Labotronics

USER MANUAL

High Performance Liquid Chromatography

LB-21HPLC

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Index

1. Safety Measures

Safety Operating Practices:

The three types of safety signals here are separated based on the degree of risk and injury:

Warning: There could be major harm or property and health damage if the warning signs and measures are not appropriately followed. The instrument and its surroundings are included in the property damage.

Caution: If the precautions and instructions on this notice are not followed, there could be minor harm or property and health. If the harm is minor, the patient does not require hospitalization. The instrument can be recovered with basic maintenance if there is only minor property damage.

Note: To guarantee the instrument operates at its best, the symbol is utilized wherever information is provided.

1.1 Precautions for usage

Warning: Liquid chromatography is the only application for LB-21HPLC. Never use it for anything else. This instrument does not have an explosion-proof feature unless otherwise instructed.

1.2 Ambient Conditions

Warning: It is advised that firework be prohibited and that the inside be thoroughly ventilated when using organic solvents. Additionally, a basin or eye wash station should be placed close by in case the organic solvent gets into the skin or eyes.

Note: Keep the device away from caustic gases, dusty areas, and strong magnetic fields to guarantee optimal performance. The worktable needs to be sufficiently sturdy and spacious. The ideal temperature range is between 10°C and 30°C, with some variation, and the ideal humidity range is 20% to 80%. Keep it out of direct sunlight and away from sources of heat or cold. The instrument should not be directly exposed to air conditioners or other devices.

1.3 Precautions for installation

Warning: Professionals should install the instrument according to the instructions precisely; ensure that the power socket voltage matches the power supply voltage shown on the instrument. Using the incorrect power voltage could lead to fire and hazard.

The pump should be connected to the power outlet using the accessory power cable. Do not use any other cable.

To avoid static electricity and electric leaks, make sure the line cord is plugged into a power outlet that is correctly grounded.

Caution: Because the instrument is so heavy, you should move it gently and keep an eye on your hands.

Note: The instrument should be connected carefully according to the directions. A communication issue could be caused by an incorrect connection.

1.4 Precautions for use

Warning: Avoid using the instrument in areas where there is or may be fire, heat, magnetic resources, or significant vibrations. It is forbidden to place combustible objects close by. To avoid the risk of negative pressure within the bottle, the cap of the bottle used to store the mobile phase should have a pore.

To keep the waste bottle from bursting when it is overfilled, there must be a space between the waste tubing and the cork. However, to stop dangerous solvents from evaporating, the distance should be small. Nevertheless, the garbage must be cleaned up right away.

Caution: Wear safety goggles, specialized lab coats, gloves, a mask, and other protective gear when working with organic solvents. If you inadvertently come into contact with a poisonous solvent, wash your body right away and seek medical attention at a hospital.

Note: Utilize solvents of HPLC grade or similar while making the mobile phase. If you want to avoid small particles scratching the plunger rod, seal ring, or obstructing the tubing, you should filter the eluent using a membrane filter (0.45μm). Additionally, you will need an online filter. Additionally, before utilizing any mobile phase, degas it thoroughly. This is a good way to avoid chromatogram noise and incorrect indication.

Rinse the entire pipe system in accordance with the manual's instructions before using it for the first time. The tube is likely to become blocked by direct use.

Prior to the sample test, make sure the system's tubing is filled with mobile phase and free of bubbles, as this will compromise the accuracy of the test findings.

Ensure you follow the manual's instructions when replacing an eluent with an insoluble one, such as a positive mobile phase (hexane) or reverse phase (methanol). Failure to do so could result in a severe tubing jam or even system paralysis.

Avoid using a mobile phase that contains halogen ions if your system has stainless steel tubing and fittings, since halogen ions are bad for stainless steel. If you are unable to avoid it, reduce the amount of content and immediately rinse the system with water after completing the analysis.

You should be aware of the following if your system contains peek tubing:

- **Do not use the following solvents:** Chloroform, dimethyl sulfoxide, acetone, tetrahydrofuran, concentrated sulfuric acid, nitric acid, dichloroacetic acid, dichloromethane, trichloromethane, etc. The PEEK material may become weaker and more brittle because of certain solvents. However, the short-term effects of using an acetone aqueous solution (less than 0.5%) on gradient performance are tolerable.
- The system pressure should be lower than the peak material's tolerance pressure when utilizing PEEK tubing to prevent bursts.
- PEEK tubing should have a bending radius greater than 10 mm so that it can bend organically during installation.
- A professional tubing cutter should be used to cut the PEEK tubing to smooth it out. Be mindful that the tube should be free of any cutting debris.

1.5 Repair, maintenance, and parts replacement

Warning: In the event of a leak or electric shock, please cut off the power before doing any repairs, maintenance, or replacing any parts.
When doing routine maintenance and repairs, the host cover does not need to be opened.

To lessen the electrostatic charge, you should routinely clear the dust off the power cord plug. After that, dry it before using it to prevent an electric shock.
Wipe the instrument with a dry cloth. To prevent deleting text or color on the panel, do not use alcohol or thinner.

1.6 Precautions for static electricity

Warning: Any spark or flame could result in a fire or explosion since the instrument may employ a lot of combustible, explosive organic reagents that could pollute the laboratory air. Avoid using the pump close to any hot or fire resources, and be mindful of electrostatics. Take these actions to lessen static electricity:

- Make the instrument grounded. It is very important, pay attention to it.
- Keep the atmosphere clean and maintain the right amount of indoor humidity (more than 65% can avoid static electricity).
- It is necessary to ground metal waste bottles that are externally conductive (no ground insulation). One end of the wire can be inserted into the liquid in the bottle, and the other end earthed when using a different material container.
- Replace a larger I.D. tubing when the flow of mobile phase is higher than usual.
- It is forbidden for people or items that contain static electricity to come into contact with the instruments.

1.7 Warning label information

For any device with this symbol, the user should refer to the instruction manual to prevent putting the equipment and the operator in danger.

Symbols	Descriptions
	If you are not completely educated and meet the requirements, do not operate outside the scope of prudence.
Warning	There may be casualties. If you have not fully comprehended and fulfilled the necessary conditions, do not act beyond the warning's scope.
Caution	There could be equipment damage or data loss. If you have not fully comprehended and met the requirements, do not operate beyond the area of prudence.
Note	Instrument failure and unsatisfactory experimental results could show up. Do not proceed beyond the note's scope unless you have comprehended and fulfilled all requirements.

2. Introduction

High Performance Liquid Chromatography LB-21HPLC delivers a flow rate range of 0.001 to 10.000 mL/min, ensuring stable and accurate liquid transfer. It operates within a temperature range of 0 to 40°C to maintain optimal functionality. It features stepper motor interpolation to enhance precision and consistency at low flow rates. Our HPLC system includes real-time pressure monitoring with high and low-pressure alarms for improved safety.

