passage behind the nozzle tip which connects to the slurry outlet port. The unpacking position is illustrated by the bottom nozzle in the image. Positioning the bottom nozzle in the **UNPACK** position is mostly used for unpacking the column. Positioning the top nozzle in the **UNPACK** position is mainly used for venting purposes.	

Packing

The packing phase of the process is illustrated below. The top nozzle is in the **PACK** position, which is halfway inserted into the column.



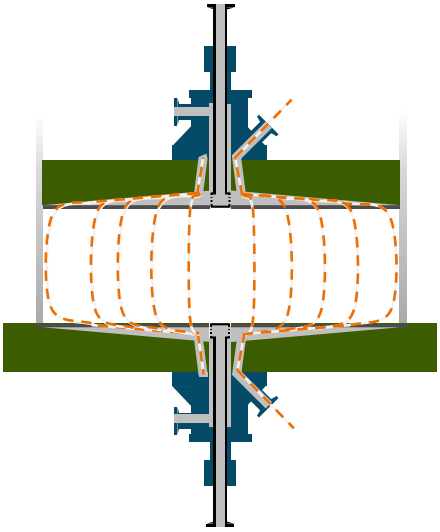
When packing from the top the slurry is pumped in via the top nozzle (**SIT**), as shown in the illustration above. The nozzle sprays the slurry directly into the column. The bottom nozzle is set in the **RUN** position, thus fully retracted. The media beads cannot pass through the bed support. Therefore the media is trapped in the column and the bed is built up with excess fluid (water or buffer) flowing out through the bottom mobile phase port (**MPB**).

The column is filled from the top and media is retained by the lower bed support.

The bed builds from the bottom up. When sufficient media has been pumped into the column the pump is stopped and the top nozzle (**SIT**) is set to the **RUN** position.

Running (chromatographic phase)

The running phase (the chromatographic phase) of the process is illustrated below. Both top and bottom nozzles are set in the **RUN** position, which is fully retracted.

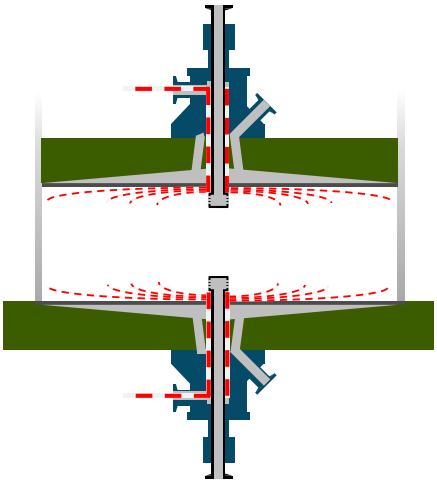


Depending on the direction of the flow selected, the mobile phase is pumped in via **MPB** or **MPT**.

The sample passes through the stationary phase and purification takes place. The mobile phase passes through either **MPT** or **MPB** depending on the flow direction.

Unpacking

During the unpacking phase the nozzles are fully extended in the **UNPACK** position, as shown in the illustration below.



Media can be rinsed out of the column when the nozzles are in their unpacking positions. The slurry will flow out of the column through **SOT** or **SOB**, depending on the direction of flow.

3.4 Additional equipment

Chromaflow Packing Station

The operation of standard Chromaflow columns with automatic nozzles also requires a packing station, or similar equipment, to pump liquids to and from the column and adjust the position of nozzles.

Different packing stations are available. Packing stations are selected depending on the size of the column and the chromatographic operation to be performed.

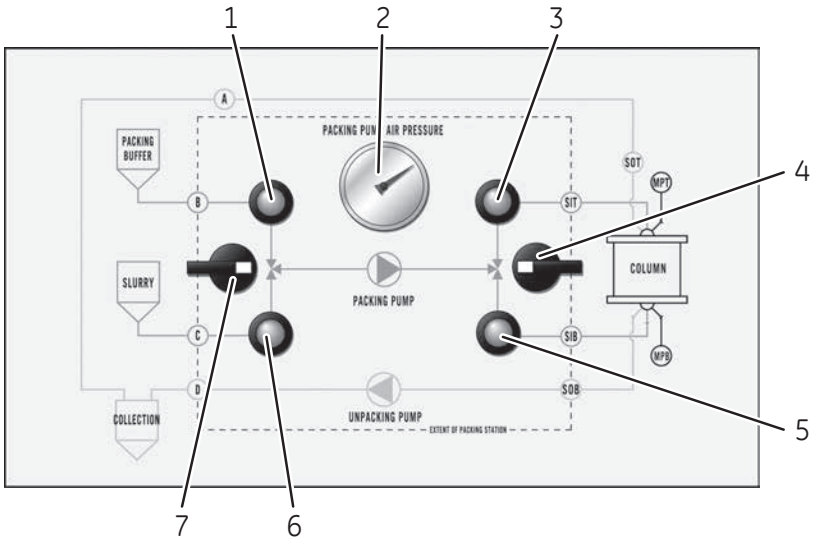
Note: *For detailed information on the packing station, see the Chromaflow Operating Instructions provided with the respective packing station.*

Chromaflow packing stations are available in four sizes, Chromaflow Packing Station 50, 100, 200, and 400, depending on the packing requirements.

All packing station sizes have the same general design, but have different pumping capacities.

Packing station controls - upper control panel

Upper and lower control panels on the packing station are used to adjust the position of automatic nozzles and control the pumping of liquid into and out from the column.

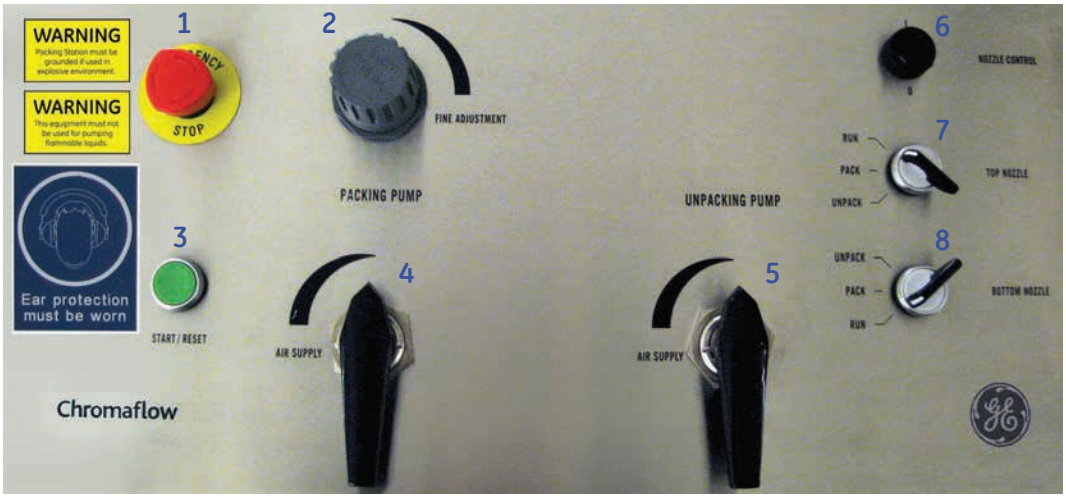


Part	Description	Function
1	Packing buffer flow indicator	The indicator displays when buffer is flowing from buffer tank to column.
2	Packing pump air pressure meter	The analogue indicator displays the packing pump air pressure.
3	Top nozzle indicator	The indicator displays flow through the top nozzle.
4	Three-way valve controller switch	The switch is used to control the valves to select the top or bottom nozzle. The mid position (as illustrated) isolates the valves.
5	Bottom nozzle indicator	The indicator displays flow through the bottom nozzle.
6	Slurry flow indicator	The indicator displays when slurry is flowing from slurry tank to packing pump.

Part	Description	Function
7	Three-way valve controller switch	The switch is used to control the valves to select either buffer or slurry. The mid position (as illustrated) isolates the valves.

Packing station controls - lower control panel

The image and table below shows an example of the lower control panel functions for a Chromaflow Packing Station.



No.	Part	Function
1	**EMERGENCY STOP**	Press this red button to stop operation immediately in an emergency situation. For more information see *Section 2.3 Emergency procedures, on page 29*.
2	**FINE ADJUSTMENT PACKING PUMP**	Use this control knob when packing the column, to make fine-scale adjustments to the packing pump air pressure.
3	**START/RESET**	Press this button to restart after an emergency stop or after loss of pneumatic feed.
4	**AIR SUPPLY PACKING PUMP**	Use this control to adjust the amount of air supply to operate the packing pump. This control should never be used when packing the column. ***Note:*** *The control overrides the **FINE ADJUSTMENT** knob.*
5	**AIR SUPPLY UNPACKING PUMP**	Use this control to adjust the amount of air supply to operate the unpacking pump.

No.	Part	Function
6	**NOZZLE CONTROL**	Use this switch to activate (**I**) or deactivate (**0**) automatic nozzle control from the packing station. ***Note:*** ***NOZZLE CONTROL*** *should always be set to* ***0*** *when the nozzle control function is not used.*
7	**TOP NOZZLE**	Use this switch to set the top nozzle to the following functions: • Run (set the switch to the **RUN** position) • Pack (set the switch to the **PACK** position) • Unpack (set the switch to the **UNPACK** position) ***Note:*** *This feature is only applicable for automatic nozzles.*
8	**BOTTOM NOZZLE**	Use this switch to set the bottom nozzle to the following functions: • Run (set the switch to the **RUN** position) • Pack (set the switch to the **PACK** position) • Unpack (set the switch to the **UNPACK** position) ***Note:*** *This feature is only applicable for automatic nozzles.*

4 Installation

About this chapter

This chapter contains information about Chromaflow installation, site requirements, how to set up Chromaflow 400-1000 columns and how to make compressed air connections to the Chromaflow packing station that is used to operate the Chromaflow 400-1000 columns.

Precautions

WARNING

Before attempting to perform any of the procedures described in this chapter, you must first read and understand the safety precautions in this document. Refer to *Section 2.1 Safety precautions, on page 15*.

In this chapter

This chapter contains the following sections:

4.1 Site requirements

Introduction

This section provides a brief description of the site requirements for the installation of Chromaflow columns.

Space and floor load

For space and floor load requirements, refer to the dimensions and weight information provided in the documentation package.

Note: *Make sure that the floor can handle the weight of Chromaflow column at fully loaded conditions. Observe that for the weight to be equally distributed, the floor must be level and without irregularities.*

Note: *In order to allow convenient working conditions for the operator, sufficient space should be provided on all sides of Chromaflow column when installed at the intended production location.*

Ambient environment

The production location must have adequate ventilation.

The following should be avoided:

- Direct sources of heat, such as sunlight
- Strong magnetic or electric fields
- Vibrations
- Corrosive gas
- Dust

4.2 Unpack the column from the crate

Introduction

This section provides a brief instruction for taking the Chromaflow column out of the transport crate. Unpacking instructions are normally attached to the outside of the transport box.

Remove the Chromaflow column from the crate as stated on the unpacking instructions, or in the absence of these instructions, follow the instructions provided in this section.

Precautions

WARNING

Heavy object. Because of the significant weight of Chromaflow 400-1000 columns, great care must be taken not to cause squeezing or crushing injuries during movement.

WARNING

Note that crates may not be marked with a center of gravity symbol. Make sure that the crates are properly balanced and centered over the forks of the lifting equipment so that they will not accidentally tip when moved.

WARNING

Move transport crates. Make sure that the forklift has capacity to safely lift the crate weight. Make sure that the crate is properly balanced so that it will not accidentally tip when moved.

WARNING

Packing crates and columns shall only be moved by personnel with appropriate training, and in adherence with local regulations.

Even if the instructions in the Chromaflow *Operating Instructions* are followed, it is the responsibility of the customer to guarantee the safety of personnel while working with the column.

CAUTION

To prevent bacterial growth, the product may be filled with denatured alcohol (18% C_2H_5OH (ethanol), 2% C_3H_7OH (isopropanol) and 80% H_2O (water)) at delivery.

The denatured alcohol mixture can be hazardous to humans if consumed.

Flush out the denatured alcohol before assembling, testing or integrating the product into the intended process context.

NOTICE

Chromaflow 400-1000 columns are usually prepared and installed by GE personnel. Contact GE if you require reinstallation at a new site.

NOTICE

The column must be unpacked from the crate and washed as soon as possible after delivery, preferably within 3 months. Storage temperature must be 4°C to 24°C.

Procedure

Step	Action
1	Place the transport crate on an even floor surface.
2	Remove the side of the transport crate that has been fastened with screws marked with black paint.

Step	Action
3	Remove the top of the transport crate.
4	Remove the wooden supports that have been used to keep the column steady in the crate.
5	Remove the column from the transport crate using a forklift or hoist and slings.

WARNING

Move column. When using a pallet truck or fork lift, do not lift the column higher than required for clearance. There is an increased risk that the column might accidentally tip when moved if the column is lifted more than 10 cm from the floor.

Note:

Use wooden blocks between the column and the forks of the forklift or pallet jack during lifting to protect the bottom of the column.

6	Use a pallet truck to transport the column to its working location.
7	Level the column as instructed in *Section 5.2.1 Level the column, on page 68*.
8	Carefully clean the column surface from particles that might have ended up on the column following transportation and unpacking to prevent any damage.

Visual inspection

When the column is unpacked, carefully check for signs of damage that may have occurred during transportation. Report any damage immediately to your local GE office and to the Transport Company concerned.

- Check that the packing list and the contents delivered are consistent.
- Check that the labels correspond to those in the documentation package.

4.3 Set up associated equipment

Introduction

Chromaflow requires connection to associated equipment for packing, unpacking, and control of the automatic nozzles, if fitted. Refer to the user documentation for detailed setup instructions of the following equipment.

- Packing station
- Slurry vessel
- Water vessel

CAUTION

Do not expose hard piping to mechanical stress. Placing nozzle connections under strain may result in damage to column ports. Use flexible couplings and/or supports to avoid strain stress.

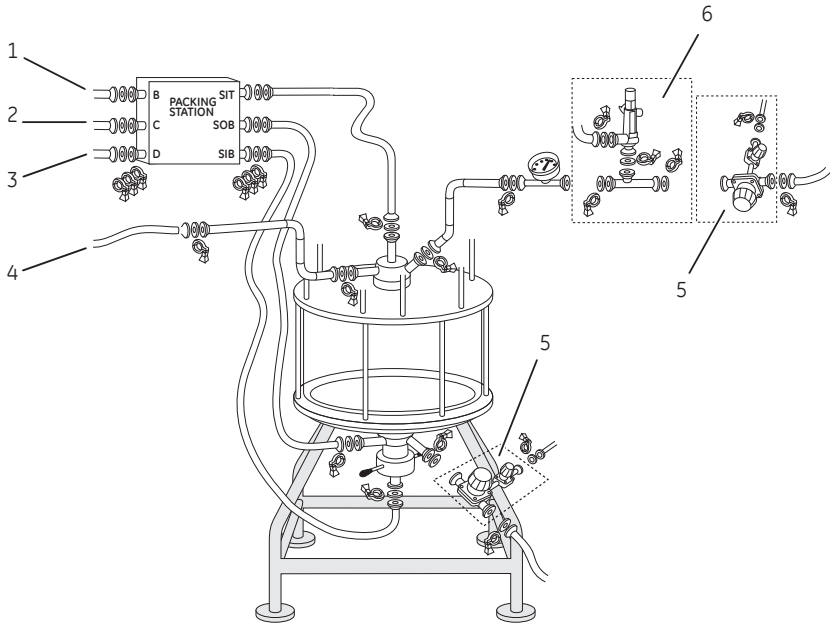
NOTICE

Media supply connections must be arranged so that piping dimensions, piping lengths, valves and height differences do not obstruct processing.

For specifications regarding media supply and connection requirements, refer to the documentation package.

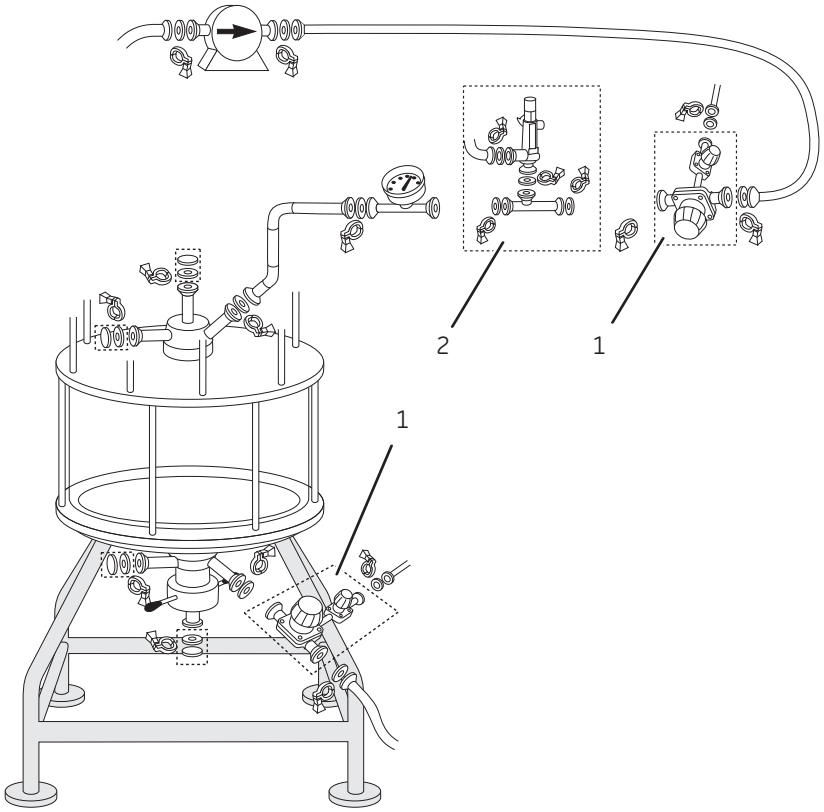
Example illustrations of setups

Packing setup



Part	Function
1	Inlet connection to packing buffer tank
2	Inlet connection to slurry tank
3	Inlet connection to collection tank
4	Outlet connection
5	Isolation valve
6	Pressure relief valve or equivalent pressure safety equipment

Running setup



Part	Function
1	Isolation valves
2	Pressure relief valve or equivalent pressure safety equipment

Procedure

Before connecting to an air supply, check the specific hose connection requirements for your column. This information is stated in the documentation package.

NOTICE

The air supply must conform to the compressed air specifications in the packing station. Refer to the relevant user documentation.

Step	Action
1	Position the associated equipment so that it is easy to operate and access.
2	Always use tubing with the same inner diameters and the same clamp sizes as the connection ports of the nozzle, valves and pump or packing station. Gaskets must have the correct bore diameter to avoid cavitation or flow restriction. Choose the largest dimension for best flow.

CAUTION

Make sure that all tubing, hoses and vessels are placed so that the risk of tripping accidents is minimized.

4.4 Set up a column

Introduction

This section explains how to set up Chromaflow columns.

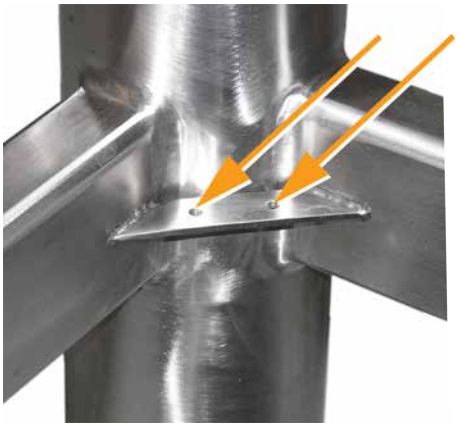
Ground connections

WARNING: EXPLOSION HAZARD

When the product is operated or maintained in a potentially explosive atmosphere, it **MUST be properly grounded** to avoid static discharge.

The following instruction describes how to ground a column.

Step	Action
1	Connect one end of a grounding cable to a grounding terminal in the plant grounding network and the other end to the grounding terminal located on the inside of the column leg (marked with a grounding symbol).



Step	Action
2	Connect one end of a second grounding cable to the grounding terminal on the inside of the column leg and the other end to the grounding terminal on the associated equipment.

Attach Mobile Phase connections

CAUTION

Make sure there is adequate over-pressure relief for the Chromaflow column.

It is recommended to attach a T-connector with a pressure sensor and one valve on **MPT** and a second valve on **MPB** to isolate the column after packing.

Note: *For packing upflow attach the pressure sensor on **MBP**.*

5 Operation

About this chapter

This chapter provides general instructions for working with Chromaflow columns. Specific information about individual columns can be found in the documentation package provided with your column.

In this chapter

This chapter contains the following sections:

Precautions

WARNING

Before attempting to perform any of the procedures described in this chapter, you must first read and understand the safety precautions in this document. Refer to *Chapter 2 Safety instructions, on page 14*.

WARNING

Only properly trained personnel may perform operation and user maintenance of the product.

WARNING

Do not operate the product in any other way than described in the Chromaflow user documentation.

5.1 Overview of column operation

Introduction

This section presents a general overview of normal operation using a Chromaflow column once it has been removed from the transport crate and installed according to installation instructions provided in *Chapter 4 Installation, on page 50*.