3. Features

- Dual plunger pump
- Long-term stability
- SPI communication system
- Microcontroller integration
- Vacuum fluorescent screen
- Compact design

4. Specifications

Model No.	LB-21HPLC
Flow Range	0.001 to 10.000 mL/min (step: 0.001 mL/min)
Temperature Range	0 to 40°C
Flow Accuracy	± 0.2% (1.0 mL/min, 8 ± 2 MPa, water, ambient temperature)
Flow Precision	RSD ≤ 0.075% (1.0 mL/min, 8 ± 2 MPa, water, ambient temperature)
Maximum Pressure	45 MPa (0.001 to 5.000 mL/min)
Pressure Accuracy	± 3% or ± 0.5 MPa
Pressure Pulsation	≤ 1.0%
Sealing	Pressure drop ≤ 1.5 MPa (40 MPa, 10 min)
Communication Mode	RS- 485
Power Consumption	80 W
Power Supply	AC 220 V, 50 Hz
Tray Packaging Dimension	580 × 470 × 370 mm
Flow Pump Dimension	420 × 260 × 160 mm
Flow Pump Packaging Dimension	600 × 440 × 380 mm
Detector Dimension	420 × 300 × 175 mm
Detector Packaging Dimension	600 × 440 × 380 mm
Tray Net Weight	6 kg
Flow Pump Net Weight	14 kg
Detector Net Weight	14 kg
Tray Gross Weight	7 kg
Flow Pump Gross Weight	15 kg
Detector Gross Weight	15 kg

5. Applications

High Performance Liquid Chromatography LB-21HPLC is used to analyze mixtures, detect impurities, and verify compound composition with high precision. It is widely used in pharmaceutical manufacturing and research laboratories for accurate analytical testing.

6. Instrument Introduction

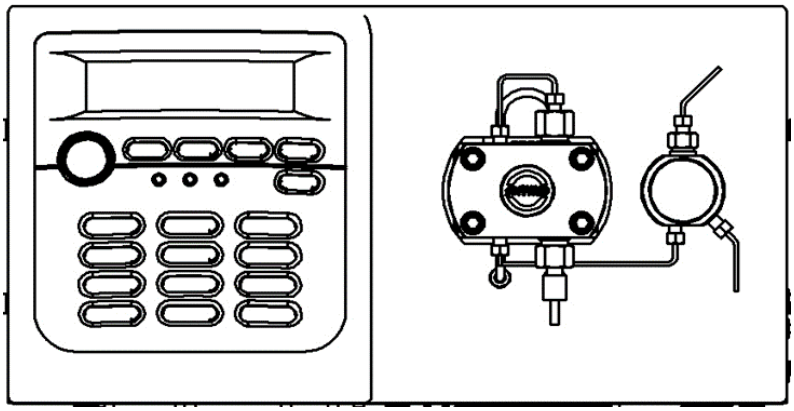

Figure-1 Front view of the instrument

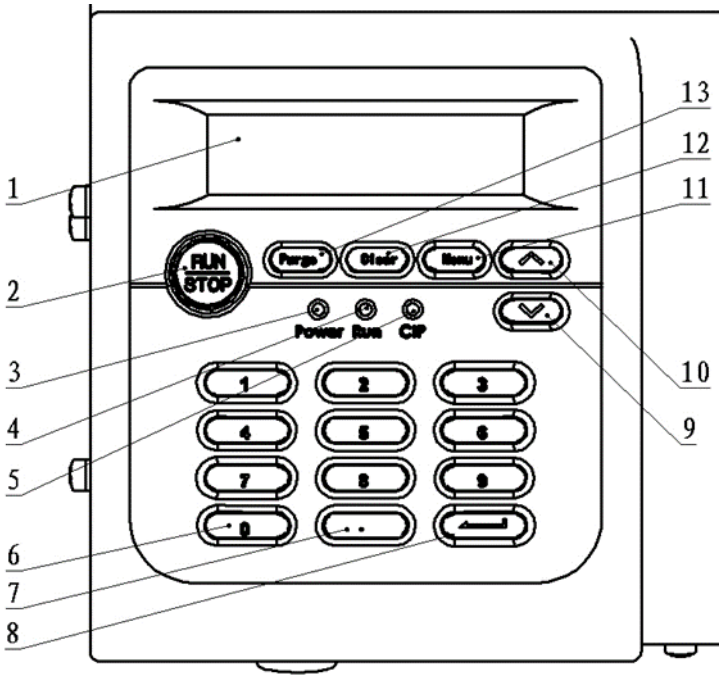

Figure-2 Keypad and LCD display

Table: Keypad Function

No.	Key	Function
1	**VFD-Display**	VFD displays operational status, menu, sub-menus, and parameters
2	**RUN/STOP**	**Local mode:** To start and stop the pump. Press once, and the pump indicator light will be on and the pump will operate according to a pre-set flow rate. **Remote mode:** Press the **RUN/STOP** key to pause the pump operation.
3	**POWER**	Power is on when the indicator is illuminated

4	**RUN**	When the run indicator is illuminated, the pump is in run mode
5	**CIP**	**0(Light is out):** The plunger rod will not be cleaned. **1(Light is green):** The plunger rod will always be cleaned. **2(Light is yellow):** The plunger rod will be cleaned for 2 minutes for 10 minutes.
6	**0 – 9**	Numerical keys
7	**.**	Decimal point
8	**ENTER**	To confirm values and selections. Upon pressing the **ENTER** key, the cursor will automatically move to the next parameter.
9	↓	Press ↓ the key to move between different menus and sub-menus.
10	↑	Press ↑ the key to move between different menus and sub-menus.
11	**MENU**	To access to function menu. Press the **MENU** key once, to access to the respective MENU1. Press again, to MENU2.
12	**CLEAR**	To delete wrong data input or to disarm an alarm.
13	**PURGE**	To flush the LB-21HPLC pump system at a high flow rate. It can be applied to alter the mobile phase or remove trapped air bubbles.

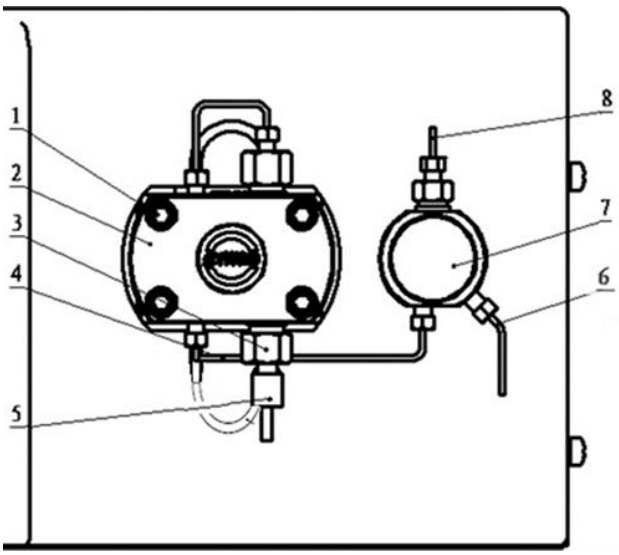

Figure-3 Fluid contact components

Table: Fluid contact components

No.	Component	Function
1	Mounting screws	To fasten and lock the pump head
2	Pump head	To draw and deliver the mobile phase
3	Check valve	Prevent backflow and allow the mobile phase to flow in one direction
4	Connecting tube	To connect the pump head with the relief valve
5	Inlet of the pump	Connect with the mobile phase pipe

6	Relief tube	Release air and waste liquid from the pump head
7	Relief valve	Loosen the relief valve knob to release pressure from the pump head. It can be used for purging and priming purposes
8	Outlet of the pump	Mobile phase comes out from here

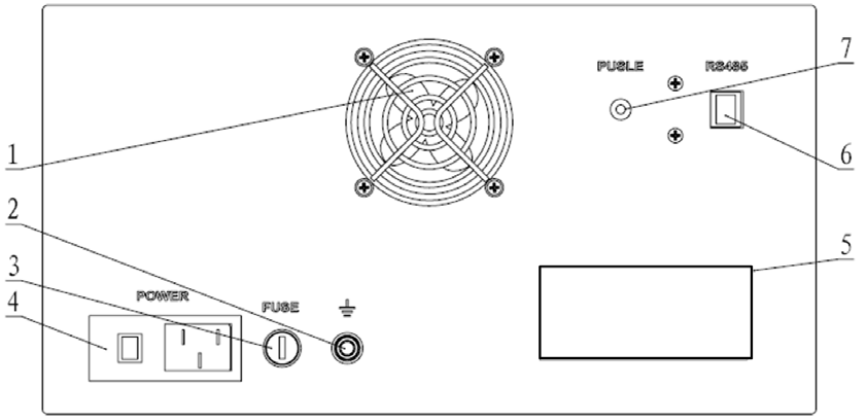

Figure-4 Rear panel

Table: Rear panel

No.	Components	Function
1	Cooling fan vent	Cool the instrument.
2	Ground terminal	Ground the main body of the pump.
3	Fuse	Fuse is in it
4	Power connector and Power switch	The power cable is connected to a grounded power outlet. Turn on / turn off the power.
5	Instrument panels	Identify the information about the instrument.
6	RS485 interface	This socket is connected to a detector or workstation for remote control.

7. Installation

7.1 Unpacking inspection

A foam-lined framework and corrugated cartons are used to package LB-21HPLC. Examine the instrument's packing as soon as you receive it to look for any damage.

Dismantling the packaging:

With the front of the packaging box facing up, place the pump on a level surface. Remove the pump and accessories packaging, cut the tape on top, and set them on the table. After that, open the instrument protective film and take off the foam.

Warning: It is advised that at least two individuals be present throughout the installation process due to the weight of the pump to keep the instrument from slipping.

7.2 Stack Order

It is advised that the instruments be stacked as seen in **Figure 5** and **Figure 6** to ensure the greatest possible functioning condition.

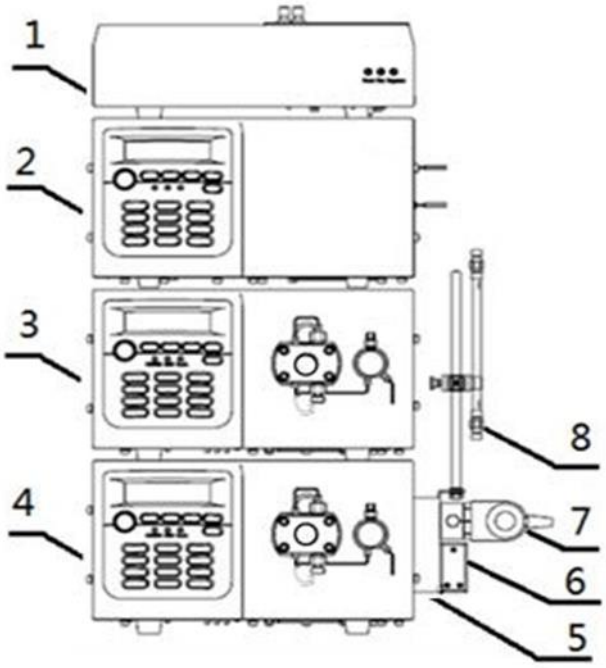

Figure-5 Stack order 1

1) Solvent manager
2) UV-Visible detector
3) Pump A
4) Pump B
5) Valve bracket
6) Mixer
7) Manual sampling valve
8) Column

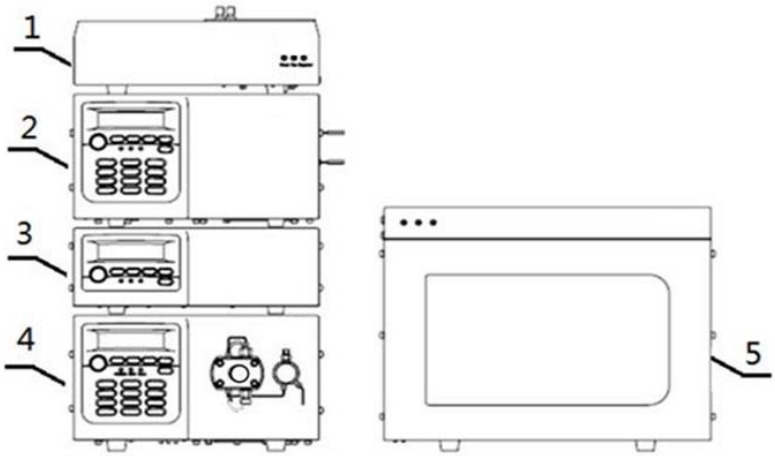

Figure-6 Stack order 2

1) Solvent manager
2) UV-visible detector
3) Pump A
4) Pump B
5) Auto-sampler

7.3 Installation requirements

7.3.1 Site requirements

1) Environment

LB-21HPLC needs to work under ambient conditions in the Table below:

Table: Environment requirements

Items	Specifications	Requirements
1	Work environment	The room should be free of dust, inflammable and explosive materials, also good ventilation is also important
2	Electromagnetic field	No electromagnetic noise nearby
3	Operating temperature	4-40°C (39-104°F)
4	Humidity	20%-80%, non-condensing

Caution: Avoid using the pump when there are temperature swings. Make the interior temperature rise gradually if the outside temperature is too low to prevent condensation from fast heating.

2) Bench space

Because of its size, the LB-21HPLC can be placed on nearly any lab bench. If you wish to have the entire 3100 system on show on the bench, ensure sure the table can support the weight of all the parts. It requires an extra 50 mm on the left, 150 mm on the right, and 150 mm on the back to allow for electrical connections and air circulation.

Warning: There is a chance of falling if the instruments are not positioned horizontally.

7.3.2 Power and power line

Ensure that the instrument may be used safely and routinely by using a dedicated power line that falls within the designated voltage range.