Important

It is essential to read through the complete sequence of this chapter before starting to operate Chromaflow columns.

Workflow example

The sequence of operations described below is the typical workflow for normal column chromatography. The workflow described assumes that the column is a new Chromaflow column or that it is a column that has been in storage. make sure the following prerequisites are fulfilled:

- The column is fitted with an adapter.
- The column is filled with storage solution.
- The column is empty of media.

NOTICE

It is recommended to replace plastic bed supports at each maintenance.

New plastic bed supports must be wetted before operation.

Phase	Function	Actions
1	**Preparing** Refer to *Section 5.2 Prepare the column, on page 66* for instructions.	1 Level the column. 2 Connect the column to the packing station (if applicable), and the required services (water, buffer, slurry, and cleaning solution). 3 Empty the column of storage solution and clean it. 4 Prime the column (including priming stainless steel bed supports or wetting the plastic bed supports). 5 Position the adapter (if applicable). 6 Perform a leakage test.
2	**Packing** Refer to *Section 5.3 Pack the column, on page 99* for instructions.	1 Prepare the media. 2 Prime the slurry lines. 3 Pack the column. 4 Rinse the slurry and packing lines.
3	**Testing** Refer to *Section 5.4 Test the packed bed, on page 113* for a brief overview. Refer to the media documentation for details.	Equilibrate and test the packed bed.
4	**Running** Refer to the user documentation for the chromatography system used.	Connect the column to a process system and run chromatography. Then proceed to one of the following steps: ***Note:*** *The next step is optional:* • *To test the packed bed, go to step 3.* • *To run chromatograhy again, go to step 4.* • *To clean the column with the packed bed (CIP, Cleaning-In-Place), go to step 5.* • *To unpack and clean the column, go to step 6.*

Phase	Function	Actions
5	**Cleaning (CIP)** Refer to the user documentation for the chromatography system used.	Clean a column with a packed bed for further production. ***Note:*** *The next step is optional:* • *To test the packed bed, go to step 3.* • *To run chromatograhy again, go to step 4.*
6	**Unpacking and cleaning** Refer to *Section 5.5 Unpack the column, on page 115* for instructions.	Unpack the bed and clean the column. ***Note:*** *The next step is optional:* • *To prepare the column for packing, go to step 1.* • *To perform maintenance on the column, go to step 7.* • *To store the column, go to step 8.*
7	**Maintenance** Refer to *Section 6.5 Maintenance procedures, on page 129* for instructions.	Perform maintenance and change all O-rings, change plastic bed supports or clean stainless steel bed supports. ***Note:*** *The next step is optional:* • *To prepare the column for packing, go to step 1.* • *To store the column, go to step 8.*
8	**Storage** Refer to *Section 6.6 Storage, on page 177* for instructions.	Prepare the column for storage. After storage, go to step 1.

5.2 Prepare the column

Introduction

This section describes the how to prepare a Chromaflow column to make sure it is ready for packing media.

Precautions

NOTICE

When moving a column with an acrylic tube from a warm environment to a cold room it is advisable to control bolt tensions. Adjust to recommended bolt torque settings if necessary.

NOTICE

Throughout the following instructions, mobile phase top (**MPT**) should be closed unless otherwise stated.

Note: *When using automatic nozzles and a packing station **NOZZLE CONTROL** should always be set to **0** when the nozzle control function is not used.*

In this section

5.2.1 Level the column

Introduction

This section describes how to level a column after it has been moved to the process location.

Note: *The column must be leveled before use so that any discrepancy from the horizontal does not exceed 5 mm.*

Tip: *If the column is moved regularly, but has one dedicated position for operation, it is recommended to mark its exact position on the floor at the dedicated place of operation after leveling. Then it is easy to return it to the exact same position, and re-leveling is not necessary.*

Level the column

Follow the instructions to level the Chromaflow columns.

WARNING

Do not adjust the feet more than 160 mm out from the base of the column stand to the floor. There is a risk of the column tipping over if the feet are screwed out too far.

NOTICE

Relieve the pressure on the column feet by raising the column using jacks or similar lifting and supporting equipment while adjusting the feet. Screw threads may be damaged if uneven pressure is placed on the column feet.

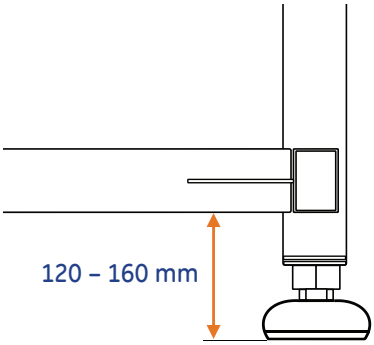
NOTICE

It is very important that the adjustment is done without the weight of the column resting on the leg being adjusted. Failure to do so *will* result in damage to the threads on that leg.

NOTICE

Make sure that the column is level after it has been moved. If the column is not level, column performance may be negatively affected.

Step	Action
1	Place two spirit levels diagonally across the top of the column flange.
2	Place jacks or similar suitable lifting equipment under the column stand.
3	Use the jacks or the lifting equipment, to carefully relieve the pressure on the column feet. Make sure that uneven load is not placed on the feet. ***Note:*** *Do not lift the column more than is absolutely necessary to avoid risk of tipping.*
4	Move the spirit level to different places around the column flange, changing the angle measured by 90 degrees each time, to make sure that the column is level in all directions.
5	Loosen the locking nuts on the column feet.
6	Adjust the level of the column by screwing the column feet away from the column stand as required until the feet touch the floor and the column stands level on the floor. Measure to make sure that the feet are extended between 120 to 160 mm from the floor and the base of the stand.



7	Move the spirit level around the column flange as described in step 4 to make sure that the column is level in all directions.

Step	Action
8	When the column the column is level and all four feet are resting on the floor, finger-tighten the locking nuts on the feet and then tighten the nuts to a torque of 70 Nm using a calibrated wrench.

9	Remove the jacks or the lifting the lifting equipment.

Tip: *To make sure that the column is level before beginning priming or packing procedures, the column can be filled with water and the water level measured around the circumference of the column tube. If the distance between the surface of the water and the top of the column tube deviates by more than 5 mm the column must be leveled.*

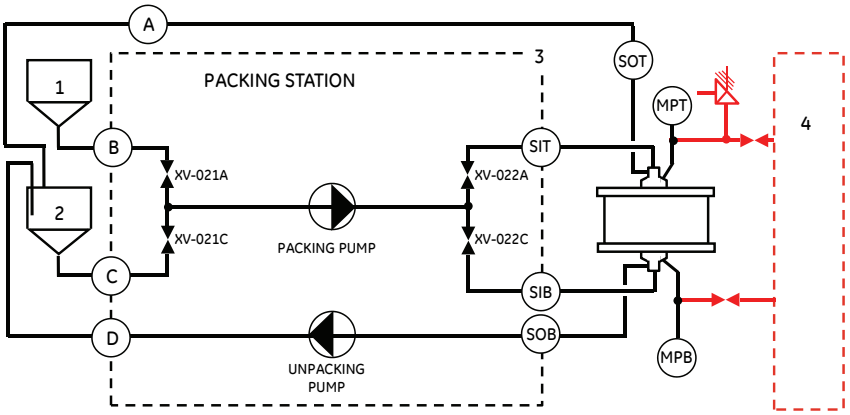
5.2.2 Connect a packing station to the column

Introduction

A packing station is used to pump liquids in and out of the column and to control the automatic nozzles. Other packing equipment may be used. This section describes specifically how to connect the column to a Chromaflow packing station. However, the general principles can be applied to other types of packing equipment.

Connections between column and packing station

The illustration below shows the connections between a Chromaflow column, a Chromaflow Packing Station and vessels.



Part	Function
1	Buffer vessel
2	Slurry vessel
3	The extent of the packing station
4	The extent of the chromatography system
Packing pump	Packing pump
Unpacking pump	Unpacking pump
A	Slurry outlet line from the top nozzle

Part	Function
B	Packing buffer inlet connection [1]
C	Slurry inlet connection[1]
D	Slurry outlet line from the bottom nozzle
MPT	Mobile Phase Top
SIT	Slurry Inlet Top
SOT	Slurry Outlet Top
MPB	Mobile Phase Bottom
SIB	Slurry Inlet Bottom
SOB	Slurry Outlet Bottom[1]
XV-021A	Valve for water/buffer inlet to the packing station
XV-021C	Valve for slurry inlet to the packing station
XV-022A	Valve for buffer/slurry inlet to column via the top nozzle (**SIT**)
XV-022C	Valve for buffer/slurry inlet to column via the bottom nozzle (**SIB**)

1 Tubes should be reinforced to prevent collapse.

Connect the column to the packing station

Note: *When connecting a packing station to a Chromaflow column refer to the documentation provided with the packing station.*

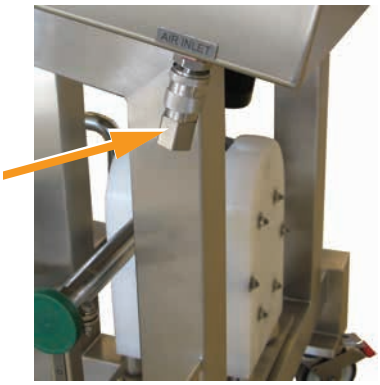
CAUTION

Do not strain nozzle connections. Connect tubing carefully. Failure to do so may damage nozzle components and cause leakage.

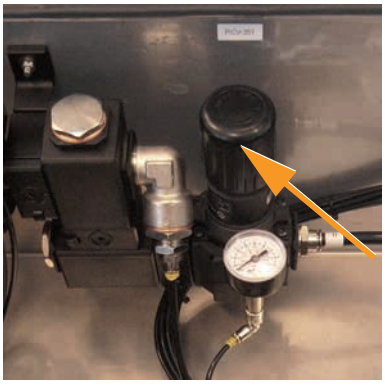
To connect Chromaflow Packing Station to the column:

Step	Action
1	Ground the packing station to process earth. Refer to the instructions in *Chromaflow Packing Station Operating instructions (29-0462-28)* for details.

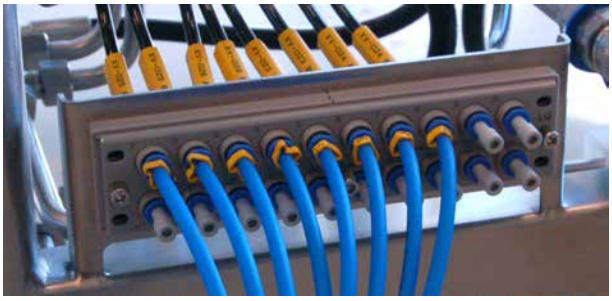
Step	Action
2	Connect the compressed air supply to the packing station air inlet (**AIR INLET**).



3	Make sure that the pressure regulator for the compressed air located inside the cabinet is set to the correct bar g pressure for your operation. Adjustments are made by turning the knob that is indicated below.



Step	Action
4	Connect the panel of pneumatic nozzle control hoses to the connection panel on column.



5	Connect packing station outlet **SIT** to column **SIT**.
6	Connect packing station outlet **SIB** to column **SIB**.
7	Connect packing station outlet **SOB** to column **SOB**.

Connect vessels to the packing station

Make the following connections to couple vessels to Chromaflow Packing Station.

CAUTION

Make sure that all tubing, hoses and cables are placed so that the risk for tripping accidents is minimized.

Step	Action
1	Connect inlet **C** to the slurry vessel.
2	Connect inlet **B** to the water/buffer vessel.
3	Connect outlet **D** either to a collection vessel, drain or slurry vessel. This connection is operation dependent.

Connect the column to vessels

The majority of column connections to vessels are made via the Chromaflow packing station, as described in the previous section. The only connections to and from column nozzle ports that are not made via the Chromaflow packing station are the line **A** (**SOT**) direct connection to an appropriate collection vessel, and the **MPT** and **MPB** connections to a process system.

When pathway **A** is connected to **SOT** and led, depending on the application to be performed, to either the water/buffer or slurry vessel, it is essential that the end of the tubing does not become submerged below the surface level of the liquid in the vessel. This is because pathway **A** also perform a venting function in some operations, as air leaves and enters the column via the line A tubing, to prevent a vacuum forming in the column.

NOTICE

Make sure that the end of the tube connection **A** remains above the level of the liquid in the collection tank at all times.

Fit pressure safety equipment to column

CAUTION

Make sure there is adequate over-pressure relief for the Chromaflow column.

Adequate pressure safety relief must be provided during column operation. Pressure safety relief alternatives are customer specific and are therefore not included in the Chromaflow delivery. Contact your local GE representative if further information is needed.

Step	Action
1	Fit pressure sensing safety equipment on the inlet mobile phase port (recommended range 0 to 6 bar). The inlet mobile phase port is normally **MPT**.
2	Fit one isolation valve to the inlet mobile phase port, after the pressure sensor on the process system side and mount a second isolation valve on the outlet mobile phase port. The valves should have the same inner diameter as the column inlet/outlet.
3	Connect tubing to the pressure safety equipment on the **MPB** and **MPT** ports and lead the tubing to a suitable drain or collection vessel.

5.2.3 Empty the column

Introduction

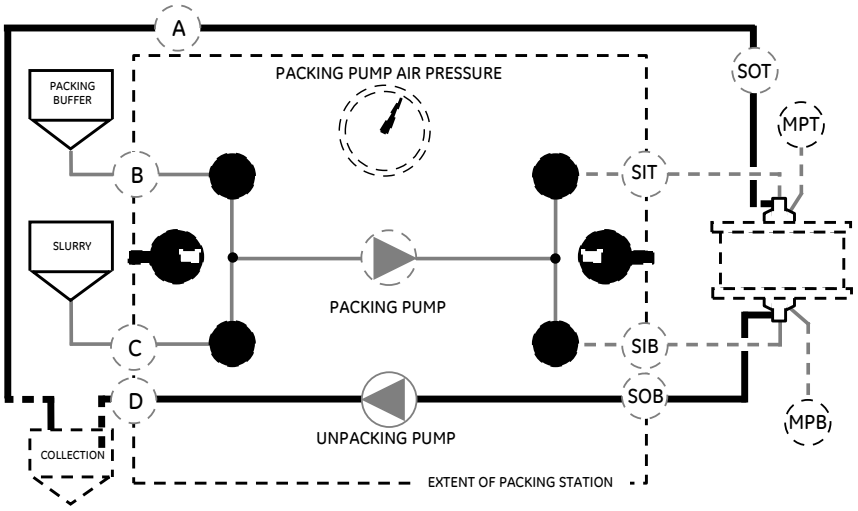
Emptying the column is relevant when the column contains storage liquid. All new columns must be emptied and cleaned before operation.

Empty the column

Follow the instructions to empty the column before use, when appropriate.

NOTICE

Make sure that the tube connection **A** (see the illustration below) connecting hose to the drain remains above the level of the liquid in the collection tank at all times. This is necessary to allow air to enter the tubing and the column.



Step	Action
1	If a packing station is used, activate the automatic nozzle control by setting the **NOZZLE CONTROL** switch to **I**.
2	Set the top and bottom nozzles to **UNPACK**.

Step	Action
3	Start the unpacking pump. The storage solution is removed from the column via outlet **D**, and air enters the column via tube connection **A** as illustrated above.
4	Stop the unpacking pump when the column appears to be empty of storage solution.
5	Proceed immediately to cleaning.

5.2.4 Cleaning the column

Introduction

Before use the assembled column must be cleaned. This section describes the recommended procedures for cleaning the column prior to packing.

NOTICE

Avoid high flow rates and rapid pressure or flow changes that can result in damage to the bed supports. This is especially important to consider when the column is empty and when the bed support is unsupported.

Clean the column

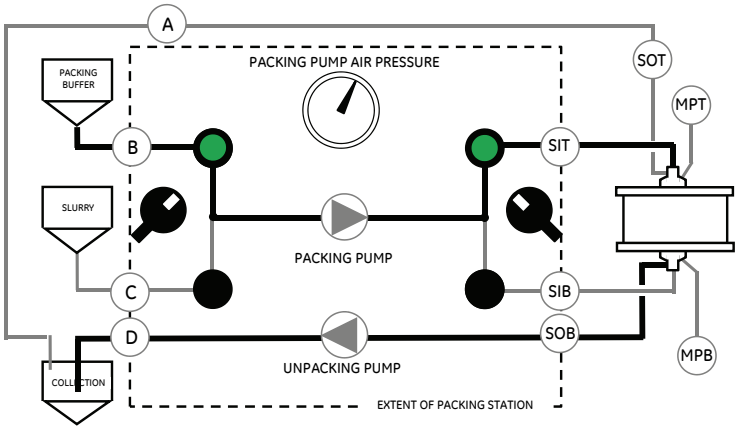
After the column is empty, follow the instructions below to clean it. Refer to *Section 8.2 Chemical resistance, on page 195* for suitable cleaning solutions.

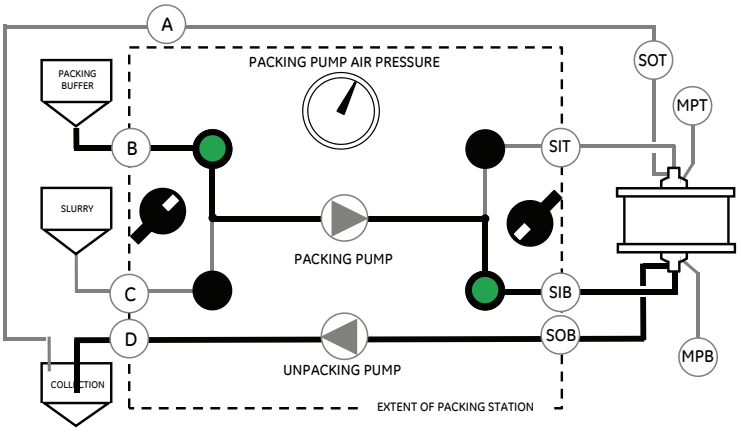
NOTICE

Make sure to clean the column after each unpacking to remove substances that can have a negative effect on column performance.

Step	Action
1	Fill the packing buffer vessel with cleaning solution.
2	Set the top and bottom nozzle controls to **RUN**. This will prevent spilling in the next step.
3	Connect the pathway **D** (from **SOB**) to waste/collection vessel. Connect the pathway **A** (from **SOT**) to waste/collection vessel. Make sure that the end of the tubing is not submerged in liquid in the vessel.
4	Set both nozzles to **UNPACK**.
5	Open the pathway **B**.

Step	Action
6	Start the packing pump and toggle between **SIT** and **SIB** with 3 to 5 seconds interval. Adjust the pump to full flow rate so that cleaning fluid reaches the column wall with sufficient force to wash the exposed surface of the O-ring.



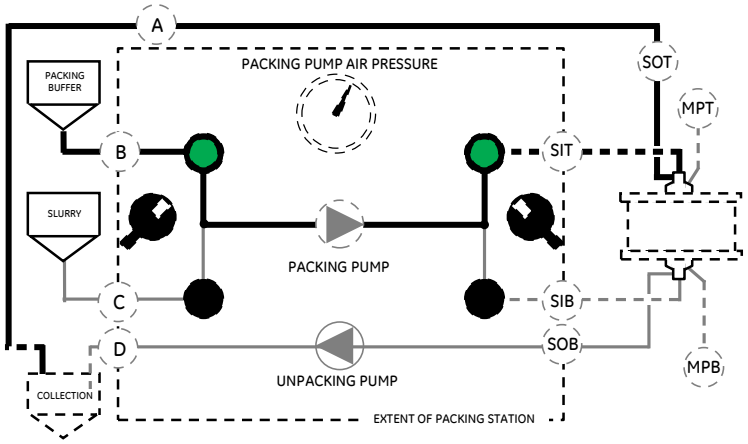


Cleaning solution will spray out from the top and bottom nozzles, respectively, cleaning the column and exit via line **D**.

Continue this procedure for some time, making sure the column is not completely filled.

7	Stop the packing pump.
8	Start the unpacking pump and pump until all cleaning solution is removed from the column.
9	Set the top nozzle to **PACK**.

Step	Action
10	Start both pumps, packing pump and unpacking pump and run simultaneously. Stop the pumps when the top bed support appears to be clean.
11	If cleaning liquid remains in the column, remove this by setting the bottom nozzle to **UNPACK** and use the unpacking pump to empty the column.
12	Set the top and bottom nozzle to **RUN**.
13	Open the pathway to **SIB**.
14	Start the packing pump to rinse the bottom slurry lines.
15	Open the pathway to **SIT** to rinse the top slurry lines.



16	Stop the pump.
17	Remove any remaining cleaning solution from the packing buffer vessel.
18	Proceed to rinse the column.

Rinse the column

Step	Action
1	Fill the packing buffer vessel with water.
2	Set the top nozzle to **UNPACK** and the bottom nozzle to **PACK**.
3	Start the packing pump. Water will flow into the column via the bottom nozzle. Once the column is full, liquid starts to exit via **SOT**.

Step	Action
4	Rinse the column with at least 5 column volumes of water.
5	Stop the pump.
6	Proceed to prime and purge the column using clean water. Follow the instructions in *Section 5.2.5 Prime the column and bed supports, on page 84*.