1) Grounding, AC power to 220V ±10%, 50 Hz.
2) Choose T1.25 A (250V) fuse.

Warning:

- The instrument should be connected to the power socket using the auxiliary power line. If the instrument is in danger or is damaged, other cables should not be utilized.
- The device may cause electrical shock or harm to staff and equipment if it is connected to a grid above its range of use.
- To prevent an electric shock, unplug the power cord before changing the fuse. The instrument's rear is where the external fuse is mounted.

7.3.3 Computer requirements

Hardware requirements:

- An Intel Core 2 CPU, 2 GB of internal storage, and more than 1 GB of hard drive space are the minimal hardware requirements.
- **The lowest resolution of display:** 1024×800,64K (16-bit image).
- **Others:** USB interface for communication, CD-ROM driver for software installation.
- Windows 7 or higher version

7.4 Fluid Connection System

7.4.1 Solvent pipeline system diagram

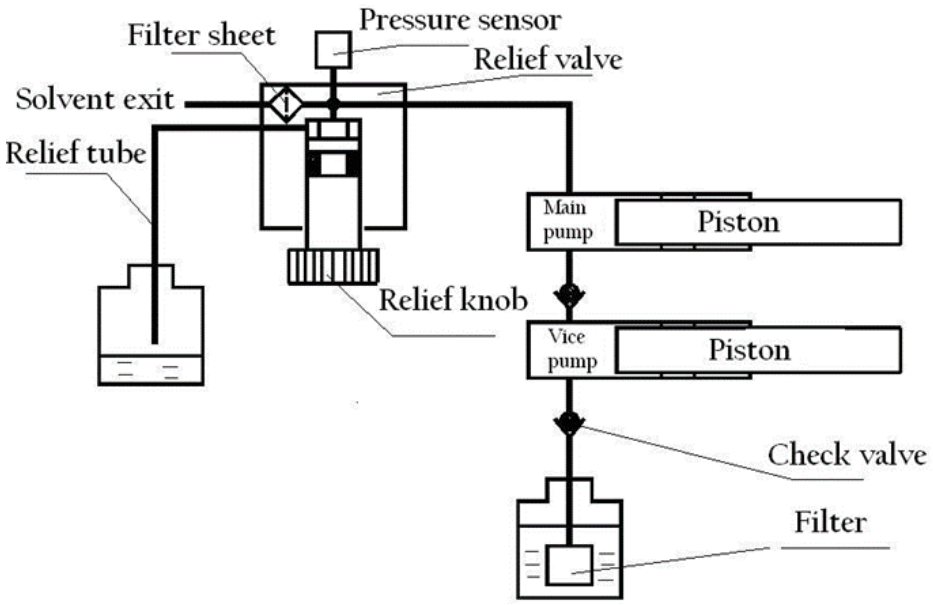

Figure-7 Fluid Connection Diagram

7.4.2 Installation Preparations

A solvent reservoir of at least 500 milliliters is required. The cap should have two holes that are 3–4 mm in diameter. The pump outlet us seal cap above the vent valve should be removed. Attach the O.D./16"× I.D.0.02" stainless steel tube to the pump port; the injection valve will be attached to the other end of the tube.

7.4.3 Connecting the solvent reservoir to the pump

Place the solvent tube within the solvent reservoir after attaching the solvent inlet filter to one end of the tube. The tube should be connected to the pump's inlet at the other end.

7.4.4 Connecting the injection valve to the pump

Use stainless steel tube (with a screw connection and a sealing edge ring) to connect the pump's output to the injection valve's inlet (the hole with the second number is typically the mobile phase's inlet).

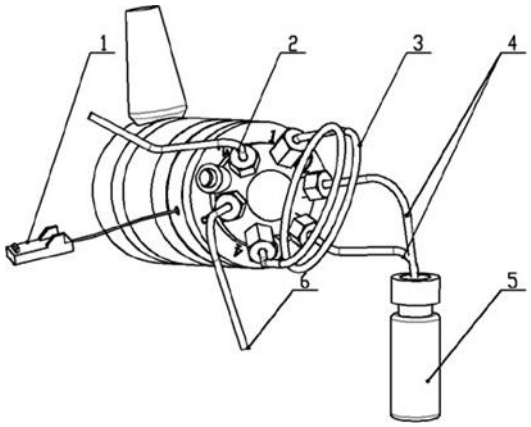

Figure-8 Fluid connection of manual filling valve

1) Automatic trigger interface
2) Mobile phase entrance
3) Quantitative loop
4) Waste tube
5) Waste bottle
6) Mobile phase export

Port #3 of the injection valve should be linked to the entry of the column.
The injection valve's port #2 must be attached to the pump's outlet.
The ferrule may be harmed if the connection screw is overtightened.
Replace the ferrule if there is a leak.
The pipe length between the injection valve and the column, as well as between the column outlet and the detector input, should be as small as feasible to minimize the loss of column efficiency (ID:0.007" is advised).
To lower the system's dead volume, ensure the ends of all the stainless steel tubes are smooth. It is advised that you connect the column inlet and outlet using the universal column connector.

7.4.5 Connecting the pump to the mixer

When there are multiple pumps in the pump system, such as in a gradient system, a mixer is necessary. Before the mobile phases enter the HPLC systems, the mixer makes sure they are well mixed after coming from various pumps.

The mixer is situated externally. The mixer's inlet is connected to the pump outlet via a stainless steel tube. Attach the outflow of the mixer to the injection valve's port #2.

An alternative mixer (mix volume: 1.5 mL, 2.5 mL, and 3.0 mL) can be selected based on the analysis.

7.4.6 Solvent waste bottle

The solvent waste bottle must be positioned lower than the apparatus.

7.5 Verification

In most cases, the instruments that consumers receive have been verified and tested. In the factory, the performance satisfied our needs. It is not necessary for users to test and confirm. Refer to the following procedures to confirm the pump's performance if you are unsure:

Check the sealing performance:

Step 1: Make sure the chromatographic column is correctly connected, turn on the pump, and check to see if the pressure shown is steady. Otherwise, please scrub the pump head's relief bubble or check valve.

Step 2: A maximum alert pressure of 40 MPa should be set. Shut off the injection valve's output.

Step 3: The pressure will gradually increase as the pump is turned on. Upon reaching 40 MPa, the pump will automatically shut off. Take note of the screen's pressure drop.

Step 4: Pressure drops less than 1.5MPa in 10min, sealing of the pump is qualified.

Step 5: When the pressure drops more than 1.5 MPa in 10 minutes, a number of things need to be taken into account, including air bubbles in the pump head or loose seals on the check valve, sampling valve, or pipe junction.

7.6 Transportation

Because the LB-21HPLC is a precision instrument, exercise caution when transporting it over long distances. The internal components of the instrument are prone to sustaining damage from severe vibration and drops. The instrument can be adequately protected by its original packaging. Use these packaging instructions if the instrument needs to be moved or returned for maintenance.