Prime the flushing ports

The flushing ports must be primed to lubricate the O-rings. Sterilizing/sanitizing solution is flushed through these valves, by pump or syringe, and into the chamber between the adapter seals when the adapter is lowered into the column. It is recommended to sanitize the barriers by changing the liquid in the chamber at least once every three months, and before each new packing.

As a sanitizing solution 20% ethanol is recommended.

NOTICE

Do not use a higher concentration than 20% v/v ethanol in the sanitizing solution as this may damage the acrylic tube.

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Follow these instructions to lubricate the O-rings and the chamber.

Step	Action
1	Connect a syringe or a pump to one of the valves and use the other as an outlet valve.
2	Flush and then fill the chamber with 20% ethanol.
	
3	Close the flushing port valve.

When the chamber is filled with liquid it serves two functions:

- It acts as a sanitary barrier.
- It provides lubrication to the two O-ring seals on the adapter.

5.2.5 Prime the column and bed supports

Introduction

This section describes the recommended methods for removing air from the Chromaflow column prior to column packing.

NOTICE

Avoid high flow rates and rapid pressure or flow changes that can result in damage to the bed supports. This is especially important to consider when the column is empty and when the bed support is unsupported.

Wet bed supports

Always wet plastic bed supports before column priming and operation:

- Always wet plastic bed supports when plastic bed supports are new or have been replaced. See *Wet the new bed support, on page 153* for instructions.
- Always prime plastic bed supports when the column has contained little or no liquid for any period of time.
- Always prime plastic bed supports when you suspect that the plastic bed supports are not hydrophilic.

Note: *The buffer recommended for wetting plastic bed supports is 20% 1-propanol. Water or 20% v/v ethanol can be used for columns with stainless steel bed supports.*

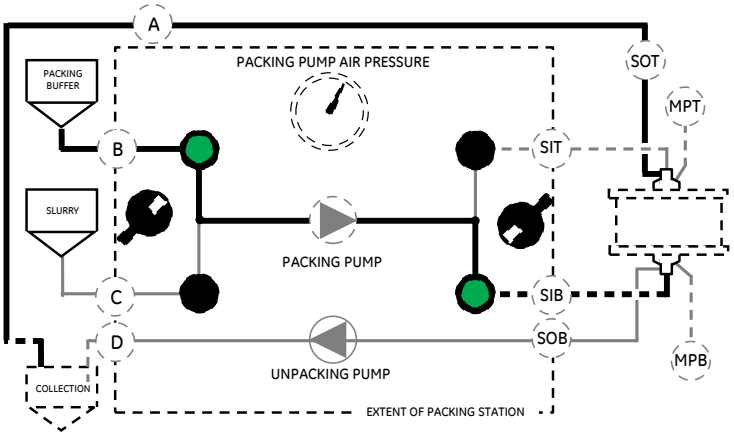
CAUTION

When working with 20% v/v 1-propanol solutions follow these instructions:

- Work in a well-ventilated area.
- Wear appropriate personal protective equipment.
- Keep storage containers tightly closed.

Priming and purging procedure

Step	Action
1	Set the bottom nozzle to **PACK** position and the top nozzle to **UNPACK**.
2	Open the pathway **B** from the packing buffer and open the pathway to **SIB**. Make sure **SOT** is open and that the line **A** is not submerged in the vessel.



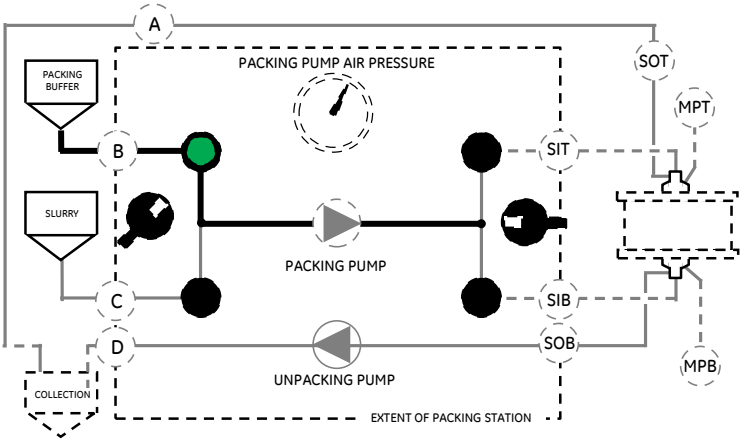
3	Start the packing pump to allow liquid to enter the column via **SIB** and air exit through **SOT**. Fill the column slowly until the column is completely filled with liquid.
4	Stop the pump and switch the top nozzle to **RUN**.
5	Start the packing pump once more and slowly increase the column pressure. ***Note:*** *Use the **FINE ADJUSTMENT** knob on the packing station to increase pressure.*

NOTICE

Be careful not to exceed the column pressure rating when increasing column pressure during the procedure.

If the pressure should rise too rapidly and exceed the pressure rating of the column, press the **EMERGENCY STOP** button on the packing station. Then open one of the mobile phase ports to release pressure inside the column.

Step	Action
6	When the column pressure is about 90% of the pressure rating, close the pathway to the column. On the packing station this is done by switching the **SIT**/**SIB** control to neutral (the mid position). See the illustration below. ***Note:*** *Do not touch the **FINE ADJUSTMENT** knob after shifting the **SIT**/**SIB** switch to neutral.*



7	Quickly open the bottom mobile phase port **MPB** to drain.
8	Once the pressure decreases to 0.3 bar g, close **MPB**. It is normal for air bubbles to rise from the bottom screen during this procedure.
9	Open the pathway to **SIB** and allow the pressure to rise again. ***Note:*** *Do not touch the **FINE ADJUSTMENT** knob. The pressure will adjust to the same value as before.*
10	Repeat steps 7-9 (the pressurization and purging procedure) 4 to 5 times until no more bubbles rise from the bottom screen.
11	Stop the packing pump.
12	Set the top nozzle to **UNPACK**.
13	Start the packing pump and continue to fill the column.
14	When the column is completely filled, set the top nozzle to **RUN**.

Step	Action
15	Allow the pressure to rise again. When the column pressure is about 90% of the pressure rating, set the column controller switch (**SIT**/**SIB**) on the packing station to close (the mid position).
16	Quickly open the top mobile phase port **MPT**. Liquid will exit through the bed support and air will escape through the port.
17	Once the pressure reaches 0.3 bar g, close the **MPT** port and allow for the pressure to rise again.
18	Set the column switch back to **SIB** and allow the pressure to rise again.
19	Repeat steps 16-18 (the pressurization and purging procedure) 4 to 5 times.
20	Quickly open the top mobile phase port **MPT** and let the pressure drop to 0.3 bar g, then close the **MPT** port .
21	The column and bed supports are now primed.

NOTICE

Do not allow the bed supports to dry out before priming the column. This may damage the bed supports and impair column performance.

5.2.6 Prepare and position the adapter

Introduction

This section describes how to prepare and position the adapter of a Chromaflow column to the correct bed height before packing.

Precautions

WARNING

Never use compressed air to raise the adapter.

CAUTION

To avoid unwanted pressure, make sure there is an opening via the nozzle mobile phase port or the safety valve whenever the adapter is moved.

NOTICE

The adapter is moved up or down by pressurizing the column with liquid, usually water. DO NOT raise/lower the column by hand as this may damage the threads on the adapter locking rods.

Lubricate the adapter

Before using or adjusting the adapter, it is critical to make sure that all contact surfaces are clean, free from particles and lubricated. To lubricate the contact surfaces:

Step	Action
1	Moisten the column tube top inside with 20% ethanol.
2	Moisten the top O-ring with 20% ethanol.

Note: *Refer to Section 8.2 Chemical resistance, on page 195 for alternative lubrication liquids.*

Level the adapter

Follow the instructions to make sure the adapter is level before moving it.

Step	Action
1	The column must be primed as described in *Section 5.2.5 Prime the column and bed supports, on page 84* and the the flushing port must be primed to lubricate the seals as described in *Prime the flushing ports, on page 82*.
2	Measure the distance between the column flange and the adapter flange at three points in the same position as the wing nuts using a vernier or steel rule. If the adapter is level go to the last step in this instruction.

3	Determine the lowest point of the adapter flange. Secure the wing nut and the nut under the flange at this position.
4	Loosen all other nuts and position them some distance away from the flange to allow the adapter to move downwards.
5	Open the **MPT** to waste.
6	Use the wing nuts to slowly lower the adapter until it is level.
7	Secure all nuts.

Step	Action
8	Read the bed height indicator. Compare the measured height to the target bed height. If there is a difference, this is the height the adapter needs to be adjusted.

Position the adapter

The adapter is moved to the desired bed height by using the packing station pumps to add or remove liquid from the column. Select the relevant instruction for leveling and lowering or raising the adapter below.

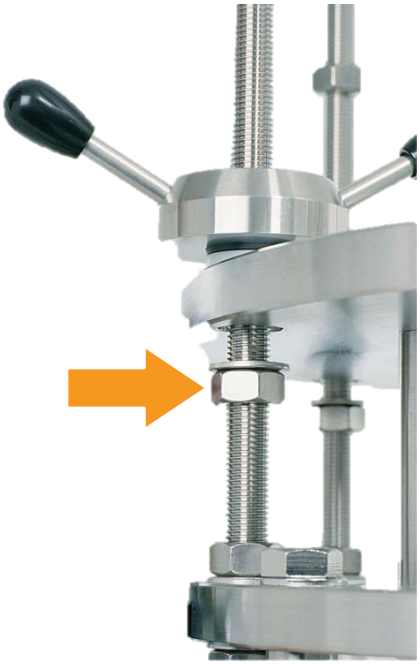
CAUTION

Make sure there is adequate over-pressure relief for the Chromaflow column.

Lower the adapter

Follow this instruction to lower the adapter down to the desired bed height.

Step	Action
1	Adjust the nuts positioned below the flange by the difference between the measured bed height and the target bed height by setting the lower adapter nuts and clamp nuts on the adapter locking rods to the desired position below the flange.



Step	Action
2	Make sure that the nuts are at the same distance from the flange. Use a caliper or steel rule.

3	Set the bottom nozzle control to **UNPACK** and the top nozzle control to **RUN**.
4	Close **MPT** and **MPB**.
5	Start the unpacking pump at a very low flow rate, thereby removing liquid from the column. The adapter will move down slowly and horizontally.

NOTICE

If the adapter is not moving horizontally, then stop the pump and re-level, refer to *Re-level the adapter, on page 135* before proceeding.

Step	Action
6	Allow the adapter to move down towards the preset level, stopping just above it.

NOTICE

Stop the pump just before the adapter reaches the desired level.

7	Confirm that the adapter flange is still parallel with the column flange.

NOTICE

If the flanges are not level, refer to *Re-level the adapter, on page 135* before proceeding.

8	Adjust the remaining distance to the preset level manually.
9	Adjust the adapter nuts and clamp nuts to just above and below the flange until the adapter is secured in position.

Raise the adapter

Follow this instruction to level and to move the adapter up to the desired bed height.

Step	Action
1	Adjust the nuts and clamp nuts positioned above the adapter flange by the difference between the measured bed height and the target bed height by setting the adapter nuts and clamp nuts on the adapter locking rods to the desired position above the flange.

Step	Action
2	Make sure that the nuts are at the same distance from the flange. Use a caliper or steel rule.

3	Set the bottom nozzle to **PACK** and the top nozzle to **RUN**.
4	Close **MPT** and **MPB**.
5	Start the packing pump at a very low flow rate, thereby filling the column with liquid. The adapter will move up slowly and horizontally.

NOTICE

If the adapter is not moving horizontally, then stop the pump and re-level before proceeding. Refer to *Re-level the adapter, on page 135* for instructions.

Step	Action
6	Allow the adapter to move up the preset level, stopping just under it.

NOTICE

Stop the pump just before the adapter reaches the desired level.

7	Confirm that the adapter flange is still parallel with the column flange. If not level, refer to *Re-level the adapter, on page 135* before proceeding.
8	Adjust the adapter nuts and clamp nuts to just above and below the flange until the adapter is secured in position.

5.2.7 Perform a leakage test

Introduction

A leakage test must be performed before the column is packed with chromatography media. The purpose of the test is to check the column for leakage and verify that the column pressure can be stably maintained over a longer period.

CAUTION

Only liquid should be used in the column during the leakage test. Do not perform a leakage test on a column containing media.

Leakage test procedure

CAUTION

Make sure there is adequate over-pressure relief for the Chromaflow column.

Step	Action
1	Make sure that the column has been primed as described in *Section 5.2.5 Prime the column and bed supports, on page 84*. Keep the same column set up for the leakage test as is used for priming.
2	Make sure that adequate pressure relief is provided, that a pressure gauge and isolating valve is mounted between the column and the upper **MPT** port valve, and an isolating valve is mounted on **MPB**. ***Note:*** *The pressure in the column during the leakage test is monitored using the **MPT** port pressure gauge.*
3	Set the bottom nozzle to **PACK** and set the top nozzle to **RUN**.
4	Isolate the **MPT** and **MPB**.
5	Start the packing pump, and use the **FINE ADJUSTMENT** knob (or equivalent) to build up pressure in the column by slowly pumping water into the column through the **SIB**.

Step	Action
6	Continue pumping water into the column until the pressure reaches between 1.5 bar g and 2.0 bar g. Then stop the pump and isolate the column by setting the bottom nozzle to **RUN**. ***Note:*** *The pressure used for the leakage test should be set below the range limit for the safety valves/rupture discs that are used.*
7	Wait for the pressure drop to stabilize before checking the column for leakage. The pressure in the column is likely to fall initially due to settling of seals and flexing of components.
8	Monitor the column pressure for 30 minutes after stabilizing. The pressure should not drop by more than 0.2 bar g over the test period and there should be no visible liquid leakage. ***Note:*** *If the pressure does drop by more than 0.2 bar g and continues to drop after the 30 minutes have passed, there is a substantial risk that the column is leaking.* *If no leak is found but doubt remains about if the column is leaking, redo the test before releasing the pressure. Note also that a drop in pressure could be due to a leaking valve and not the column itself.*
9	Release the column pressure slowly at the end of the test by carefully opening either **MPT** or **MPB** port and allowing the pressure in the column to drop completely. Once the pressure has been released, close the isolating valve.

5.3 Pack the column

Introduction

Each media has clearly defined and documented methods for optimal packing. When packing Chromaflow columns with media it is also important to understand the importance of controlling the following factors:

- Media volumes
- Slurry concentrations
- Compression factor
- Flow rates

This section explains these factors and describes how to calculate volume, how to prepare slurry, and how to pack the column.

For additional information about methods to meet your specific needs, contact your local GE representative.

In this section

5.3.1 Factors for media preparation

Calculating slurry volumes

The required volume of media can be calculated as follows:

Settled media required (liters) = **packed bed volume** (liters, as shown in table below) **× compression factor**

The following table illustrates the packed bed slurry volumes for corresponding bed heights and Chromaflow column diameters.

Column diameter (cm)	Cross-section area (cm^2)	Bed height (cm)						
		10	15	20	25	30	40	50
40	1260	13 L	19 L	26 L	32 L	38 L	51 L	63 L
60	2830	30 L	44 L	58 L	72 L	86 L	115 L	143 L
80	5030	53 L	78 L	104 L	129 L	154 L	204 L	255 L
100	7850	84 L	124 L	163 L	202 L	241 L	320 L	398 L

Note: *Volumes indicated are the total column volume and include the volume for the two conical distributor plates in the columns.*

Optional formula

The amount of media required for packing the column can also be calculated using the following formula:

$$A = (\pi \times r^2 \times H \times Cf/1000) + X$$

Where:

A = volume of media needed, liter
π = 3.14
r = radius of column (half the diameter), cm
H = bed height in column, cm
Cf = Compression factor of media
X = added volume due to the conical design of the distributor plate, liters (see the table below)

Column diameter (cm)	Conical area volume
40	0.46 L
60	1.4 L
80	3.0 L
100	5.8 L

Slurry concentrations

The media is normally diluted with buffer and mixed into a slurry. The percentage of media in the slurry, the slurry concentration, is necessary in the calculation of slurry volume required. You should always check the documentation provided with the media for recommendations.

Example calculation:

To pack a 400 mm column to bed height of 10 cm with media with a compression factor of 1.1 and a slurry concentration of 50%:

Settled media required (liters) = **packed bed volume** (liters) **× compression factor**

(13 × 1.1)/0.5 = 28.6 L

Result: 28.6 liters of slurry at 50% concentration is required to pack the bed.

Note: *As all media will compress to some extent, and pipes, valves, and pump will contain some amount of slurry, extra slurry must be allowed for and added to the calculated slurry volume.*

Compression factor

The media needs to be compressed slightly to maintain a stable packed bed. The multiplication factor to convert packed bed volume to settled media volume is the compression factor.

compression factor CF = (gravity settled bed height)/(packed bed height)

Depending on the type of media used, a compression factor between 1.1 to 1.25 is recommended. You should always check the documentation provided with the media for recommendations.

Flow rates

For some packing operations, optimal packing flow rates can be used to create beds suitable for production.

Contact your local GE representative for optimal flow rates for your operations.

5.3.2 Prepare slurry

Calculate slurry volume

Note: *Prepare the slurry at least two hours before usage in order for it to properly degas. The slurry can be prepared up to one day ahead.*

Calculate the required volume of media as follows:

Settled media required (liters) = (packed bed volume (liters, from the table provided in *Calculating slurry volumes, on page 100*) × compression factor)/ slurry concentration

Add a safety factor of slurry to allow for slurry volume of pipes, valves, and pump.

NOTICE

Always prepare an excess of slurry. An excess of slurry will help to prevent air from entering the system.

Prepare slurry

Step	Action
1	Re-slurry the sedimented media in the slurry tank, by using the plastic stirrer rod (or the automatic stirrer in the vessel).
2	Pour the slurry into a graded vessel (slurry vessel).
3	Take a sample for slurry concentration determination by transferring 100 ml of the slurry to a graduated cylinder.
4	Keep the slurry as a homogeneous suspension before starting the packing.

Final checks

Check that the actions listed below are completed before continuing with the packing procedure.

- Make sure that the slurry vessel, buffer vessel and column are correctly connected to the packing station.
- Make sure that the column has been prepared for packing and primed as described *Section 5.2.5 Prime the column and bed supports, on page 84*.
- Make sure that the slurry is prepared and available in the slurry vessel.

- Stir the slurry, using a plastic stirrer rod or automatic stirrer, until a homogeneous suspension is obtained.

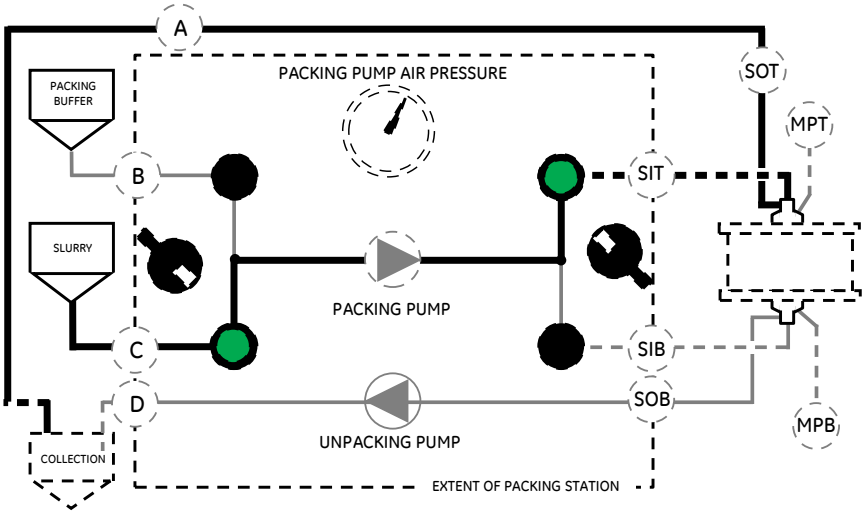
5.3.3 Prime slurry lines

Introduction

The slurry lines must be primed before beginning to pack the column with chromatography media. This is done to remove the air from the slurry lines in order to minimize the risk of introducing air into the primed column.

Procedure

The illustrations below shows the pathways and upper control panel settings used for top nozzle slurry line tubing priming.



NOTICE

It is important to make sure that no air is introduced into the column via any of the nozzle ports to keep the wetted plastic bed supports adequately hydrophilic.

Follow this procedure to prime the **SIT** line.

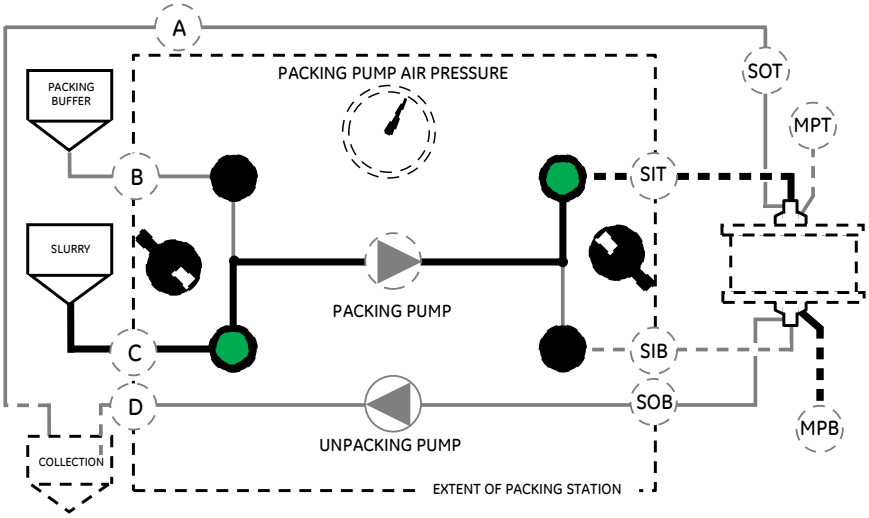
Step	Action
1	Close **MPT** and **MPB** ports.
5	Set the top nozzle and bottom nozzle to **RUN**.

Step	Action
6	Open the pathway to **SIT**.
7	Open the pathway **C**, the slurry vessel valve.
8	Start the packing pump and use the **FINE ADJUSTMENT** knob (or equivalent) to increase the speed to approximately 50% to pump slurry through **SIT** line and back to slurry vessel, ensuring homogenous slurry is achieved.
9	Take a slurry sample.
10	Having primed the lines, slow the pump down using the **FINE ADJUSTMENT** knob, and continue immediately to pack the column without stopping the pump.

5.3.4 Pack the column with media

Procedure

The following illustration shows the pathways used for packing the column using downflow.



Follow the instructions to pack the column using downflow.

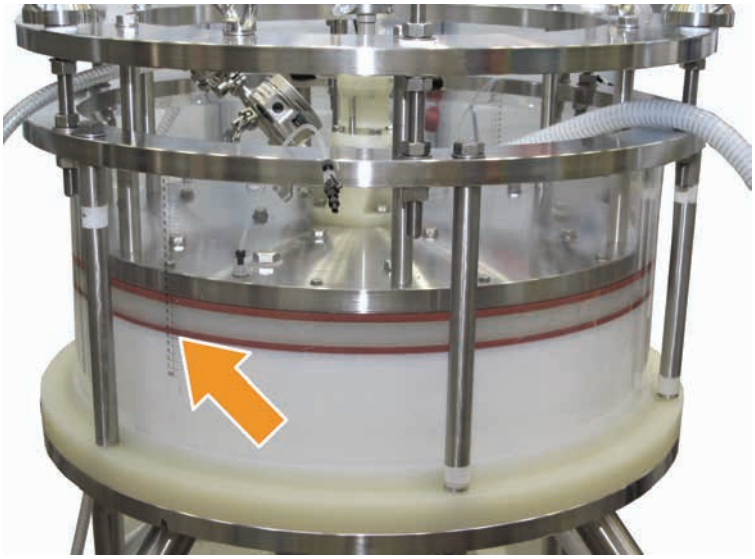
Step	Action
1	Set the top nozzle to **PACK** and set bottom nozzle to **RUN**.
2	Open the pathway **C**, the slurry vessel valve.
3	Open the pathway to **SIT**.
4	Open **MPB** to drain (or collection vessel).
5	Carefully increase the speed of the packing pump to maximum flow rate, or use a defined flow rate dependent on the medium used. ***Note:*** *Use the **FINE ADJUSTMENT** knob on the packing station to increase pressure.* *Result:* The packed bed starts to build in the column from the bottom, and the internal pressure increases.

Step	Action
6	Monitor the column pressure. If the pressure reaches above 2.75 bar reduce the pump speed to maintain approximately 2.75 bar.

NOTICE

Monitor the column pressure carefully to make sure the column is not subjected to over-pressure.

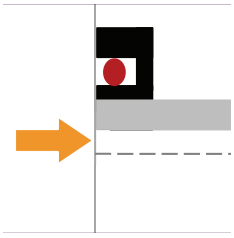
Step	Action
7	Monitor the level of the packed bed.



Step	Action

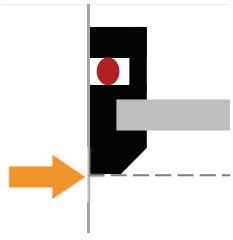
- If the column has a stainless steel bed support then stop the pump when the packed bed reaches 0.5 cm from the top bed support, or when a defined compression factor is reached.

  See below the column illustrated from the side, where the packed bed level (dotted line) is 0.5 cm below the bed support (grey).



- If the column has a plastic bed support then stop the pump when the packed bed reaches the lowest visible part of the adapter, below the bottom O-ring, or when a defined compression factor is reached.

  See below the column illustrated from the side, where the packed bed level (dotted line) reaches the adapter (black).



Note:

A badly or loosely packed bed may show as a larger gap between the packed bed and the bed support. This can lead to unsupported flow which can cause damage to the bed support.

Tip:

The defined compression factor can be controlled by measuring the amount of transferred slurry into the column, and correlating it to the slurry concentration.

8 Set the top nozzle to **RUN**.

9 Close the packing station **SIT** valve by turning the switch to the mid position.

Step	Action
10	Close **MPB** when **MPT** pressure is lower than 0.1 bar, but just above 0, to avoid draining of the column by siphoning.

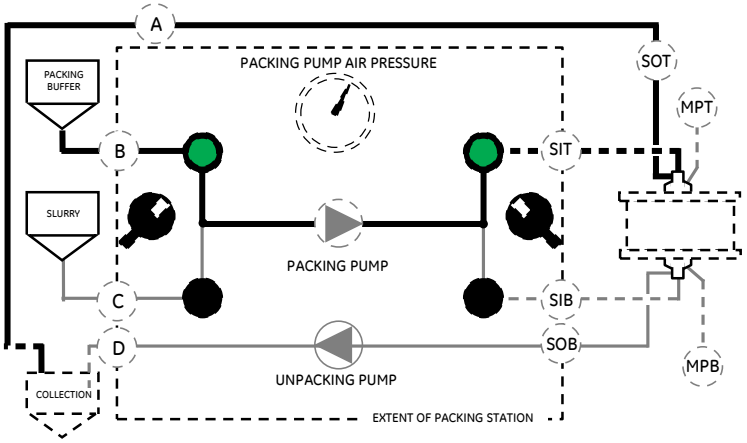
The packed bed with the correct compression will provide adequate support for the bed support under all normal running conditions.

5.3.5 Rinse slurry lines

Procedure

Follow the instructions to rinse the slurry lines.

Step	Action
1	Open the pathway to a vessel containing water (in the illustration below this is the vessel labeled **PACKING BUFFER**), which will allow the tubing and piping to be cleaned from media.
2	Open the pathway to **SIT**.



3	Start the packing pump and pump until the liquid received in the collection vessel is free from media.
4	Stop the packing pump.

5.4 Test the packed bed

Introduction

After packing the bed it is advisable to test the efficiency of column performance to make sure the packed bed is within the required specifications for performing chromatography separations. This will require a chromatography system and a suitable test protocol. Refer to HETP (Height Equivalent to a Theoretical Plate) testing protocols and pass criteria for the relevant media.

This section briefly describes the concept of efficiency testing and gives examples of testing criteria.

Tip: *For a theoretical background and a brief overview of column efficiency testing an Application note (28-9372-07) is available for download from the GE website.*

Testing column efficiency

Efficiency testing is the analysis of the residence time distribution for a tracer substance passing through the column. Typical test signals applied to the column are pulse or step signals.

In order to characterize the chromatography column without interference, tracer substance and eluent conditions are selected such that chemical interactions with the medium and disturbances of the fluid flow are avoided.

Testing criteria

Specifications for qualification of optimal column efficiency may be defined as:

- Reduced plate height $h \leq 3$
- Asymmetry factor $0.8 < As < 1.8$

These specifications rely on the use of the following optimized test conditions and parameters:

- An inert tracer substance to avoid interaction with the medium
- Optimal liquid velocity enabling highest theoretical efficiency
- Low external volume
- A low sample volume in the pulse test (1% of CV)

Specifications for column qualification as well as test procedures should always be reviewed with regard to specific application needs as well as practical constraints that may not allow for application of optimal test conditions. The specification for reduced plate height and asymmetry may very well be different for different purification steps in the process.

5.5 Unpack the column

Introduction

This section outlines a general procedure for unpacking and cleaning a column.

The unpacking procedure consists of the three following phases:

1 Loosening the packed bed, see *Section 5.5.1 Loosen the packed bed, on page 116*

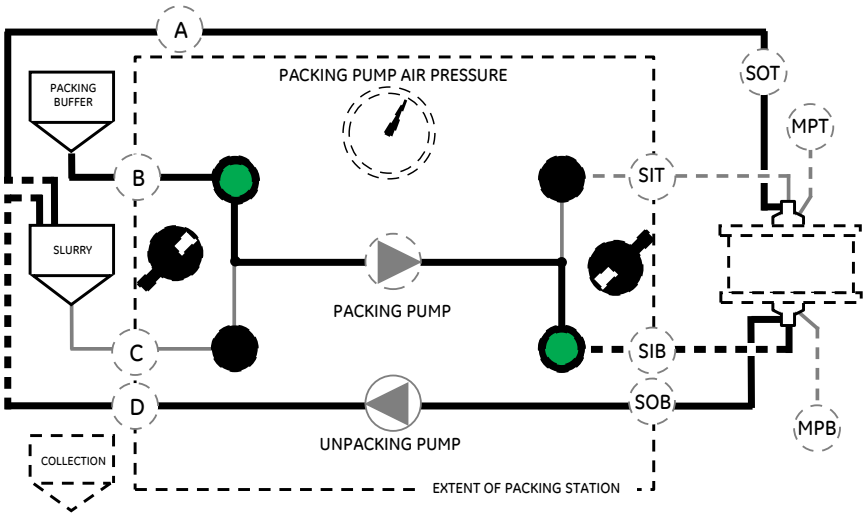
2 Emptying the column of slurry, see *Section 5.5.2 Empty the column of slurry, on page 119*

3 Clean and prime the column after unpacking, see *Section 5.5.3 Clean and prime the column after unpacking, on page 121*

5.5.1 Loosen the packed bed

Loosening procedure

The illustration below shows the pathways used when loosening the packed bed.



Follow the instructions below to begin to unpack the column by loosening the packed bed.

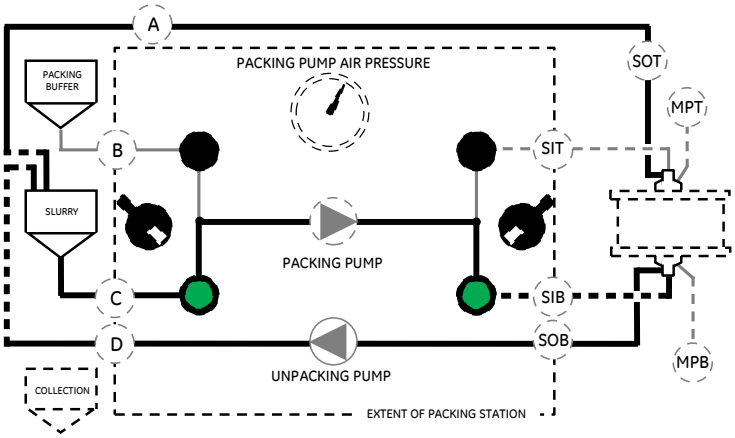
Step	Action
1	Make sure that the slurry vessel, buffer vessel and column are correctly connected to the packing station.
2	Connect outlet **A** and **D** to the slurry vessel.

NOTICE

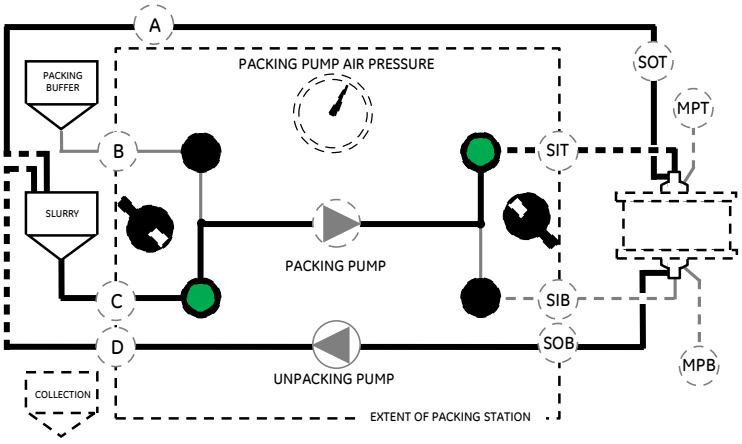
Make sure that the outlet **A** connecting hose to the slurry tank remains above the level of the liquid in the tank at all times. This is necessary to allow air to enter the tubing and the column.

3	Fill the buffer tank with at least two times the column volume of buffer for unpacking.
4	Set both and top and bottom nozzles to **UNPACK**.

Step	Action
5	Check that the **MPB** and **MPT** ports are closed.
6	Open **SIB**.
7	Open pathway **B** (packing buffer or water).
8	Start the packing pump at maximum flow rate and pump until approximately a half column volume (0.5 CV) of slurry has collected in the slurry vessel.
9	Open pathway **C**. Slurry will now be recycled into the column and back to the slurry vessel.



Step	Action
10	After around 3 to 5 minutes, open **SIT** to redirect the flow entering the column.

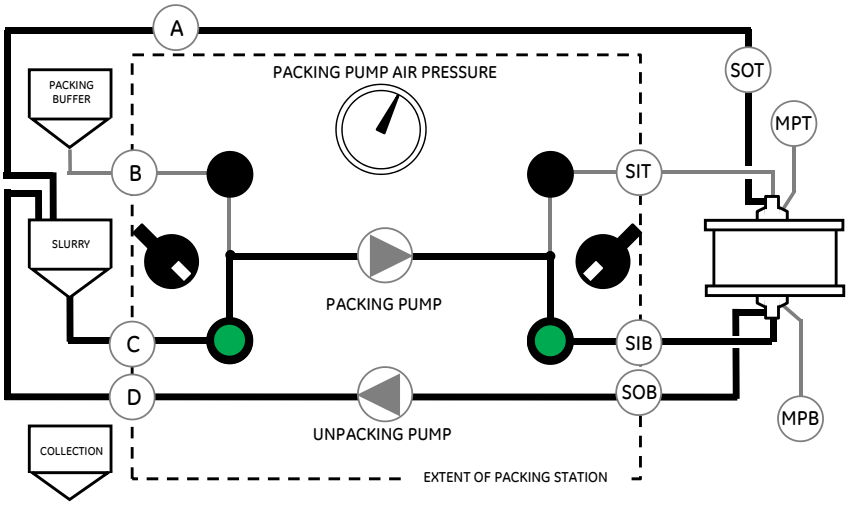


Step	Action
11	After around 3 to 5 minutes, open **SIB** to redirect the flow entering the column.
12	If necessary, repeat steps 9 to 10 to toggle between **SIT** and **SOB** until all of the recycling media is slurry, then stop the packing pump.
13	Proceed to the next section to empty the column of slurry.

5.5.2 Empty the column of slurry

Emptying procedure

The illustration below shows the pathways used when emptying the column of slurry.



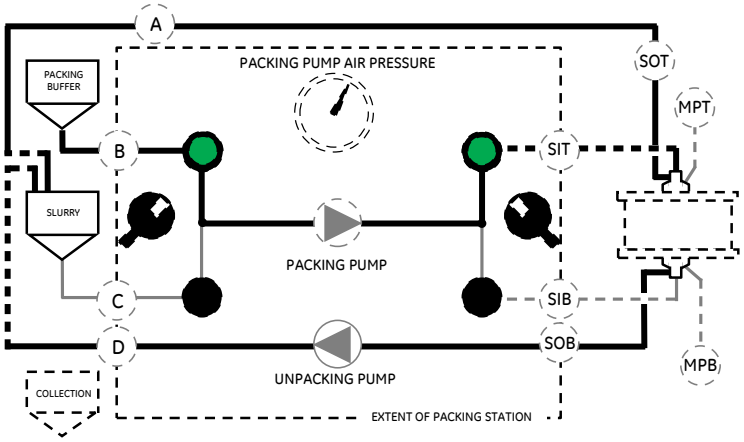
Note: *When emptying the column of slurry, the **A** and **D** outlet tubing connections can be directed to the slurry tank as the collection tank, if this is of a suitable size to accommodate the volume to be emptied from the column.*

Follow the instructions below to complete the unpacking of the column by emptying the column.

Step	Action
1	Make sure that the packed bed has been loosened according to the instructions above.
2	Set the top nozzle to **UNPACK**.
3	Start the unpacking pump. Slurry is removed from the column via **SOB** and the **D** line, and air enters the column via **SOT** and line **A**.

NOTICE

Make sure that the outlet **A** connecting hose to the collection tank or slurry tank remains above the level of the liquid in the tank at all times. This is necessary to allow air to enter the tubing and the column.

Step	Action
4	Stop the pump when the column is empty of slurry. ***Note:*** *If the chromatography media does not fully re-slurry, repeat steps 8 to 10 in the instruction for loosening the packed bed.*
5	Change to pathway **B** and start the packing pump, and toggle between the **SOT** and **SOB** to flush out the remaining media. Be careful to limit the amount of unpacking buffer or water used so that slurry dilution is avoided. 
6	When the column is empty, stop the pump.
7	Set the top nozzle to **PACK** and open the pathway through **SIT**. Run the packing pump and unpacking pump simultaneously for a final rinse of the bed supports and the complete column. **NOTICE** Monitor the column pressure to make sure the column is not subjected to over-pressure.
8	Set both nozzles to **RUN**.
9	Proceed to clean the column and lines.

5.5.3 Clean and prime the column after unpacking

Cleaning procedure

Step	Action
1	Clean the empty column, see *Clean the column, on page 79*.
2	Rinse the column after cleaning, see *Rinse the column, on page 81*.
3	Clean the sanitary barrier using the flushing ports, see *Prime the flushing ports, on page 82*.
4	Prime and purge the column using clean water. Follow the instructions in *Section 5.2.5 Prime the column and bed supports, on page 84*.

If required, replace the water with storage solution (for storage) or packing buffer for operation.

6 Maintenance

About this chapter

Regular cleaning and maintenance of Chromaflow 400-1000 columns is essential to maintain the column in a good condition and extend the operational lifetime of column components and to achieve reliable results. It is important that the column is kept free from contamination such as microbial growth and adsorbed proteins in the liquid handling system.

This chapter provides an overview of general cleaning, maintenance, and storage information applicable to Chromaflow columns.

In this chapter

This chapter contains the following sections:

Precautions

WARNING

Before attempting to perform any of the procedures described in this chapter, you must first read and understand the safety precautions in this document. Refer to *Chapter 2 Safety instructions, on page 14*.

Note: *After any maintenance or service has been performed on the column it is strongly advised that a leakage test is performed to make sure of the column integrity prior to packing media. Refer to instructions in Section 5.2.7 Perform a leakage test, on page 97.*

6.1 Service

Introduction

Regular service and preventive maintenance of the column is necessary to maintain the column in optimal condition and extend the operational lifetime of column components.

Service and preventive maintenance work should be performed according to GE recommendations.

WARNING

Only properly trained personnel, or personnel with equivalent knowledge of similar equipment, are allowed to perform maintenance work on the column. Only personnel authorized by GE are allowed to perform service on the Chromaflow column.

Service frequency

The requirements for frequency of service and preventive maintenance depend on the frequency of use of the column and the specific applications performed.

A general recommendation is one service and preventive maintenance visit every two years, but if columns are in continuous operation an annual service and preventive maintenance visit is recommended.

Service agreements

Contact your local GE representative for information about frequency of service requirements to suit individual application needs and for details of the GE service agreement options available for Chromaflow columns.

6.2 Cleaning

Introduction

Cleaning and sanitizing are essential elements of any process to ensure that biological contamination is kept within acceptable limits. This section describes when to clean and aspects relevant to cleaning.

During a normal operation cycle cleaning can be required for the following reasons:

1 Before and after servicing the column. Firstly to ensure the vessel is clean to perform work upon, and secondly to ensure the vessel is clean for return to operation.

2 During normal process cycles with a packed column.

3 Before storing a column with or without a packed bed.

4 After storage in preparation for packing and running.

5 Before decommissioning and removal from the process environment.

Cleaning methods

There are two methods for performing cleaning on Chromaflow columns. Both methods involve pumping solutions through the column whether it is packed with media or not. One method is performed with a packing station and and one method without (CIP).

If the column is packed and in use then usually the system attached to the column is sufficient for CIP during normal operation. Most other cases require a packing station to be connected to the column to operate the nozzles and clean the various pathways as well as the interior of the column itself.

For CIP cleaning method using other systems, refer to the relevant user documentation.

For cleaning after unpacking, refer to instructions in *Section 5.5.3 Clean and prime the column after unpacking, on page 121*.

For cleaning stainless steel bed supports, refer to instructions in *Section 6.3 Cleaning stainless steel bed supports, on page 127*. Plastic bed supports are not cleaned but exchanged.

NOTICE

To retain sanitary surfaces, it is recommended that clean gloves be worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Biohazardous substances

The cleaning procedures described in this section do not apply to columns that have been exposed to biohazardous substances. If your column or associated equipment has been contaminated with a biohazardous substance, contact your local GE service representative for further information about decontamination procedures.