1) Turn off the power.
2) Unplug the power cord and RS-485.
3) Remove the connecting pipe and other elements between components.
4) Take the chromatography system's pump out. Place it on a large platform in a special sealed bag.
5) Put the system into the original packaging foam and fix it.
6) Place the fixed system and other accessories into the original packaging carefully.
7) Seal the box with tape to keep liquid out. It is advised to use plastic wrap to cover the packaging box.
8) Transport the packaged instrument.

8. Operations

8.1 Power ON

1) The constant current pump can only be turned on when the system is properly connected and its power source satisfies the instrument's specifications.

Caution: At this point, the power switch ought to be turned off (**"I"** denotes power on, and **"O"** denotes power off).

2) Turn on the power switch.
3) Power light is on, display screen is bright, and LB-21HPLC begins to self-test. The VFD display sequence is as follows:

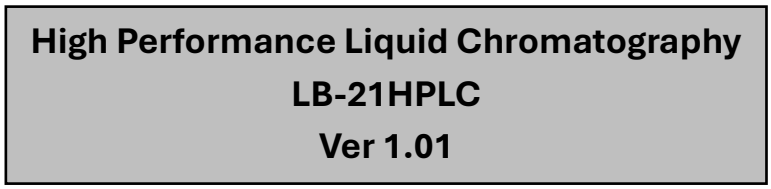

Figure-9

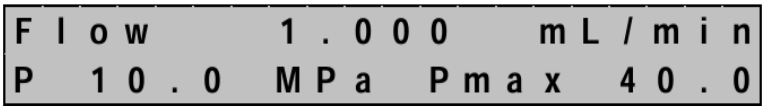

Figure-10

Caution: The options shown on the main screen are the default settings if you are using LB-21HPLC for the first time. Prior to the latest closure, all the pump's parameters were kept at their previous levels.

Caution: The default parameters of LB-21HPLC are listed as follows:
Flow: 1.000mL/min
***Pmax:** 40.0MPa
Pmin: 0MPa
Pressure unit: MPa

8.2 Menu 1: Basic Operation

In the main interface, you can modify the maximum alarm pressure and the flow rate. If you want to modify a parameter, first press the "↵" Enter key, and then press **"↓, ↑"** key to move the cursor to the parameter that needs to be modified. After typing the new parameter value, press "↵" button to confirm. Press the "**Run / Stop**" button directly, and the pump will run according to the set value. If you want to stop the pump, press the "**Run / Stop**" button again.
If the setting value of a flow rate is greater than the limit range, the pump will sound an alarm after confirmation and restore the value before the modification. Press the "**Menu**" button once to enter the "**MENU-1 Basic Operation**" functional state. You can move into the setting interface of the flow rate, the maximum limit of the pump pressure, and the minimum pressure by **"↑, ↓"** key.

You can modify them in the corresponding interface. You can type a new value directly when the cursor is flashing on the parameter you want to modify. Press "↵" button to confirm.

Figure-11

Caution: Flow rate range: 0.001-10.000mL/min

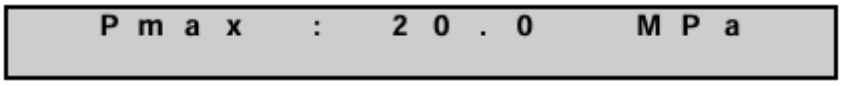

Figure-12

Caution:

Pressure limit set value:

Pmax: ≤45MPa (flow rate: 0.001-5.000mL/min)

Pmax: ≤20MPa (flow rate: 5.001-10.000mL/min)

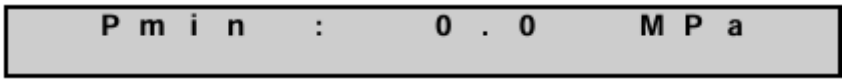

Figure-13

Caution: Real-time detection of the pump's work pressure is possible. The pump will sound an alert and cut off if the pressure drops below Pmin or reaches Pmax within a minute of starting. If the setting parameters for any of the parameters are outside of the parameters' scope, LB-21HPLC will reject them and keep the original ones.

After modifying the parameters in MENU-1, press **"Menu"** again to enter MENU-2. Press the **"Menu"** twice to return to the main screen.

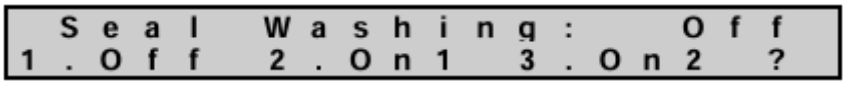

Figure-14

Caution:

The input number of the plunger rod washing menu indicates:

1(Light is out): Stop to clean the plunger rod

2(Light is green): Always clean the plunger rod

3(Light is yellow): Intermittently clean the plunger rod.

8.3 Advanced Operation

From the main interface, press the **MENU** button to access **"MENU-2 Advanced Operation**". Use ↑ and ↓ access the setting interface of various parameters in MENU2, including the coefficient of the mobile phase adjustment, the velocity correction factor, and the pressure unit setting. Key the new value in and press "↵" to alter the parameters.

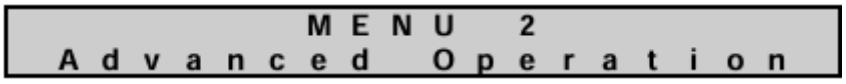

Figure-15

8.3.1 Setting LB-21HPLC A/B in Gradient system

Both the front panel and our workstation software allow you to operate the LB-21HPLC from a computer. To satisfy the needs of users in the production and research fields, the workstation has additional gradient analysis, flow time program analysis, and other features.

The setting of the A/B pump can be changed in "**MENU2 Advanced Operation**". Press the **"↑/↓"** button to enter the A/B pump select interface. Set it before analysis. The cursor flashes where it can be modified. Enter the correct number, then press "↵" button to confirm.

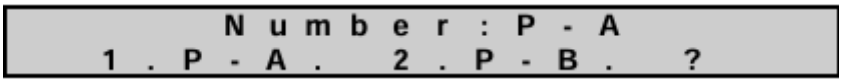

Figure-16

8.3.2 Flow compressibility

The compressibility properties of various solvents vary. Retention time will be impacted by the solvents' compressibility. LB-21HPLC offers a compressibility correction option that maximizes flow stability based on solvent type to reduce this effect. Using degassed distilled water as the mobile phase, the flow compressibility is initially set to 100% and can be adjusted via the user interface.

8.3.2.1 Measurement of the flow compressibility

Equipment: 10mL volumetric flask, stopwatch, degassed solvent.

Procedure:

1) Disconnect the column and eliminate the backpressure of the system.
2) Select a proper flow rate according to the analysis method. Press the "**RUN/STOP"** key to start the pump.
3) Start the stopwatch and begin to collect the mobile phase from the pump outlet in a 10mL volumetric flask.
4) Record the time it takes to reach 10mL, t.
5) Repeat steps (2) to (4) three times to get the average, t1.
6) Stop the pump and reconnect the column. Then repeat steps (2) to (5). Calculate the mean time with the column attached to the system, t2.

$$\text{Relative error} = (t2-t1)/t2\times 100\%$$

If the relative error is allowable, you do not need to change the compression coefficient. If the error is obvious, adjust the compression coefficient following the steps (**8.3.2.2 Adjusting the compressibility**).

8.3.2.2 Adjusting the compressibility parameter

Enter the "**MENU2 Advanced Operation**" to adjust the compressibility parameter, input a new compressibility parameter, and press " ↵ " to confirm.

Figure-17

Caution: The flow compressibility ranges from 85 to 115%. The cursor will blink, and the value will revert to 100% if the entered flow compressibility value is greater than the range.

8.3.3 Flow correction

The accuracy of the flow will be impacted by several issues, including a dirty block filter, a leaking seal, a dirty valve, and a clogged capillary tubing connection. Before adjusting the flow calibration, make sure to verify these regions.

The flow compressibility measurement and flow accuracy correction use the same methodology. You should modify the calibration parameter as follows if the flow accuracy exceeds the allowed standard:

Enter the "**MENU2 Advanced Operation**", press **"↑, ↓"** button to get into the flow correction interface. Then input a calibrated parameter and press " ↵ " to confirm.

Caution: The flow correction has a range of 85 to 115%. The cursor will blink, and the value will revert to 100% if the entered flow correction value is greater than the range.

8.3.4 Pressure unit

LB-21HPLC can display pressure in three different units: MPa, bar, and psi.

1 MPa = 10^6 Pa = 10 bar = 145.04 psi

Enter the "**MENU2 Advanced Operation**", press **"↑, ↓"** button to get into the unit interface. Then choose the unit you need and press " ↵ " to confirm.

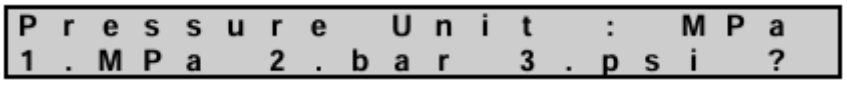

Figure-18

Caution: Select "1" for MPa, "2" for bar, and "3" for psi.

9. Maintenance

The HPLC system needs to be maintained and repaired to function normally. Simple repairs are the definition of maintenance. These repairs can be performed from the pump's front panel. Removing the pump from the stacked structure is not necessary. Additionally, repairs are primarily defined as those that require disassembly, removal of the pump body from the stacked structure, and replacement of internal components.

9.1 Maintenance of solvent filters

Blockage of the solvent filter is likely to be caused by a dirty reservoir and contamination of the mobile phase. Long-term use of a buffer can also result in flow impairment and filter contamination. Pump functioning will be impacted by impassability, pollution, and blockage. Adhering to recommended methods can ensure the pump operates normally and prolong the filter's service life. To effectively stop fungi from developing, swap out a colorless reservoir with a brown one.

- Filter all solvents through a 0.45 micron (or smaller) filter.
- The aqueous mobile phase needs to be replaced or filtered often.

Adding 0.0001 to 0.001 mole of sodium azide may stop fungal growth under the test's permitted parameters.

Even if the mobile phase was degassed, air bubbles will still show up in the infusion tube if the solvent filter is severely blocked. Now is the time to clean the solvent filter. Cleaning steps are as follows:

1) After taking the solvent filter out of the bottle-head assembly, submerge it in a beaker filled with 30% v/v nitric acid. Spend fifteen minutes sonicating.
2) Use distilled water to thoroughly rinse the solvent filter. Spend 10 minutes sonicating the solvent filter in distilled water.
3) Blow-dries the filter.
4) For 10 minutes, sonicate the solvent filter once again in distilled water.
5) Blow-dries the filter again.
6) After reassembling the solvent filter, place it inside the container of mobile phase.

9.2 Cleaning the check valve

Check valves are installed at the pump's input and exit. A check valve component can be utilized at either end of the pump because the parts are identical. **Figure 19** depicts the check valve's appearance.

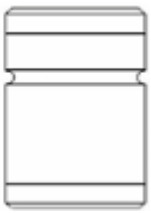

Figure-19 Appearance of the check valve

Check valves typically do not require cleaning. Cleaning should be done if there is sediment in the valve or if the valve ball and seat are sticky.

9.2.1 Overall cleaning

The adhesion condition can be improved by general cleaning once the check valve has been fully assembled.

1) Remove the check valve located at the entry of the pump: Take off the check valve parts, unload the entrance pressure cap with a wrench, and remove the infusion tubing from the cap.
2) Remove the check valve from the pump's outlet: Using a wrench, remove the infusion tubing above the pump head, then remove the component by unloading the pressure cap.
3) Put them in ethanol, clean them twice for 15 minutes using ultrasonics, and then blow off the solvent within.
4) Pay attention to the check valve's direction as you assemble the cleaned check valve correctly.

Caution: There is a ring mark next to the check valve's entrance. Deep cleaning is required if general cleaning is unable to resolve the issue.

9.2.2 Deep cleaning

1) Using a wrench, remove the pressure cap. Remove the check valve.
2) Place the wallpaper knife in the space between the valve's gasket and the coat. Carefully remove the valve ball, seat, and seal parts. Submerge them in ethanol and use ultrasonic cleaning.
3) Use a magnifying glass to examine the valve seat and ball. Replace the damaged valve seat or ball with a new one.
4) Using **Figure 20**, assemble the cleaned check valve. The valve seat's various sides have varying degrees of roughness. Before installation, carefully identify them (the smooth side is seated by the gem ball).
5) Blowing is a useful technique for verifying that the installation is accurate. blowing the check valve from the inlet where there is a ring mark close by with a dust ball. The breeze on the opposite side is palpable. Air cannot go through otherwise.

Caution: To prevent the valve components from being lost during cleaning, it is advised to use a compact container.

The components cannot mix between valves since each set of the valve ball and seat is ground in pairs.

If no one uses the pump for more than a month, the system should be cleaned with methanol and deionized water in succession, as problems could arise when the pump is operating.

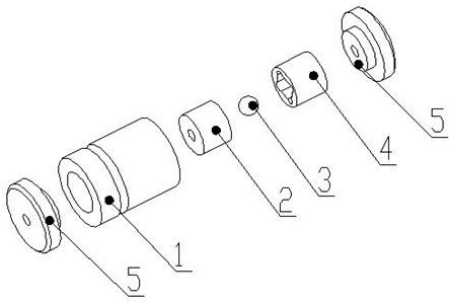

Figure-20 Assembly of the check valve

1) The marked valve jacket
2) Valve seat
3) Valve ball
4) Seal with a limit set
5) Sealing gasket

9.2.3 Cleaning the online solvent filter

To filter the mobile phase that leaves the pump outlet and keep the particles out of the chromatographic system, the pump incorporates an online filter built into the vent valve. Regularly clearing the internet filter is recommended for users. Clean the pressure cap after using a wrench to remove the sealing ring and the sintered stainless-steel filter.