Cleaning agents

All column parts can be cleaned with the agents most commonly used in column chromatography, such as detergents, 20% ethanol, weak acids, sodium hydroxide and salt solutions. For details refer to the chemical resistance guidelines in *Section 8.2 Chemical resistance, on page 195*.

CAUTION

Do not use chemicals harmful to the column.

NOTICE

For acrylic-tubed columns, do not use solutions containing more than 20% ethanol.

Rinse the column after cleaning

Water is the preferred rinse solution due to the solubility properties of sodium chloride in water compared to other possible rinsing solutions such as ethanol. It is recommended that a water rinse of at least five column volumes is used.

6.3 Cleaning stainless steel bed supports

Stainless steel bed supports may clog if not cleaned regularly. The supports should be cleaned at least after 5 to10 packings, but preferably after each packing. If the supports cannot be cleaned properly using the method below, contact your local GE representative for advice.

Note: *The compressed air that is used for cleaning bed supports must be completely free from lubricating oils and particles.*

Step	Action
1	Remove the bed support from the column.
2	Flush the bed support free from entrapped media using water.
3	Place a shut-off valve on a compressed air supply hose. Fit a 0.5 to 1 inch plastic TC-clamp connector or similar to the valve.
4	Use a shallow container to soak the bed support in 0.5 to 1 M NaCl solution for a maximum of 5 minutes.
5	Place the bed support with the coarse side up on a clean non-scratching (plastic) support, that allows air and media to flow through the bed support.
6	Open the valve and start blowing air though the bed support from a close distance. Make sure the whole surface of the bed support is properly blown and that special care is used at the outer rim and at the center.
7	Rinse the bed support thoroughly in water immediately after the above procedure is finished.
8	Repeat the procedure, this time using at least 20% ethanol solution. If 20% ethanol cannot be used, use water. Finish by carefully rinsing with water.

6.4 Maintenance schedule

The table below provides recommendations for the frequency of regular maintenance procedures for columns in continuous use.

Note: *Make sure that the product file is kept updated and together with the product documentation. The file should contain information about inspections, maintenance procedures that have been performed, spare parts that have been exchanged and any other relevant information for safe operation.*

Procedure	Frequency
Change all O-rings	At least once every two years
Clean O-ring grooves	Every time O-rings are changed
Change liquid in flushing channels	Every two months and preferably before each packing
Check all seals for leakage	Regularly
Change O-rings and seals on moving parts (e.g. adapter and nozzles)	Every 5 to 10 packings, more frequently if required
Clean stainless steel bed supports	Every 5 to10 packings, more frequently if required
Replace plastic bed supports	Every 5 to 10 packings, more frequently if required

NOTICE

Make sure that cleaning of O-ring grooves is performed each time O-rings are removed and replaced. Clean O-ring grooves manually and gently with 20% ethanol, taking great care not to damage the sealing surface.

6.5 Maintenance procedures

Introduction

This section describes the specific maintenance procedures when cleaning or replacing parts and provides information about service.

Maintenance work shall be performed according to GE recommendations. Contact your GE representative for advice and before performing any maintenance procedure you are not sure about.

In this section:

Precautions

WARNING

Decontaminate the equipment before maintenance or decommissioning to ensure that hazardous residues are removed.

WARNING

Do not perform any type of maintenance work on the column while the adapter is in motion. Do not reach inside the column while moving the adapter.

WARNING

Do not perform any type of maintenance work on the column while the column is pressurized. Note that the column can be pressurized even when the system is closed down.

CAUTION

Make sure to protect adapter sealing surfaces. Sealing surfaces should always be clean and free from scratches, blemishes, wear or any other type of damage.

Spray the column tube wall above the top piston seal with a 20% ethanol solution. Failure to do so may cause damage to the column wall and seals.

CAUTION

Do not leave O-rings or seals in hot water for longer than the time period recommended in GE product documentation.

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

NOTICE

Lubricate all O-rings and seals thoroughly with 20% ethanol.

NOTICE

Make sure that tools are properly calibrated before use. Use of uncalibrated tools such as torque wrenches may result in over-tightening or under-tightening of bolts and result in damage to column components.

NOTICE

When performing maintenance it is recommended to exchange the plastic bed supports.

6.5.1 Remove the adapter

Introduction

When removing the adapter, it may be helpful to refer to the exploded views in *Section 8.3 Exploded drawings: Adapter, bottom distributor and bed support, on page 199*.

NOTICE

When working on columns fitted with automatic nozzles, a packing station must be used as the nozzles are controlled from this unit. Columns with manual nozzles can use a pressurized water supply with a shut off valve.

NOTICE

When performing maintenance it is recommended to exchange the plastic bed supports.

Tools required

The following tools are required for removing the adapter:

- A crane or hoist, with suitable lifting capacity
- Three slings
- Three adapter legs
- Stainless steel wrenches

Adapter removal procedure

WARNING

During removal of the adapter, or any other movement performed through applying hydrostatic pressure, a pressure gauge must be connected to the system so that the internal column pressure can be monitored.

CAUTION

Make sure to protect adapter sealing surfaces. Sealing surfaces should always be clean and free from scratches, blemishes, wear or any other type of damage.

Spray the column tube wall above the top piston seal with a 20% ethanol solution. Failure to do so may cause damage to the column wall and seals.

NOTICE

The adapter is moved up or down by pressurizing the column with liquid, usually water. DO NOT raise/lower the column by hand as this may damage the threads on the adapter locking rods.

The procedure contains three steps:

1 Prepare the adapter, raising it to its highest position within the column.

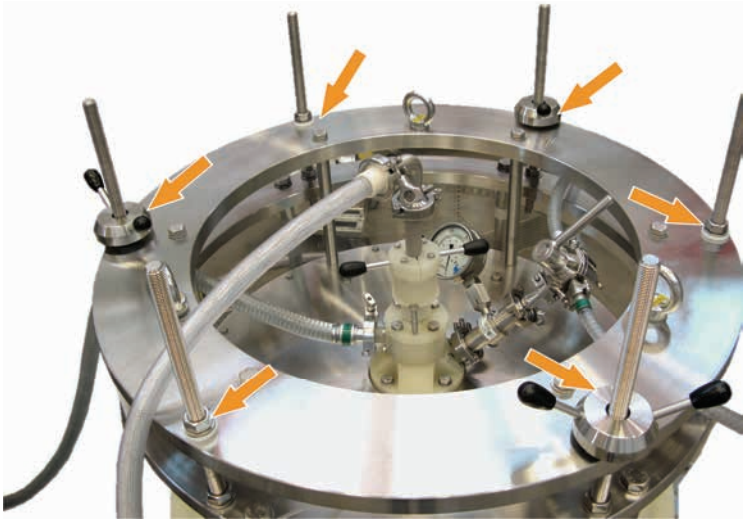
2 Re-level the adapter (if it is tipped out of alignment during raising).

3 Lift the adapter out of the column.

Prepare the adapter

Step	Action
1	Before removing the adapter the column must be full of liquid (usually distilled water). The column tube wall above the top piston seal must be perfectly clean and sprayed with 20% ethanol solution. The seals must be lubricated (see *Lubricate the adapter, on page 88*) and the flushing ports must be primed (see *Prime the flushing ports, on page 82*.
2	Prime to remove all the air in the column, packing station, pipes and vessels. See instructions in *Section 5.2.5 Prime the column and bed supports, on page 84*.

Step	Action
3	Remove all fasteners on the adapter flange.



4	Set the bottom nozzle to **PACK** and the top nozzle to **RUN**.
5	Open the pathway to **SIB**.
6	Start the packing pump and fill the column to move the adapter upwards. The pressure needed to move the adapter should be less than 0.5 bar. ***Note:*** *If the pressure rises above 0.5 bar, or if the adapter is tilting, then stop the pump and repeat the procedure to lubricate the seals (see Lubricate the adapter, on page 88) and prime the flushing ports (see Prime the flushing ports, on page 82.* *If the adapter is tilting, then re-level it according to instructions in Lift the adapter, on page 135 before proceeding to the next step.*
7	Check to see if anything other than the seals are preventing movement, or if the adapter has tipped out of alignment. ***Note:*** *Adapter disalignment of a few mm is acceptable and will normally even itself out.*
8	Stop the pump as the top O-ring of the adapter approaches the upper edge of the column tube.

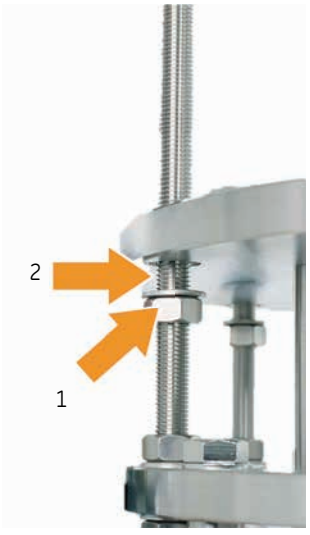
Re-level the adapter

If leveling is required follow these instructions.

Step	Action
1	Stop the packing pump or inflow of water.
2	Lubricate the O-rings.
3	Fit a nut and washer to the clamp rod at the highest point on the adapter flange.
4	Start the pump and watch the adapter as it straightens itself out. Once the adapter is level, stop the pump and remove the nut and washer.

Lift the adapter

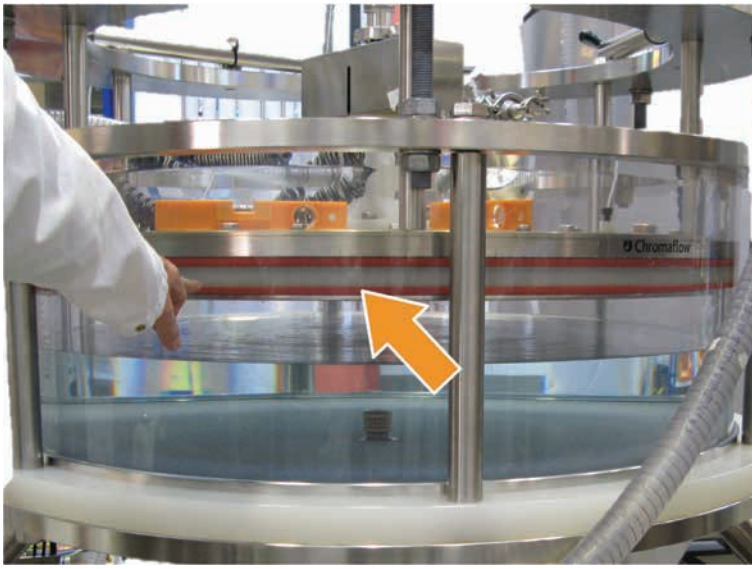
Step	Action
1	To provide additional safety during the following steps, position the lower nuts and washers equally spaced just below the lower face of the adapter flange on all adapter locking rods. The nuts (1) must remain in contact with the flange (2).



Step	Action
2	Pass three slings from an overhead crane or hoist through the three eyebolts on the adapter flange.

3	Carefully lift the the column just off the floor and then lower it again. This will position the center the column aligned with the hoist.

Step	Action
4	Raise the adapter further by packing the column with water very slowly until the lower O-ring is just clear of the upper edge of the tube (i.e. the column begins to leak). Then stop the pump.



Note:

Refer to the assembly drawing in the documentation package provided with the column for additional information on lifting.

5	Keep the hoist steady in a position where it holds the adapter and prevents it from tipping.
6	Set the top nozzle to **RUN**, then turn off the packing station.
7	Disconnect the multi-connector lines from the top nozzle (these are attached at the quick connection points). On automatic nozzles, disconnect all air supply lines and piping from the nozzle assembly.
8	Tension the slings using the hoist to begin to move the adapter slowly upwards. Continue the gradual upward movement of the adapter and carefully lift the adapter free from the column.

Step	Action
9	Mount the legs and bolts of the adapter legs provided with the column to the underside of the adapter. The adapter legs are mounted in the positions otherwise occupied by the locking rods. Make sure to use washers between the top of the adapter legs and the adapter flange. 
10	Gently lower the adapter, now fitted with adapter legs, to the floor to rest on the adapter legs.

6.5.2 Remove automatic nozzles and exchange O-rings

Introduction

Nozzles in a Chromaflow column are either automatic or manual. This section describes how to remove an automatic nozzle from a Chromaflow column, how to replace O-rings and how to refit the nozzle in the column again. There is an option of fitting a pre-assembled new nozzle tip unit to the Chromaflow nozzle. These can be ordered separately. Refer to the Spare Part List, or www.gelifesciences.com for further information.

Note: *When performing maintenance on nozzles it is recommended to use a table with an approximately 40 mm diameter hole or to use supports with a central gap of 40 mm (for example two support blocks that the nozzle is balanced on). This will allow you to place the nozzle assembly upright.*

Nozzle O-ring tool (option)

The replacement of nozzle tip O-rings can be performed by either:

1 fitting a preassembled new nozzle tip unit to the Chromaflow nozzle, or

2 removing and replacing the existing nozzle tip O-rings.

If the second method is chosen, an optional tool is available for expanding and fitting the new nozzle tip O-rings into place.

Remove an automatic nozzle

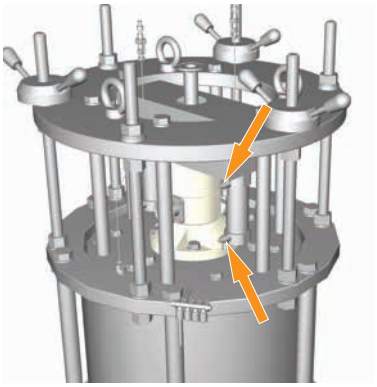
NOTICE

Before disassembling the nozzle unit, check that all associated air connections and piping are marked correctly - this will ensure proper re-connection.

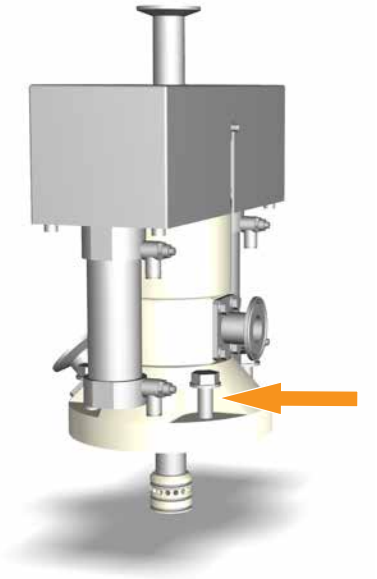
NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Set the nozzle you are servicing to **RUN**.
2	Make sure the unit controlling the automatic nozzle is set to **OFF**.
3	Disconnect the multi-connector lines from the nozzle attached at the quick connection points.
4	Disconnect all air supply lines and piping from the nozzle assembly.



Step	Action
5	Remove the four bolts and washers that secure the nozzle assembly to the column backing plate.



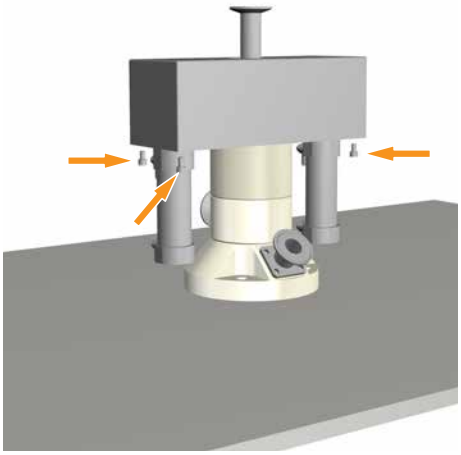
6	Carefully pull the entire nozzle assembly straight out.

Exchange automatic nozzle O-rings

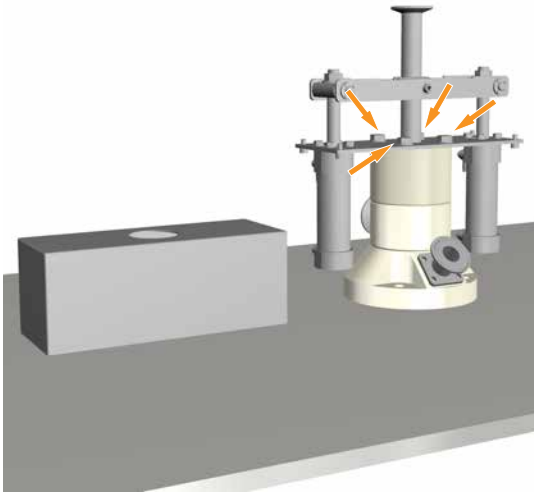
When exchanging the nozzle O-rings, it may be helpful to refer to the exploded view of nozzle parts in *Section 8.4 Exploded drawings: Automatic nozzle, on page 201*.

Step	Action
1	Unscrew the nozzle tip from the nozzle tube.

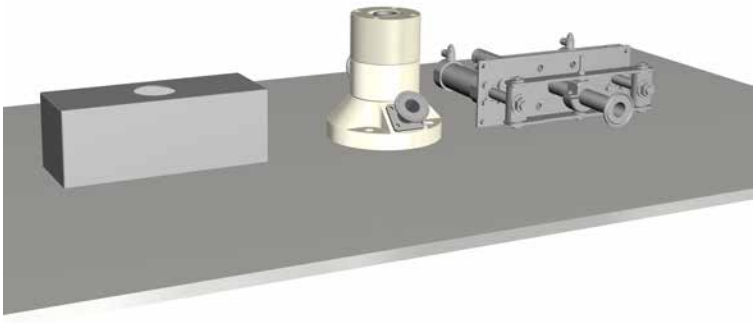
2	Place the nozzle assembly in the upright position on a table with a hole or on supporting blocks.
3	Remove the four screws securing the cover to the nozzle assembly.



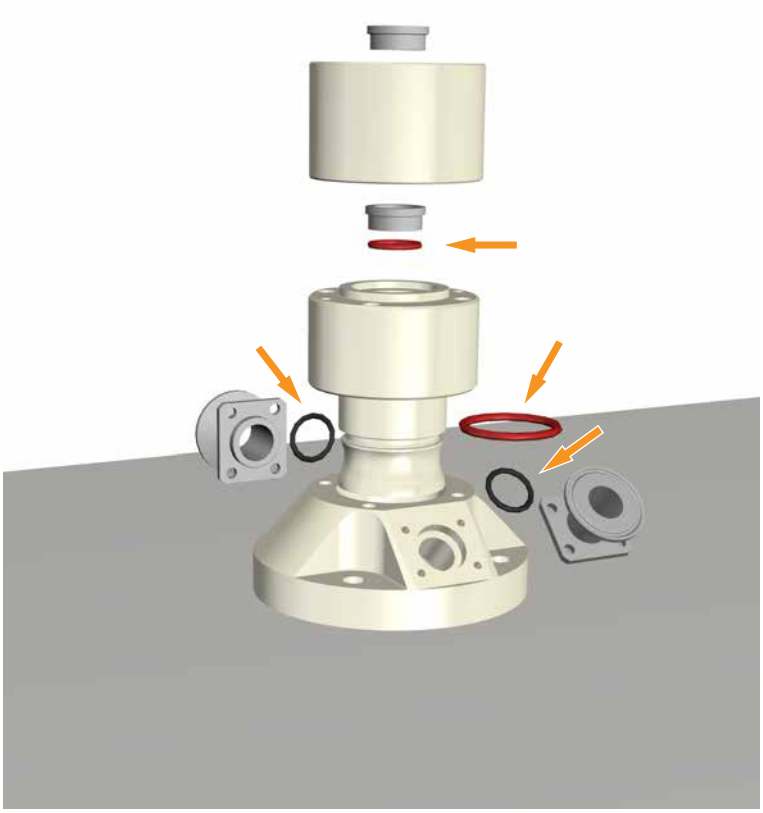
Step	Action
4	Remove the cover.
5	Remove the four bolts holding the mounting plate.



6	Lift and remove the mounting plate assembly,



Step	Action
7	Disassemble the remaining nozzle parts and remove the O-rings, as illustrated below, from the tube.



Step	Action
8	Exchange the nozzle O-rings using the assembly drawing (found in the documentation package) as a guide.

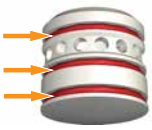
NOTICE

Visually inspect all O-rings for signs of handling damage before mounting.

NOTICE

Lubricate all O-rings and seals thoroughly with 20% ethanol.

9	Exchange the three seals on the nozzle tip.



10	Reassemble the nozzle in the reverse order of steps.

6.5.3 Remove manual nozzles and exchange O-rings

Introduction

Nozzles in a Chromaflow column are either automatic or manual. This section describes how to remove a manual nozzle from a Chromaflow column, how to exchange O-rings and how to refit the nozzle in the column again. There is an option of fitting a pre-assembled new nozzle tip unit to the Chromaflow nozzle. These can be ordered separately. Refer to the Spare Part List, or www.gelifesciences.com for further information.

Note: *When performing maintenance on nozzles it is recommended to use a table with an approximately 40 mm diameter hole or to use supports with a central gap of 40 mm (for example two support blocks that the nozzle is balanced on). This will allow you to place the nozzle assembly upright.*

Nozzle O-ring tool (option)

The replacement of nozzle tip O-rings can be performed by either:

1 fitting a preassembled new nozzle tip unit to the Chromaflow nozzle, or

2 removing and replacing the existing nozzle tip O-rings.