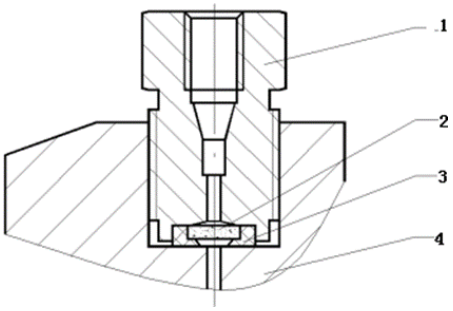

Figure-21 Assembly of the online filter

1) Pressing the cap
2) Filter disc
3) Sealing ring
4) Release valve

Step 1: Take off the filter disc. Put it in a beaker filled with 25% v/v nitric acid. For 15 minutes, sonicate.
Step 2: Use distilled water to thoroughly rinse the solvent filter. Spend 10 minutes sonicating the solvent filter in distilled water.
Step 3: Blow-dries the filter.
Step 4: For 10 minutes, sonicate the solvent filter once again in distilled water.
Step 5: Blow-dries the filter again.

9.2.4 Cleaning the pump head

When buffer salts are used for an extended period, crystalline salt may form. Crystalline salt damages the plunger rod and seals. Regular cleaning of the sealing ring and flow route is necessary to prevent this circumstance. Consumers can make an appropriate cleaning solution (e.g., 10% methanol and distilled water, 20% ethanol aqueous solution, etc.). The high-pressure constant flow pump comes with a plunger rod cleaning device as standard. Choose an appropriate cleaning technique and conduct a "**Normal operation**" based on the situation.

Caution: Add and replace the cleaning fluid in time.

9.2.5 Pump head assembly

When removing the pump head, the case cover does not need to be opened. The following are the procedures for disassembling and installing the pump head:

1) Turn off the pump power.
2) Remove the infusion line connected to the pump.
3) Remove the pump head body by unscrewing the four pump head screws with a 3# hexagonal key (Allen key).
4) When installing the pump head, pay attention to evenly tighten the four fixed screws, referring to **Figure 22**.
5) Connect the infusion line removed in step 2 above.

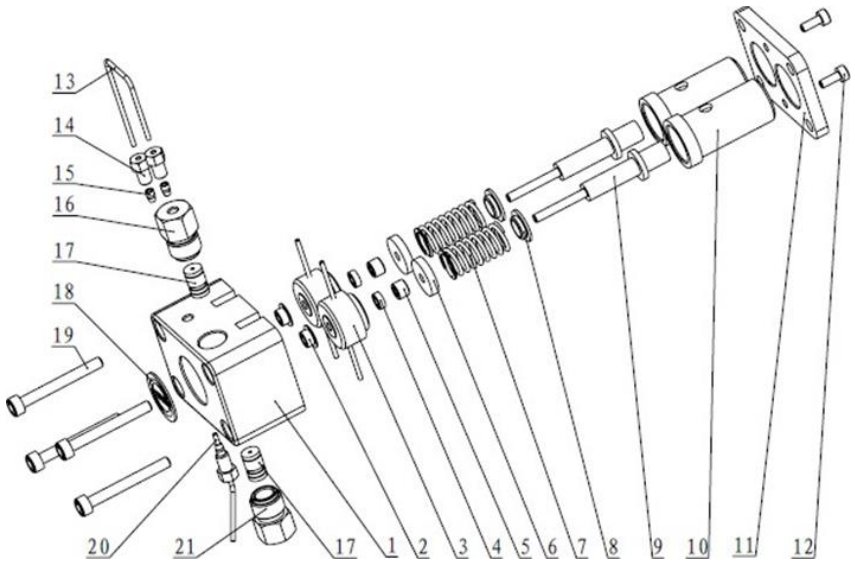

Figure-22 Pump head assembly

1) Pump head body
2) Plunger seal
3) Support ring with plunger rinsing
4) The secondary seal
5) The secondary seal pressure ring
6) Spring outside locating sleeve
7) Plunger spring
8) Spring inside locating sleeve
9) Plunger rod components
10) Sleeve
11) Mounting plate
12) Mounting plate screw
13) Stainless steel tube
14) Tube screw, 1/16" I.D
15) Stainless steel ferrule, 1/16"I.D
16) Export pressure cap
17) Check valve
18) Badge of the pump head
19) Pump head screw
20) Outlet pipe
21) Entrance pressure cap

Pump head disassembly and installation methods are as follows (Refer to **Figure 22**, **Figure 23**):

Step 1: Place the pump head body on a flat surface. Use 2.5# hexagonal key to unscrew the 2 mounting plate screws, #12 on **Figure 22**. Remove the clamp (10#).

Step 2: Unload the sleeve (**Figure 22** #10), plunger rod components (**Figure 22** #9), plunger spring (**Figure 22** #7), locating sleeve (**Figure 22** #3), etc.

Step 3: Observe the plunger rod surface with a magnifying glass to find whether axial wear occurs on it. If there is no axial wear, clean the plunger rod; or, a new plunger rod is needed.
Step 4: Take out the primary seal carefully (**Figure 22** #2) (the user should not remove it unless a new one is needed).
Step 5: Put the new primary seal (the openings with spring should be in the direction of the pump head) into the pump head body (**Figure 23** #1).
Step 6: Place all parts in order according to **Figure 23**. Install the pressure plate.
Step 7: Before uniformly tightening two fixe screws, place the pump head on the worktable, slowly press the pressure plate, and insert the two-pressure plunger rod into the primary sealing ring.

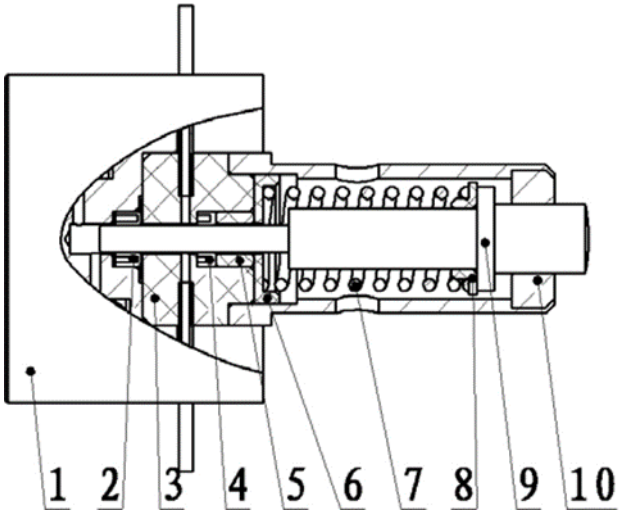

Figure-23 Cross-section of sapphire plunger

1) Pump head body
2) Plunger seal
3) Supporting ring with plunger rinsing
4) Primary seal
5) The secondary seal pressure ring
6) Spring outside locating sleeve
7) Plunger rod spring
8) Inside spring locating sleeve
9) Plunger components
10) Sleeve

Caution: Plunger rod is easy to break. Be careful while unloading and Assembling.