If the second method is chosen, an optional tool is available for expanding and fitting the new nozzle tip O-rings into place.

Remove a manual nozzle

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Set the nozzle you are servicing to **RUN**.
2	Loosen the 4 bolts attaching the nozzle to the adapter.

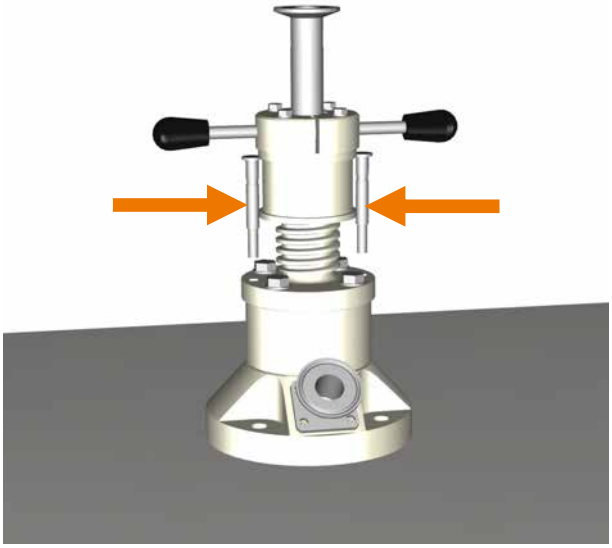
Step	Action
3	Carefully pull the nozzle straight up and out. 

Exchange manual nozzle O-rings

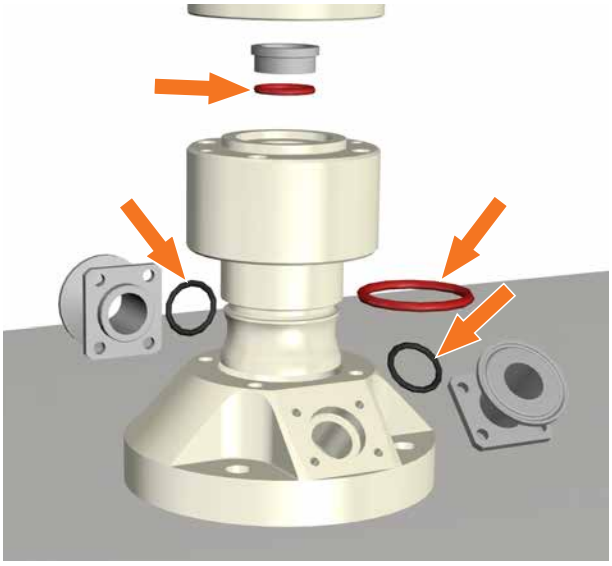
Follow the procedure below to exchange nozzle O-rings on manual nozzles.

Step	Action
1	Unscrew the nozzle tip from the nozzle tube.

2	Place the nozzle assembly in the upright position on a table with a hole or on supporting blocks.
3	Remove the nozzle posts (see below) and the 4 nozzle bolts and washers that secure the nozzle assembly and lift the assembly straight up.



Step	Action
4	Disassemble the remaining nozzle parts and remove the O-rings, as illustrated below, from the tube.



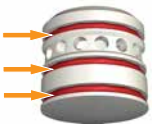
Step	Action
5	Exchange the nozzle O-rings using the assembly drawing (found in the documentation package) as a guide.

NOTICE

Visually inspect all O-rings for signs of handling damage before mounting.

NOTICE

Lubricate all O-rings and seals thoroughly with 20% ethanol.

Step	Action
6	Exchange the three seals on the nozzle tip. 
7	Reassemble the nozzle in the reverse order of steps.

6.5.4 Exchange adapter O-rings

Introduction

This section describes the general procedure for exchanging the adapter O-rings on Chromaflow columns.

Procedure

Follow the instructions below to exchange the adapter O-rings.

Step	Action
1	Remove the adapter as described in *Section 6.5.1 Remove the adapter, on page 132*.
2	Use a plastic spatula and pry both the O-rings from their grooves and remove them.

NOTICE

Do not to use a knife or other sharp-edged object as these may damage column components.

3	Inspect the O-ring grooves for damage and dirt. Exchange damaged or dirty O-rings. Clean and undamaged O-rings can be refitted.
4	Lubricate the O-rings with 20% ethanol and place them in their grooves. Make sure that the O-rings are not twisted.

CAUTION

Twisted or deformed O-rings will cause leakage.

6.5.5 Exchange plastic bed supports

Introduction

The bed support of Chromaflow columns may be made of plastic or stainless steel. This section describes how to exchange plastic bed supports.

Refer to the exploded view diagrams in *Section 8.3 Exploded drawings: Adapter, bottom distributor and bed support, on page 199* for general information when exchanging bed support units.

CAUTION

When working with 20% v/v 1-propanol solutions follow these instructions:

- Work in a well-ventilated area.
- Wear appropriate personal protective equipment.
- Keep storage containers tightly closed.

NOTICE

When performing maintenance it is recommended to exchange the plastic bed supports.

Wet the new bed support

Note: *It is recommended to start to wet new plastic bed supports the day ahead of the exchange.*

Step	Action
1	Carefully open a 20 to 30 cm hole in the plastic bag containing the new bed support.
2	Pour 1-propanol on the new bed support inside the plastic bag until it covers the bed support.
3	Make sure the liquid is evenly spread over both sides of the bed support, then seal the the plastic bag.
4	Leave the bag for at least 2 hours, but preferably overnight.

Remove adapter distributor

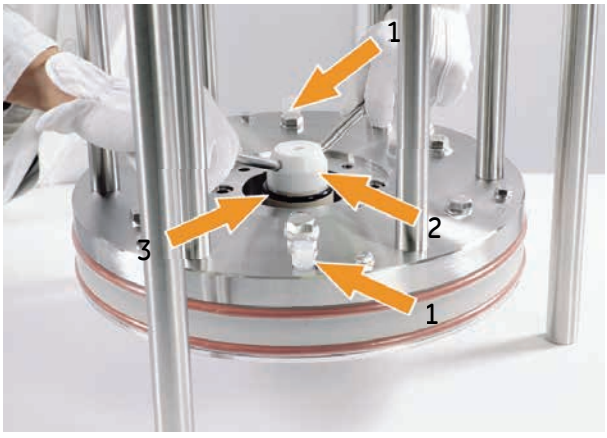
NOTICE

At least two people are required to perform this procedure.

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Remove the adapter as described, see *Section 6.5.1 Remove the adapter, on page 132*.
2	Remove the nozzle as described, see *Section 6.5.2 Remove automatic nozzles and exchange O-rings, on page 139* or *Section 6.5.3 Remove manual nozzles and exchange O-rings, on page 146*.
3	Remove the flush extension tubes (1), then remove the bed retainer nut (2) and the distance ring (3).



Tip:

Use the nut fixation tool provided in the bed support spare and accessory kit to remove the bed retainer nut. This will prevent the nut from rotating.

Step	Action
4	Remove the bolts and washers holding the bed support to the distributor plate. When removing the last bolts, make sure that the bed support is not dropped.
5	Carefully remove the adapter distributor and place it upside down on a clean surface. ***Note:*** *On Chromaflow 800 and 1000, position the adapter over a suitable flat surface that will support the bed support when it is lowered.* **NOTICE** Always make sure that the surface is a particle-free protective surface, to avoid damaging the components.

Remove and exchange bed support

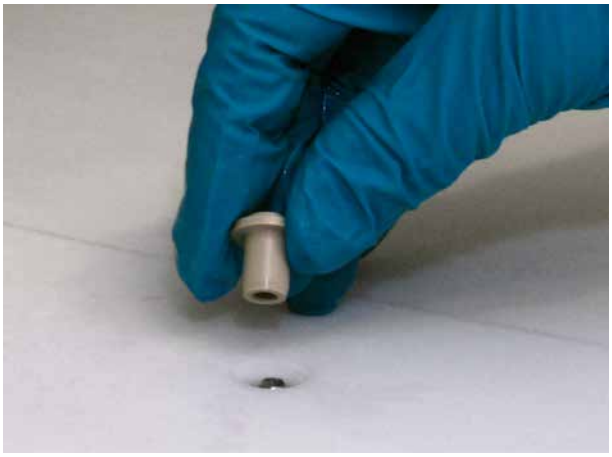
For this procedure it is recommended to be at least two persons.

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

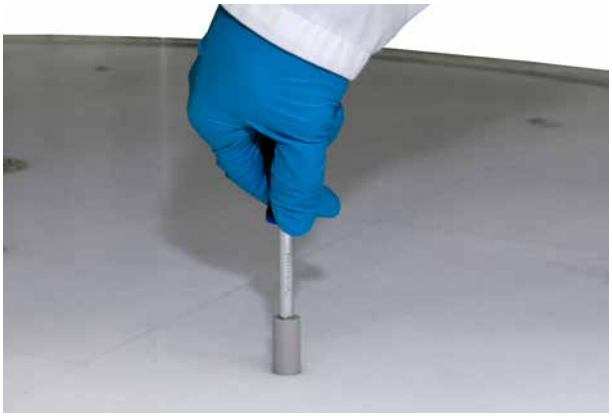
Step	Action
1	Make sure the bed supports are properly wetted according to the instructions in *Wet the new bed support, on page 153*.

Step	Action
2	On Chromaflow 800 and 1000 columns the plastic bed supports are held in with additional bed support nuts. Remove the bed support nuts.



3	With one person holding down the distributor, the other person can firmly remove the current bed support by placing a hand in the center hole and pulling upwards.
4	To insert the new bed support, bend the bed support on one edge and fit it into the distributor, working slowly around until you meet stiff resistance.

Step	Action
5	One person presses down on the portion of the bed support that is not seated, while the other person pulls up on the center of the bed support. The unseated portion of the bed support should then slide into place.
6	On Chromaflow 800 and 1000, make sure to align the holes for the bed support nuts. Then refit the bed support nuts using three or four turns at this stage. Finally, tighten the bed support nuts to a torque of 1.8 Nm.



Refit the adapter distributor

For this procedure it is recommended to be at least two persons.

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Step	Action
1	Refit the adapter O-rings.
2	Lift the adapter up and fit the bolts by hand to seat the adapter into place.

Step	Action
3	Center the distributor plate to the backing plate by placing the distance ring and the two flushing extension tubes in place.

4	Use a torque wrench to tighten the inner bolts, working around the backing plate tightening diametrically opposite pairs of bolts sequentially. Gradually tighten the bolts in increments until the recommended torque setting specified on the assembly drawing (AD) for the column is reached.
5	Remove the distance ring.
6	Use a torque wrench to tighten the outer bolts, using the same technique as for inner bolts. Refer to the AD for recommended torque settings.

NOTICE

Always recheck bolt tensions after the column has been pressurized and retighten to recommended bolt torque settings when necessary.

7	If applicable, refit the bed retainer screw.

Step	Action
8	Fit new O-rings to the bed retainer nut.

NOTICE

Lubricate all O-rings and seals thoroughly with 20% ethanol.

9	Use the bed retainer nut tool to screw in the bed retainer nut until it bottoms-out in the middle of the bed support.
10	Fit new O-rings to the distance ring and push the ring down into the adapter backing plate.
11	Refit the nozzle assembly.

The adapter is now ready to put back into the column.

6.5.6 Exchange the adapter stainless steel bed support

Introduction

This section describes how to exchange stainless steel bed supports on Chromaflow columns. Note that stainless steel bed supports can be cleaned and reused. This instruction is suitable for both new or cleaned stainless steel bed supports.

CAUTION

Handle stainless steel parts with care and use only stainless steel tools. Using inappropriate tools will cause damage to surfaces and may lead to leakage and corrosion.

NOTICE

Do not use sharp metallic tools that may scratch the stainless steel surface. If scratched, the stainless steel should be re-passivated prior to use. Contact GE for additional information

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

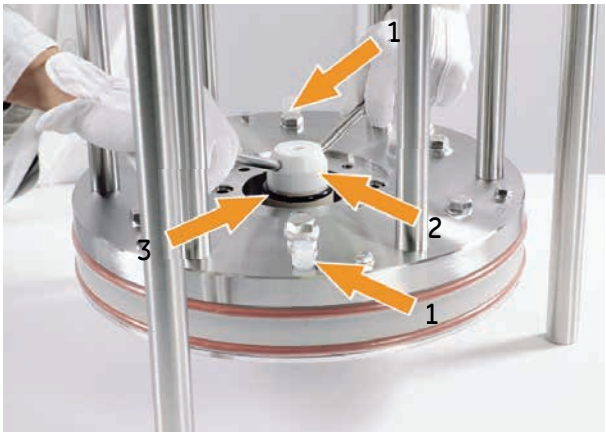
Tools required

The following tools are required to replace stainless steel bed supports on Chromaflow columns:

- 13 mm open torque wrench (calibrated)
- Bed support retainer nut fixation tool
- Table or other suitable clean surface

Procedure

Refer to the exploded view diagrams in *Section 8.3 Exploded drawings: Adapter, bottom distributor and bed support, on page 199* when replacing stainless steel bed support units.

Step	Action
1	Remove the adapter as described *Section 6.5.1 Remove the adapter, on page 132*.
2	Remove the nozzle as described *Section 6.5.2 Remove automatic nozzles and exchange O-rings, on page 139* or *Section 6.5.3 Remove manual nozzles and exchange O-rings, on page 146*.
3	Remove the flushing port extension tubes (1), then remove the bed retainer nut (2) and the distance ring (3).  ***Tip:*** *Use the bed support retainer nut fixation tool provided in the bed support spare and accessory kit to remove the bed retainer nut. This will prevent the nut from rotating.*
4	Remove the bolts and washers holding the bed support to the distributor plate. When removing the last bolts, make sure that the bed support cannot drop.

Step	Action
	Carefully remove the adapter distributor and place it upside down on a clean, protective surface.
	Note: *On Chromaflow 800 and 1000, position the adapter over a suitable flat surface that will support the bed support when it is lowered.*
	NOTICE Always make sure that the surface is a particle-free protective surface, to avoid damaging the components.
5	Exchange the O-rings listed in the Spare Parts List in the documentation package provided with the column.
6	Attach an O-ring to the welded bed retaining screw, then attach the new bed support unit.

Step	Action
7	Lift the bed support assembly into place and screw in the bolts and washers by hand. See *Fit bed support retaining screws, on page 164* for the detailed procedure of mounting the bed support retaining screws.

8	Using a stainless steel torque wrench, tighten diametrically opposite bolts in ¼-turn increments to the recommended torque setting specified on the assembly drawing (AD) for the column.
9	Use the nut fixation tool to screw in the bed retainer nut.
10	Fit new O-rings to the distance ring and push the ring down into the adapter backing plate.
11	Refit the nozzle assembly.

The bed support assembly is now ready to put back into the column.

Fit bed support retaining screws

The following tools are required to mount the bed support retaining screws to Chromaflow columns with stainless steel bed supports.

- PTFE tape
- Stainless steel 13 mm open torque wrench (calibrated)
- Stainless steel ring spanner or socket spanner for M8 bolts

Follow the instructions below to mount and tighten the adapter unit bed support retaining screws when fitting steel bed supports to Chromaflow columns.

Step	Action
1	Wrap the threads of each of the bed support retaining screws (M8 bolts) with PTFE tape, as shown in the illustration. ***Note:*** *Make sure that the tape extends far enough up the bolt so that there is PTFE tape between all metal-to-metal contact surfaces of the bolt when it is in place.* 
2	Fit the new bed support assembly to the adapter backing plate, being careful to align the screw holes.

Step	Action
3	Mount the bed support retaining screws, washers and nuts, into the bed support retaining screw holes, as shown in the illustration, and fingertighten the screws.

Step	Action
4	Screw down the nut on each of the bed support retaining screws.

Step	Action
5	Hold the top of the bed support retaining screw in place with a ring spanner or socket spanner, and then slowly tighten the nut on each of the screws using a calibrated open torque wrench, as shown in the illustration.

6	Work around the adapter crosswise and gradually tighten diametrically opposite pairs of screws in turn to the recommended torque setting specified on the assembly drawing (AD) for the column.
7	Remove the excess PTFE tape from the thread of the bed support retaining screw, until no tape can be seen on the screw thread above the nut.

6.5.7 Exchange the bottom unit O-rings and plastic bed support

Introduction

This section describes how to replace the bottom unit O-rings on Chromaflow columns with plastic bed supports.

Refer to the exploded view diagrams in *Section 8.3 Exploded drawings: Adapter, bottom distributor and bed support, on page 199* when replacing the bottom O-rings bed support unit.

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

NOTICE

Extreme care must be taken to protect the bottom surface of the column tube, as this is a sealing area and must be kept clean and scratch free.

Procedure

Note: *Make sure there is PTFE tape on the threads of all bolts before tightening. This will stop the threads from binding.*

Step	Action
1	Remove the adapter as described *Section 6.5.1 Remove the adapter, on page 132*.
2	Empty the column of water or buffer.
3	Remove the bolts and washers on the top column flange. Then remove the top column flange.
4	Remove the tie bars. Then carefully remove the column tube and place it on a soft, clean surface.

Step	Action
5	Remove the nozzle and, if applicable, the bed support nuts. ***Note:*** *Bed support nuts are only fitted on Chromaflow 800 and 1000 columns.*
6	Using the special tool, remove the bed retainer nut and then remove the distance ring.
7	Remove the bed support, then remove the O-ring in the distributor and replace with a new O-ring.

NOTICE

Lubricate O-rings thoroughly with 20% ethanol.

8	Place a new bed support assembly on the bottom distributor. ***Note:*** *On Chromaflow 800 and 1000, also refit the bed support nuts.*
9	Screw in the bed support retaining nut and then the distance ring. ***Note:*** *On Chromaflow 800 and 1000 columns, also tighten the bed support nuts with a torque wrench to the recommended torque setting specified on the assembly drawing (AD) for the column.*
10	Refit the nozzle assembly.

NOTICE

Make sure the bottom of the tube is clean and flawless.

11	Carefully lower the column tube onto the bed support assembly, centering it as best as possible.
12	Refit the tie bars and their associated bolts and washers.
13	Very carefully position the column flange onto the tie bars and refit the bolts and washers. Do not tighten at this point. Make sure that the column tube and the column flange are properly aligned.

Step	Action
14	Begin by tightening the bolts on the bottom of the tie bars by hand. Then use a stainless steel torque wrench to slowly tighten all the bolts in turn, turning diametrically opposite bolts in ¼-turn increments until the recommended torque setting specified on the assembly drawing (AD) for the column is reached.
15	Once the bolts at the bottom of the tie bars are secure continue with the top bolts of the tie bars. Use the same procedure as for the bottom bolts.

6.5.8 Exchange the bottom unit O-rings and stainless steel bed support

Introduction

This section explains what to do when performing these maintenance procedures on Chromaflow columns with stainless steel bed supports.

Refer to the exploded view diagrams in *Section 8.3 Exploded drawings: Adapter, bottom distributor and bed support, on page 199* when exchanging the bottom O-rings bed support unit.

NOTICE

Extreme care must be taken to protect the bottom surface of the column tube, as this is a sealing area and must be kept clean and scratch free.

NOTICE

To retain sanitary surfaces, it is recommended that clean, disposable gloves are worn whenever working with the column, and especially whenever replacing O-rings or working with internal column components.

Remove the stainless steel bed support

Step	Action
1	Remove the adapter according to the appropriate instructions for your Chromaflow column.
2	Empty the column of water or buffer.
3	Remove the bolts and washers on the top column flange, then remove the flange.
4	Remove the tie bars and then carefully remove the column tube and place it on a soft, clean surface.
5	Remove the bottom nozzle.

Step	Action
	Use the bed support retainer nut fixation tool to remove the bed retainer nut and then remove the distance ring.
6	Unscrew and remove the bed support retaining screws holding the bed support in place, then carefully lift the bed support assembly and set it aside on a clean surface.

Note:

Chromaflow 800 and 1000 are fitted with threaded holes on the top outer surface of the bed support ring to allow eye bolts to be screwed in, to simplify the lifting procedure.

NOTICE

Always make sure that the surface is a particle-free protective surface, to avoid damaging the components.

Remove O-rings and fit new or cleaned bed support

Note: *Make sure there is PTFE tape on the threads of all bolts before tightening. This will stop the threads from binding.*

Step	Action
1	Remove the O-ring from the bottom distributor plate and exchange it for a new, lubricated O-ring.

NOTICE

Lubricate O-rings thoroughly with 20% ethanol.

2 Place a new O-ring on the bed retainer screw.

3 Place a new (or a cleaned) bed support assembly on the adapter backing plate, being careful to align the screw holes with the ring. Screw in the bed support retaining screws.

4 Exchange all O-rings on the bed support retainer nut and the distance ring, then refit the bed retainer nut (using the nut fixation tool) and the distance ring.

5 Refit the nozzle assembly.

On Chromaflow 800 and 1000, and columns equipped with automatic nozzles, reconnect the air supply lines and any necessary pipe work.

6 Refit the column tube O-ring, ensuring that it is not twisted.

NOTICE

Make sure the bottom of the tube is clean and flawless.

7 Carefully lower the column tube onto the bed support assembly, centering it as much as possible.

Step	Action
8	Refit the tie bars and their associated nuts and washers. Do not tighten.
9	Very carefully position the column flange onto the tie bars and replace the associated bolts and washers. Do not tighten.
10	Beginning with the bolts on the bottom of the tie bars, use a torque wrench and slowly tighten all of the bolts in turn. Work sequentially around the column, tightening diametrically opposite pairs of bolts in ¼-turn increments until the recommended torque setting specified on the assembly drawing (AD) for the column is reached.
11	Once the bottom bolts are secure, tighten the bolts at the top of the tie bars.

6.5.9 Refit the adapter

Introduction

This section describes how to refit the adapter after completing service and maintenance procedures.

Refer to the exploded view diagrams in *Section 8.3 Exploded drawings: Adapter, bottom distributor and bed support, on page 199* when refitting the adapter.

NOTICE

Refitting the adapter assembly into the column requires the column to be filled with liquid.

NOTICE

Make sure that the adapter is horizontal and perpendicular to the tube. Take great care that the steel bed support edge does not contact the acrylic tube.

Procedure

Step	Action
1	If the column is not filled with water, fill it to approximately 5 cm of the top of the column tube.
2	Lubricate the adapter O-rings and/or column walls with 20% ethanol.
3	Lift the adapter using the slings and hoist as described in *Section 6.5.1 Remove the adapter, on page 132*.
4	Remove the three legs on which the adapter stood and, using the hoist, carefully move the adapter to the column tube.
5	Lower the adapter very carefully over the tie bars and into the column tube.

Step	Action
6	On Chromaflow 800 and 1000, the top nozzle may have been removed when removing the adapter. To replace the top nozzle, first lower the adapter into the column until the first seal is engaged with the column wall and then support the weight of the adapter on nuts and washers from the underside of the adapter flange. Remove the hoist and fit the nozzle. At this point, do not fit any air lines or extra piping to the top nozzle. Open **MPT**.
7	Make sure the nozzles are set to **RUN** during assembly.
8	Refit all the clamp nuts, and washers onto the locking rods.
	NOTICE Do not fully tighten the extender rod nuts until the extender rods have been fitted to the adapter.
9	Tighten down each in turn, diametrically opposite, until the first adapter O-ring is engaged. To ensure alignment, measure the gap between the column and adapter flanges at diametrically opposite points. Adjust the nuts and handles accordingly until the gap is uniform. Use a steel rule or vernier caliper.
10	On Chromaflow 800 and 1000, and columns with automatic nozzles, reconnect any air supply lines and external piping.
11	Follow the instructions for moving the adapter down that are appropriate for your type of Chromaflow column.

When maintenance procedures have been completed and the adapter refitted a leakage test should be performed to validate the integrity of the column. Refer to *Section 5.2.7 Perform a leakage test, on page 97* for instructions.

Following successful completion of the hydrostatic pressure test the column is fully assembled and ready to be prepared as described in *Section 5.2 Prepare the column, on page 66*.

6.6 Storage

Introduction

This section describes how to store Chromaflow columns and the preparation necessary for storage of columns that have previously been used in production and for continued storage of new Chromaflow columns.

The instructions vary depending on the type of bed support. Plastic bed supports need to be adequately wetted throughout the procedures.

NOTICE

The column must be unpacked from the crate and washed as soon as possible after delivery, preferably within 2 months. Storage temperature must be 4°C to 24°C.

Storage conditions

The following conditions shall be maintained while the column is in storage:

- Store the column indoors in a dust-free environment with a well-controlled climate.
- Maintain the storage area at a constant temperature, between 4°C to 24°C.
- Make sure the relative humidity is between 0% to 95%, non-condensing (preferably low humidity).
- Keep air humidity and air temperature differences as low as possible to prevent condensation and corrosion.
- Acrylic tube columns must be protected from sunlight, and other sources of UV light.
- Do not expose the column to chlorides.
- Change the storage solution regularly, at least once every three months.

Before storage

The following procedure describes how to prepare the column for long-term storage. The procedure provides that the column is set up with associated equipment as instructed in *Section 4.3 Set up associated equipment, on page 55*.

Step	Action
1	Clean the column according to your established cleaning procedure (for example using 1.0 M NaOH).

NOTICE

Make sure that the column is free of Cl residue before storage. Otherwise there is a risk of corrosion of applicable steel components (column tube and bed supports).

2	Prime the column with a suitable storage solution, for example 18% EtOH samt 2% IPA or 0.01 M NaOH. Refer to *Section 8.2 Chemical resistance, on page 195* for more information.
3	Move both nozzles to **RUN**.
4	Rinse and disconnect tubing.
5	Fit blind caps to nozzle port inlets and outlets.

Prepare a column without media for storage

Follow this procedure to prepare a column without packed media for storage.

NOTICE

Chlorides and low pH can cause corrosion of stainless steel. Rinse thoroughly with clean water after use.

Step	Action
1	Clean the column according to your established cleaning procedure (for example using 1.0 M NaOH). Refer to *Section 8.2 Chemical resistance, on page 195* for more information.

Step	Action
2	Prime the column with a suitable storage solution (for example 18% EtOH samt 2% IPA or 0.01 M NaOH).
3	Open the pathway to **SIT**.
4	Make sure that top and bottom nozzles are set to **RUN**.
5	Start the packing pump to clean the tubing pathways to **SIT** via **SOT** and to slurry.
6	Disconnect the tubing and fit blind caps to nozzle port inlets and outlets.
7	Exchange the liquid in the chamber between the adapter seals by priming the flushing ports. See *Prime the flushing ports, on page 82* for details.

Prepare a column with packed media for storage

A chromatography system should be used to fill a packed column with storage solution to provide low enough flow rate not to disturb the packed bed. Refer to the equipment's user documentation for instructions.

NOTICE

Chlorides and low pH can cause corrosion of stainless steel. Rinse thoroughly with clean water after use.

Prepare a new column for further storage

Prepare a new column for further storage

A new column can be stored as delivered for up to 3 months in recommended storage conditions. After this period, or earlier if the recommended storage conditions cannot be met, the storage solution needs replacing if the column shall be stored longer.

When replacing storage solution, it is important to use a procedure that will prevent air from being introduced into the bed supports, especially in columns with plastic bed supports.

WARNING

Hazardous substances and biological agents. When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of Chromaflow 400-1000 columns.

WARNING

For personal safety during the transportation, installation, operation, maintenance and service of columns, use protective glasses and other personal protective equipment appropriate for the current application at all times. The following personal protective equipment should always be available:

- Protective glasses
- Working gloves to protect against sharp edges
- Protective footwear, preferably with steel toe-cap
- Disposable gloves

Always use clean disposable gloves when manually handling parts.

NOTICE

Do not strain nozzle connections and be careful when connecting piping. Failure to do so may result in damage.

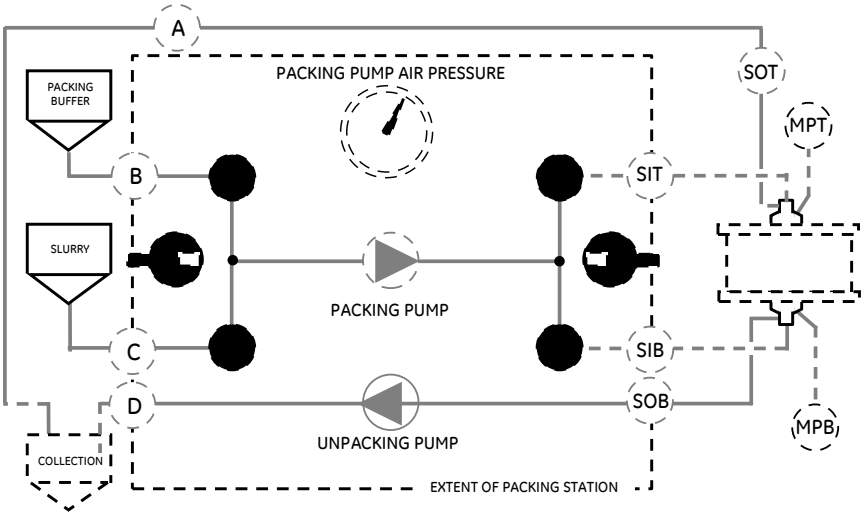
NOTICE

It is recommended to exchange the storage solution regularly, at least every 3 months.

Prerequisites

- The column is equipped with isolating valves on **MPT** and **MPB** and a pressure gauge on the **MPT** before the isolating valve.
- The column is connected to a packing station or similar equipment. Refer to *Section 5.2.2 Connect a packing station to the column, on page 71* for details.

- The column is connected to a vessel via pathway B containing suitable storage solution (see the illustration below).
- The column is collected to a collection vessel via pathway D (see the illustration below).
- The tubing A is not submerged in the collection vessel.



Empty slurry lines of air

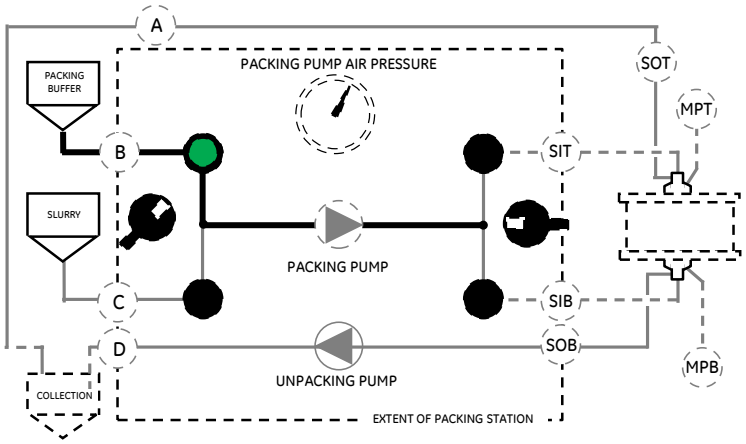
NOTICE

It is important to make sure that no air is introduced into the column via any of the nozzle ports to keep the wetted plastic bed supports adequately hydrophilic.

Follow this procedure to first empty the slurry lines of air.

Step	Action
1	Close the **MPT** and **MPB** ports.
2	Set both top and bottom nozzles to **RUN**.
3	Open the pathway to **SIT**.
4	Start the packing pump. Pump until liquid is flowing out through **SOT**. *Result:* Liquid is pumped through the top nozzle and back to the collection vessel and removes any air in the lines.

Step	Action
5	Stop the pump and open the pathway to **SIB**.
6	Start the packing pump. Pump until liquid is flowing out through **SOB**. *Result:* Liquid is pumped through the bottom nozzle and back to the collection vessel and removes any air in the lines.
7	Close the pathway to the column. On the packing station this is done by switching the **SIT**/**SIB** control to neutral (the mid position). See the illustration below.



8	Stop the pump.

Replace storage solution

Follow this procedure to replace the storage solution. Make sure the packing buffer vessel contains the relevant replacement solution.

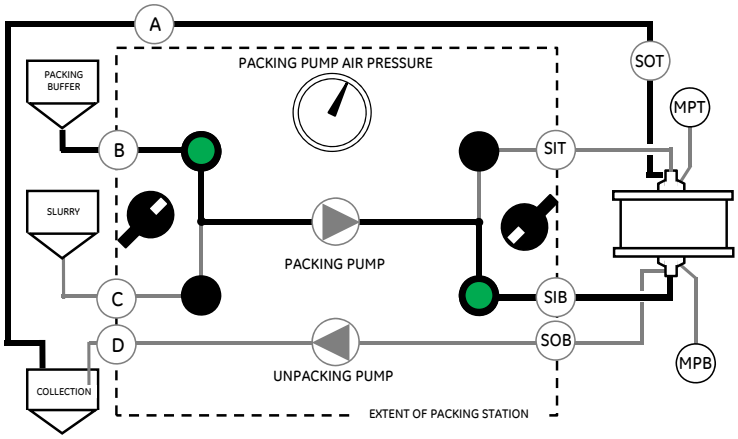
NOTICE

It is important to make sure that no air is introduced into the column via any of the nozzle ports to keep the wetted plastic bed supports adequately hydrophilic.

NOTICE

Make sure that the end of the tube connection **A** remains above the level of the liquid in the collection tank at all times.

Step	Action
1	Make sure the **MPT** and **MPB** ports are closed.
2	Set the top and bottom nozzle to **UNPACK**.
3	Start the unpacking pump carefully and start to empty the column of liquid via **SOB** into the collection tank. When the column is empty, stop the unpacking pump.
4	Set the bottom nozzle to **PACK**.
5	Open the pathway **B** from the packing buffer and open the pathway to **SIB**.



6	Start the packing pump. Pump until the column is filled and fluid is flowing back into the vessel, then stop the pump. *Result:* Liquid is pumped through the bottom nozzle (upflow) until all air is evacuated from the column via **SOT**.
7	Set the top nozzle to **RUN**.
8	Start the packing pump.

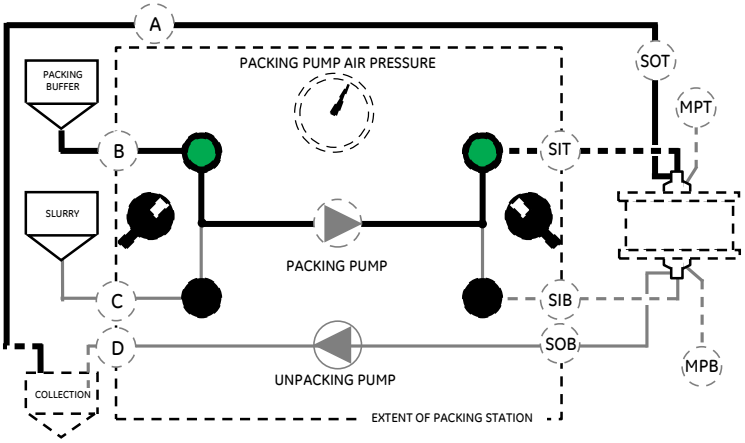
Step	Action
9	Allow the packing pump to build up 1 bar g pressure. **NOTICE** Make sure to carefully monitor the pressure on the pressure gauge.
10	Quickly open the **MPT** to drain, then close it again. *Result:* This quick release of pressure and fast flow through the bed support will purge the the air via the column top nozzle.
11	Repeat the previous two steps (steps 9 to 10) at least three times or until no more air can be noticed in the outlet when the **MPT** port is opened.
12	Allow the packing pump to build up 1 bar g pressure again.
13	Quickly open the **MPB** port to drain, then close it again. *Result:* This quick release of pressure and fast flow through the bed support will purge the the air via the column bottom nozzle.
14	Repeat the previous two steps (12 to 13 at least three times or until no more air can be noticed in the outlet when the **MPT** port is opened.
15	Stop the packing pump and set both top and bottom nozzles to **RUN**. *Result:* The column is sealed.
16	Proceed to rinse the slurry lines.

Rinse slurry lines

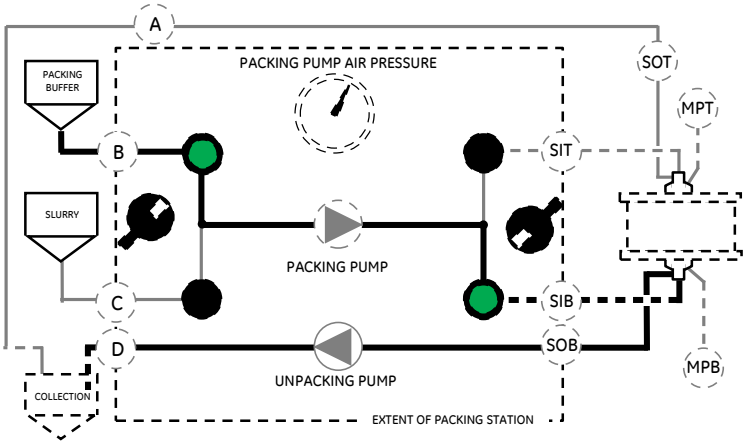
Follow the instructions to rinse the slurry lines and exchange the fluid in the flushing ports and adapter seal.

Step	Action
1	Open the pathway to a vessel containing water (in the illustration below this is the vessel labeled **PACKING BUFFER**), which will allow the tubing and piping to be cleaned from media.

Step	Action
2	Open the pathway to **SIT** to clean the tubing to and from the top nozzle.



3	Start the packing pump and pump until the tubing is clean.
4	Open the pathway to **SIB**, to clean the tubing to and from the bottom nozzle.



5	When the tubing is thoroughly rinsed, stop the packing pump.
6	Remove the tubing to the column and fit blind caps on the nozzle ports.
7	Exchange the liquid in the flushing ports and adapter seals. For instructions refer to *Prime the flushing ports, on page 82*.

The column is prepared for further storage.

7 Troubleshooting

Introduction

This chapter provides information to assist users and service personnel to identify and correct problems that may occur when operating Chromaflow columns.

The following guide presents a series of questions to direct the operator to the cause of the problem.

If the problem persists or cannot be resolved using this guide, contact your GE representative for advice.

High backpressure

- Is the pressure gauge showing correct values?
- Are all the valves between the pump and collection vessel open?
- Are all the valves clean and free from internal blockage?
- Has the correct valve for the flow rate been selected?
- Are the column bed supports blocked or clogged? Is the bed support properly cleaned of media? Air may be trapped in the bed support. Always wet/prime the bed supports before use.
- Is there any equipment in use, either before or after the column, which could generate back-pressure? For example, is an adequate size of flow cell being used?
- Are there differences in internal diameter between the tubing on the column, the tubing from the pump, or anywhere else in the system?
- Do the gaskets and tubing have the same internal diameter?
- Are the sample and collection vessels at approximately the same level as the pump or are they higher?
- Has the media been packed too to a greater compression factor than recommended?
- Could there be tightly bound sample material in the chromatography media? Perform CIP according to the procedure established for the media in use.

Leakage

- Are the connections to and from the pump correct and tightened?
- Is the adapter properly positioned with respect to the column tube?
- Is there media in the buffer return line? Check that the bed support is correctly fastened to the adapter/bottom unit. Check the bed support retaining apparatus in the center of the bed support.

Chromatograms not as expected

- Is the recorder speed and/or signal set correctly, or has the sensitivity been changed?
- Is the linear flow rate as intended?
- Is the adapter correctly positioned in close contact with the bed?
- Has back-mixing occurred anywhere in the column set-up? For example, could the sample have passed through an air trap?
- Is there a gap between the packed bed and the bed support? This may be due to the flow rate during the run being higher than the packing flow rate, the column may be too loosely packed, or the run pressure may be too high.
- Has the column been correctly equilibrated?
- Were all dead volumes correctly filled with appropriate buffers and sample before running?
- Has there been a change in how the sample has been handled prior to its application to the column? For example, are there sample batch variations? In the process, have any changes been made to the following: nets, separation media, time intervals between different operations, buffer constituents, precipitation procedures, chromatographic system hardware? Any such changes should be analyzed for their effect on the chromatogram.
- Consider the dilution, filtration, age, temperature and preparation of the sample buffers. Do all buffers comply with the defined specifications?
- Is the bed support clogged?
- Has the column bed properly cleaned?
- Does the packed bed have the wrong porosity?
- Is the media damaged?
- Is the column free from visible cracks and foreign materials? Smaller cracks, less than 5% of the bed height, may be present without affecting the packing performance.

Air trapped in the column

- Do the buffers have the same temperature as the column?
- Are all connections fitted together tightly?
- Are all closed valves tightly shut?
- If an air trap is used, is the air trap volume adequate?
- ***Note:*** *In general, if air has entered the column then the column should be re-packed and primed. If only a small amount of air has been trapped in the column, however, the air can be removed. Follow the instructions below to remove small amounts of air from the column.*

Step	*Action*
1	*Pump a solution that is a few degrees Celsius warmer than the media upwards through the column. Use the highest possible flow rate that the packed bed and chromatography skid allow.*
2	*Recycle the liquid and monitor the UV signal. If air is coming out of the column, the UV signal will be erratic. It will stabilize when the air is removed.*
3	*Test the column again before use.*

Adapter out of alignment

The most common factor contributing to the adapter moving out of alignment is insufficient lubrication of the O-rings. Follow the instructions below if the adapter is not level.

Step	Action
1	Stop the packing pump or inflow of water.
2	Lubricate the O-rings.
3	Fit a nut and washer to the clamp rod at the highest point on the adapter flange.
4	Start the pump and watch the adapter as it straightens itself out. Once the adapter is level, stop the pump and remove the nut and washer.

Note: *Some tipping of the adapter is acceptable and will normally even itself out.*

Pumps

This section outlines troubleshooting for pump related issues of Chromaflow Packing Station 50, 10, 200, and 400.

Problem	Possible cause/corrective action
Pump not working	• **EMERGENCY STOP** button has been pressed. • Air pressure too low. Check the pressure of air supply. Adjust if necessary. • No inlet or outlet valve open. Check valves. • Improper cleaning leaving media to sediment in the pump. • If none of the above, contact GE service personnel.
Little or no flow	• Check that connected inlet is actually used. • Insufficient pressure and volume of air supplied to the packing station thus starving the pumps. Increase pressure and air. • Inlet containers are placed too low relative to the pump. Check inlet containers. • No liquid is supplied to the pump. Make sure that the inlet containers are placed as close to Chromaflow Packing Station as possible and on the same floor of the building. • Tubing from inlet container causes pressure or flow loss. Reasons may be too long tubing, too small internal diameter, tube may have a narrow section or is partly plugged. • Malfunctioning valve at container battery limit. • Improper cleaning leaving media to sediment in the pump

Problem	Possible cause/corrective action
Too high outlet pressure	• Check that connected outlet is actually used. • Outlet containers are placed too high relative to the pump. Make sure that the outlet containers are placed as close to Chromaflow Packing Station as possible and on the same floor of the building. • Tubing to outlet container causes pressure or flow loss. Reasons may be too long tubing, to small internal diameter, tube may have a narrow section or is partly plugged. Check also for non-functioning valve(s). • Malfunctioning valve at container battery limit. [1]

1 Battery limit is used to indicate a delimitation point between Chromaflow and the customer process equipment. Battery limits denote the points where functional responsability is handed over from the plant to the instrument or *vice versa*.

Valves

This section outlines troubleshooting for valve related issues of Chromaflow Packing Station 50, 10, 200, and 400.

Problem	Possible cause/corrective action
Chromaflow Packing Station does not function at start	• Valves might not be working. Check that compressed air supply is sufficient. • Reduce the pump setting and press the **START/RESET** button.
Flow not as desired	Check the status of inlet and outlet valves and also that the pathway is open.

Bed supports

This section outlines troubleshooting for issues related of the bed supports.

Problem	Possible cause/corrective action
The plastic bed support released from the distributor.	• The bed support is blocked or partially blocked. This can result in an increased pressure behind the bed support and increase the load on the bed support. • The bed support is subjected to high unsupported flow. A higher flow means a higher pressure drop across the bed support and therefore an increased load. • The column is badly or loosely packed. This will manifest itself as a gap between the packed bed and the bed support resulti in unsupported flow. • The bed support is subjected to rapid pressure or flow changes. This pulsation causes flexing of the stainless steel bed supports and the structure will eventually fail. The corrective action is to exchange the bed support.
Serious deformation or breakage occurred at the center of the stainless bed support.	

Compressed air

This section outlines troubleshooting for compressed air related issues of Chromaflow Packing Station 50, 10, 200, and 400.

Problem	Possible cause/corrective action
Compressed air failure during a run	Insufficient air pressure caused by, for instance, malfunctioning air supply equipment or a leaking air hose. Rectify the air supply problem.

8 Reference information

About this chapter

This chapter contains technical data pertaining to Chromaflow 400-1000 columns and components. Exploded diagrams are also provided for reference purposes. More detailed information and specifications for individual columns can be found in the documentation package provided with your column.

In this chapter

This chapter contains the following sections:

8.1 Technical specifications

Introduction

This section contains a short summary of general technical specifications, requirements and storage conditions for Chromaflow columns. Note that the specifications for individual columns may differ. Specific information for each column can be found in the documentation package provided with your column.

General technical specifications

Specification	Columns
Dimensions	See documentation package
Net weight	See documentation package
Compressed air requirements	N/A
Air consumption	N/A
Operating temperature range	4°C to 30°C
Safety standards	See *Section 1.3 Regulatory information, on page 10*
Design pressure	See documentation package
Test pressure (ASME)	1.3 × design pressure
Test pressure (PED)	1.43 × design pressure
Maximum pressure	See documentation package
Maximum altitude	2000 m
Storage temperature range	4°C to 24°C

8.2 Chemical resistance

Introduction

This section provides guideline information about the use or avoidance of various chemicals in relation to Chromaflow 400-1000 columns.

CAUTION

Make sure that any water used with the column is particulate-free (down to 1 µm). Particles can block and damage the bed supports.

Chemical resistance of Chromaflow columns

Resistance to buffers

Chromaflow columns are resistant to chemical agents used in protein recovery, including buffer solutions for adsorption, elution and washing, and to solutions effective in cleaning, sanitization and storage.

Resistance to sodium chloride

Depending on configuration, Chromaflow columns can be equipped with wetted components of stainless steel and must therefore be appropriately maintained when exposed to sodium chloride (NaCl) during chromatographic processes.

Stainless steel bed supports have a high degree of resistance to corrosion but may corrode under certain conditions, such as extended exposure to NaCl. The degree of susceptibility varies according to conditions of use. Low pH in combination with NaCl, high temperatures and damage to the steel surface all increase the risk of corrosion.

If a stainless steel surface is damaged (scratches, impact marks etc.) it must be cleaned and passivated before being taken into operation. Refer to *Application Note 28-9433-77 Handling of stainless steel column parts in sensitive environments*. Your GE representative can provide guidance on suitable cleaning and passivation procedures if required.

It is recommended that a water rinse of at least five column volumes is used to remove NaCl from stainless steel surfaces. Water is the preferred rinse solution due to the solubility properties of sodium chloride in water compared to other possible rinsing solutions (such as ethanol solutions).

Columns should be stored with solutions free from chloride ions.

Handling of stainless steel columns

For further information refer to *Application note 28-9433-77 Handling of stainelss steel column parts in sensitive environments*.

Chemicals to avoid

When using Chromaflow columns it is recommended that the following chemicals are avoided:

- Extreme oxidizers
- Fluorine and halogenated compounds
- Chlorinated solvents (such as methylene chloride)
- Esters
- Aromatic hydrocarbons (such as toluene)
- Alcohols at concentrations higher than those specified in the table below
- Chloride solutions in combination with pH below 4. Always wash the column with at least five column volumes of a pH-neutral solution (water) between and after use of chloride solutions and low pH buffers.

NOTICE

Ethanol >20% v/v and other chemicals not recommended in the chemical resistance table may cause cracks in the acrylic materials of Chromaflow 400-1000 columns and must not be used.

Chemical resistance guidelines

WARNING

Some of the chemicals used with Chromaflow columns may be flammable under certain conditions. Make sure to use chemicals only under conditions where they are not flammable. Refer to local and/or national classifications of flammable liquids.

CAUTION

Do not use chemicals harmful to the column.

CAUTION

Do not use chemicals with temperatures above the specified limits.

NOTICE

Chlorides and low pH can cause corrosion on stainless steel. Rinse thoroughly with clean water after use.

Inspect the column regularly for signs of corrosive attacks, which may cause column damage if untreated. Note that the stainless steel bed supports are especially vulnerable to corrosion.

Note: *The following table presents a summary of information obtained from several published sources, not from individual tests on column components. It should be used only as a guide. The effect of chemicals will generally be more severe at higher temperatures. Note also that the combined effects of agents have not been taken into account in this table. The applicable chemical resistance depends on the configuration of the column and the associated materials of construction.*

The recommended temperature range for the use of each of the chemicals listed below is 4°C to 30°C, unless otherwise specified.

Chemical	Concentration [1]	Time/cycle restrictions	Comments	CAS no. [2]
1-propanol		16 h	For wetting plastic bed supports	71-23-8
Acetic acid	25%	3 h	Cleaning-In-Place (CIP)	64-19-7
Acetone	2%	1 h	Efficiency test	67-64-1
Ammonium sulphate	2 M [3]	5 h	Adsorption	7783-20-2
Benzyl alcohol	2%	12 months	Storage at room temperature	100-51-6
Ethanol	20%	2 months and max. 0.5 bar	Storage in 4°C to 24°C	64-17-5
Ethanol	70% [4]	3 h	CIP	64-17-5
Ethanol/ acetic acid	20%/ 10%	3 h	CIP	64-17-5/ 64-19-7
Guanidine hydrochloride	6 M	5 h	CIP	50-01-1
Hydrochloric acid	0.1 M (pH = 1) [5]	1 h	CIP	7647-01-0

Chemical	Concentration [1]	Time/cycle restrictions	Comments	CAS no. [2]
Isopropanol	30%	1 h	CIP	67-63-0
Phosphoric acid	5%	8 h	For passivation of stainless steel bed supports	7664-38-2
Sodium chloride	0 to 3 M [6]	3 h	Purification, CIP	7647-14-5
Sodium hydroxide	1 M (pH = 14)	24 h, room temp. to 30°C	CIP	1310-73-2
Sodium hydroxide	0.01 M (pH = 12)	12 months	Storage	1310-73-2
Sodium hydroxide/ ethanol	1 M/ 20%	3 h	CIP	1310-73-2/ 64-17-5
Sodium sulphate	1 M [3]	3 h	Adsorption	7757-82-6
Urea	8 M [3]	5 h	Purification, CIP	57-13-6
Commonly used aqueous buffers for chromatographic use	10 to 250 mM, pH 2 to 10	24 h	Equilibration, adsorption, elution	

1 When a concentration is stated as a percentage, this is v/v.

2 CAS no.: Registration number assigned by the Chemical Abstract Services (CAS), American Chemical Society.

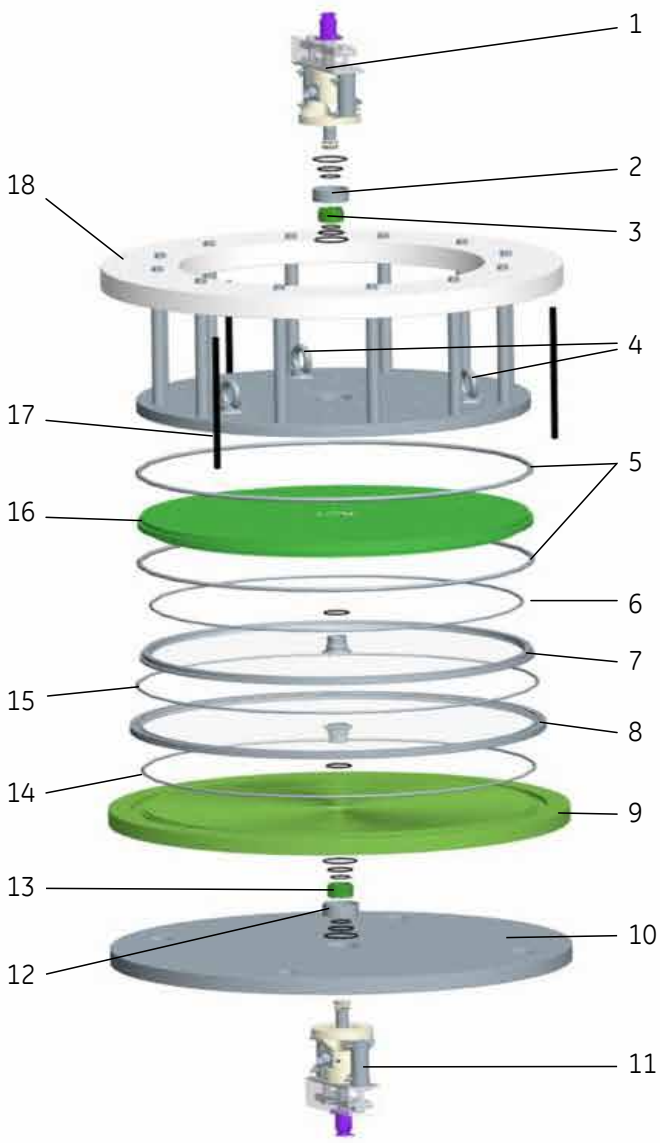
3 The pH in these solutions depends on the pH of the buffer, which can vary between 3 to 13.

4 On stainless steel parts only. Exposure of other column parts to ethanol at higher concentrations than 20% can damage the column.

5 A pH below 4 is not recommended for stainless steel.

6 For columns containing wetted stainless steel components a maximum concentration of 1.0 M NaCl is recommended. Refer to the Resistance to sodium chloride section above for further information.

8.3 Exploded drawings: Adapter, bottom distributor and bed support



Parts

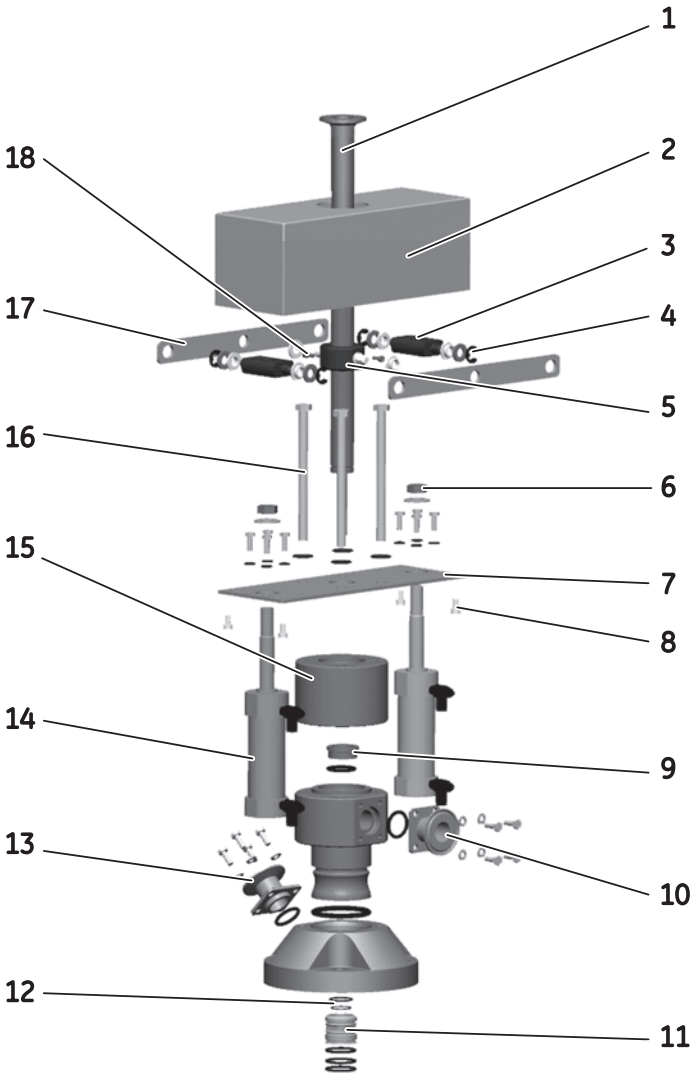
Part	Description
1	Top nozzle (automatic) [1]
2	Upper distance ring
3	Upper bed retainer nut
4	Lifting eye(s)
5	O-ring above top and below distributor plate (red)
6	O-ring between bed support and end cell [1, 2]
7	Upper bed support [1, 2]
8	Lower bed support [1, 2]
9	Bottom distributor plate
10	Bottom back plate
11	Bottom nozzle (automatic) [1]
12	Lower distance ring
13	Lower bed retainer nut
14	O-ring above distributor
15	O-ring below bed support [1, 2]
16	Top distributor plate
17	Locking rod(s)
18	Adapter flange

1 Not part of adapter

2 Bed support part

8.4 Exploded drawings: Automatic nozzle

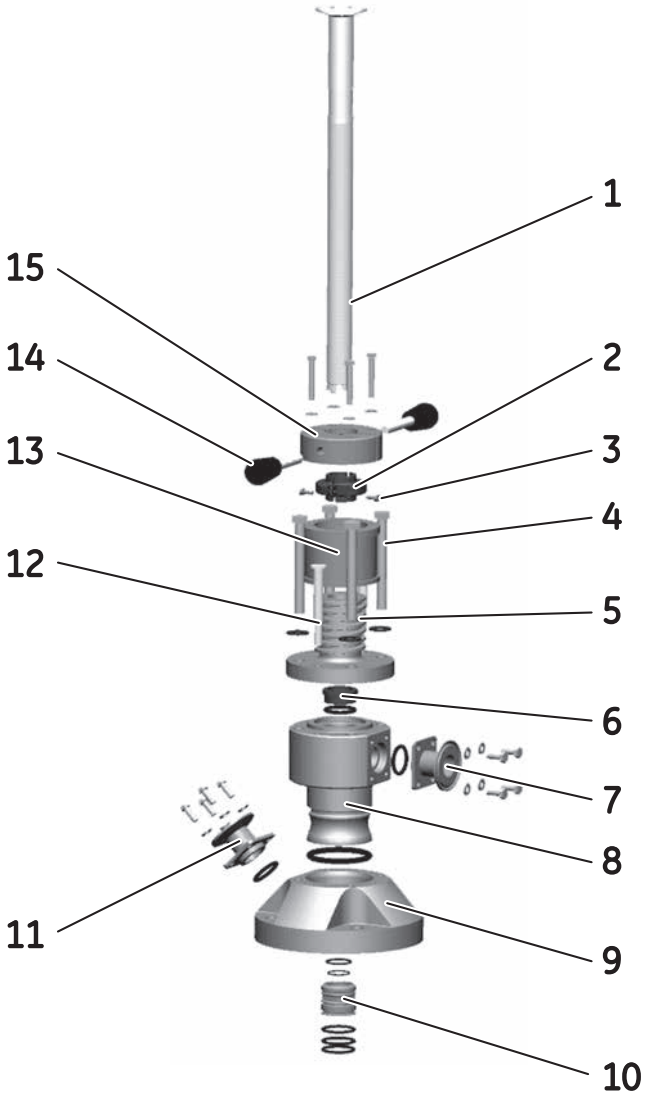
Automatic nozzle parts



Part	Description
1	**SIT**/**SIB** connection
2	Cover
3	Pivot block
4	Retaining ring
5	Block assembly
6	Rod end nut
7	Mounting plate
8	Head cap screw
9	Bushing
10	**MPT**/**MPB** connection
11	Nozzle tip
12	Inner pipe seal
13	**SOT**/**SOB** connection
14	Pneumatic cylinder
15	Spacer block
16	Nozzle bolts (4)
17	Pivot bar
18	Head cap screw

8.5 Exploded drawing: Manual nozzle

Manual nozzle parts



Part	Description
1	Nozzle tube/**SIT**/**SIB** connection
2	Tube connector
3	Head cap screw
4	Nozzle bolts (4)
5	Collar tube
6	Bushing
7	**MPT**/**MPB** connection
8	Inner nozzle body
9	Outer nozzle body
10	Nozzle tip
11	**SOT**/**SOB** connection
12	Nozzle post
13	Collar sleeve
14	Handle
15	Split collar

8.6 Health and Safety Declaration Forms

Checklist before planned maintenance/service

To ensure the protection and safety of service personnel, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts maintenance work.

If the system is going to be serviced on site, complete the checklist in the document: *On Site Service Health & Safety Declaration Form*.

If the system is going to be returned for service, complete the checklist in the document: *Health & Safety Declaration Form for Product Return or Servicing*.

Copy the document you need from the following pages.

On Site Service Health & Safety Declaration Form

Service Ticket #:	

To make the mutual protection and safety of GE service personnel and our customers, all equipment and work areas must be clean and free of any hazardous contaminants before a Service Engineer starts a repair. To avoid delays in the servicing of your equipment, please complete this checklist and present it to the Service Engineer upon arrival. Equipment and/or work areas not sufficiently cleaned, accessible and safe for an engineer may lead to delays in servicing the equipment and could be subject to additional charges.

Yes	No	Please review the actions below and answer "Yes" or "No". Provide explanation for any "No" answers in box below.
		Instrument has been cleaned of hazardous substances. Please rinse tubing or piping, wipe down scanner surfaces, or otherwise ensure removal of any dangerous residue. Ensure the area around the instrument is clean. If radioactivity has been used, please perform a wipe test or other suitable survey.
		Adequate space and clearance is provided to allow safe access for instrument service, repair or installation. In some cases this may require customer to move equipment from normal operating location prior to GE arrival.
		Consumables, such as columns or gels, have been removed or isolated from the instrument and from any area that may impede access to the instrument.
		All buffer / waste vessels are labeled. Excess containers have been removed from the area to provide access.
Provide explanation for any "No" answers here:		

Equipment type / Product No:		**Serial No:**	

I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.

Name:		**Company or institution:**	
Position or job title:		**Date (YYYY/MM/DD):**	
Signed:			

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Health & Safety Declaration Form for Product Return or Servicing

Return authorization number:		*and/or* **Service Ticket/Request:**	

To make sure the mutual protection and safety of GE personnel, our customers, transportation personnel and our environment, all equipment must be clean and free of any hazardous contaminants before shipping to GE. To avoid delays in the processing of your equipment, please complete this checklist and include it with your return.

1. Please note that items will NOT be accepted for servicing or return without this form
2. Equipment which is not sufficiently cleaned prior to return to GE may lead to delays in servicing the equipment and could be subject to additional charges
3. Visible contamination will be assumed hazardous and additional cleaning and decontamination charges will be applied

Yes	No	Please specify if the equipment has been in contact with any of the following:	
		Radioactivity (please specify)	
		Infectious or hazardous biological substances (please specify)	
		Other Hazardous Chemicals (please specify)	

Equipment must be decontaminated prior to service / return. Please provide a telephone number where GE can contact you for additional information concerning the system / equipment.

Telephone No:		
Liquid and/or gas in equipment is:	☐	**Water**
	☐	**Ethanol**
	☐	**None, empty**
	☐	**Argon, Helium, Nitrogen**
	☐	**Liquid Nitrogen**
	Other, please specify	

Equipment type / Product No:		**Serial No:**	

I hereby confirm that the equipment specified above has been cleaned to remove any hazardous substances and that the area has been made safe and accessible.

Name:		**Company or institution:**	
Position or job title:		**Date (YYYY/MM/DD)**	
Signed:			

To receive a return authorization number or service number, please call local technical support or customer service.

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