10. Troubleshooting

Pump troubleshooting may be a frustrating and occasionally enigmatic task. This section describes a straightforward procedure for pump troubleshooting.

10.1 Elimination of Air Bubbles

The following conditions increase the likelihood of air bubbles becoming caught in LB-21HPLC: The pump's first usage, prolonged inactivity, incorrectly degassed mobile phase, etc. Eliminating these trapped air bubbles is essential. Otherwise, they impact system performance in several ways, including column damage, fluctuating pressure, and unstable flow rate.

How to eliminate trapped air bubbles:

To eliminate air bubbles in the pump head and inlet lines, open the relief valve, press the **"PURGE"** key, and flush with a high flow rate.

Use a syringe to remove mobile phase from the relief valve's outlet until the liquid flows out if the pump is unable to do so after pressing the **"PURGE"** key.

If the pump is still unable to withdraw the mobile phase, it is probably caused by a dirty check valve. Follow the related content in the maintenance manual to clean the check valve.

10.2 Leakage of the pump head

Leakage from the pump head is a frequent issue. The primary source of leaks is damage to the seal in the pump head. Thus, replace worn parts as soon as the pump discharges.

10.3 Overpressure

The system for measuring pressure in real time is operating. The LB-21HPLC will automatically stop and sound an alert if the pressure rises beyond the predetermined maximum pressure. Right now, opening the vent valve to relieve system pressure is a wise decision. Then, to find the cause of the excess pressure, either reset the pressure cap or examine the entire flow line.

Caution: Setting maximum pressure should be lower than the maximum pressure the column can withstand, or the column may be damaged.

10.4 Pump not delivering solvent

The most common causes of a pump not delivering solvent are blockages in the solvent filter or air bubbles in the pump head. The issue can be resolved by cleaning the solvent filtering cup or getting rid of air bubbles. However, the issue may also be brought on by a faulty check valve. Therefore, clean the check valve if the pump does not draw liquid after the solvent cup has been filtered and cleaned. Refer to the relevant sections of the user manual for cleaning the check valve. The table below lists common failures and their fixes.

Table – A summary of the most common problems affecting system operation

Symptoms	Cause	Solutions
Infusion instability and the pressure fluctuation	Bubbles in the pump head	Eliminating air bubbles through the relief valve
		Spare bubbles with a syringe through the relief valve
	The old solvent remained in the pump cavity	Increase the flow rate to replace old solvent from the relief valve
	Bubbles in the solvent filter and the interline	Vibration the solvent filter to eliminate air bubbles
		If the solvent filters are dirty, clean them with an ultrasonic cleaner or replace the solvent filters
		Degas the mobile phase
	Something is wrong with the check valves	Clean or change the check valve
	Piston rod or seal leakage	Replace the piston rod and seal
		Replace the faulty part
	Pipeline leakage	Tighten the screw where leakage occurs
		Replace the failed parts
	Pipeline blocked	Clean or replace the tube
The pump is running, but no liquid coming out	Air bubbles in the pump cavity	Eliminating air bubbles through the relief valve
		Spare bubbles with a syringe through the relief valve
	Bubbles in the pump head entering from the infusion entrance	Tighten the cap of the pump head
	Air in the pump head	Perfuse fluid in the pump head, open the relief valve, and turn on the pump under the maximum flow rate until there is no air bubbles appear
	Incorrect check valve installation	Install the check valve correctly
	Dirty check valve or worn check valve	Clean or replace the check valve
	No solvent in the reservoir	Fill up the reservoir
The actual flow rate is lower than the set value	Abnormal check valve	Clean or replace the check valve
	Dirty filter	Clean or replace the filter
No liquid delivered by the pump (the pump is not running)	Power off	Turn on the power
	The power supply is not connected	Connect the power supply

The flow rate decreases when the pump is running	Air in the pump is gathering	Open the relief valve, let the pump run at high velocity to eliminate air bubbles
	Solvent filters plugged	Open the cap of the pump head. if there is no liquid coming out from the infusion tube quickly, it means the solvent filter is plugged. Clean or replace the filter.
	Immiscible solutions in the pump	Use a transition solvent to dissolve the Immiscible one
	Plunger seal leakage	Inspect and replace the piston rod and seal
	Compression compensation failure	Check or replace the pressure board or pressure transmitter
The flow rate is too high	The velocity compensation failure	Inspect or replace it
	P.C. board failure	Replace the P.C. board
	Compression compensation failure	Inspect or replace it
High pressure	Tubing blockage	Screening each section of the pipe to find the blocked part. Replace or cut off the block tube
	Improper pipe diameter	Replace it with a proper tube
	Online filter blocking	Clean or replace the filter sieve
	Chromatographic column plugged	Replace the chromatographic column
No/Low pressure	No solvent in the reservoir	Fill up the reservoir
	The vent valve is not tight	Tighten the relief valve
	Pump pressure sensor fault	Check or replace the pressure sensor
	Pipe joint leakage	Tighten the screw or ferrule where is leaking
		Replace the worn parts
	Seal leakage	Clean or replace the sealing ring
	Both the inlet and outlet of the pump have air bubbles	Open the relief valve and let the pump run under high velocity to eliminate air bubbles
The pump has a humming sound and cannot start properly	The motor failure	Stop the pump and check it
	The phase voltage is too low	The phase voltage is too low
The flow rate is zero	Air bubbles in the pump head	Eliminate the air
	The entrance check valve was plugged	Check and replace it

	The export check valve was plugged	Check and replace it
	The direction of the check valve is reversed	Install the check valve in the right direction
The pump stops operating	Pressure is higher than the limit pressure	To reset the highest limit pressure
		Replace the column
		Replace tubes with proper ones
	Power cut	Check the power supply
		Shut off the main power supply, and check the fuse
The pump is on, the pressure is common, but no liquid comes out	Serious leakage in the system	Repair the injection valve or pipeline and fasteners between the pump and detector
	Fluid passage blockage	Clean the injection valve or the connection between the column and detector, or particles in the flow cell
	The entrance of the column is plugged with particles	Clean or replace the column inlet filter or replace the column. Degas the mobile phase and samples properly
The column pressure increases, but the flow rate decreases	The column or the protected column is partially blocked	Clean or replace the column inlet filter, or replace the column
	The entrance tube of the detector is partly plugged	Remove and clean the flow cell and line

11. Accessories

Standard Accessories

S. No	Accessory Name	Quantity
1	High Pressure Constant Flow Pump	2 Pcs
2	Gradient Mixer	1 Pc
3	UV-Vis Detector (Excluding Sample Cell)	1 Pc
4	Sample Cell (Analytical Cell / 10 mm Optical Path / Outer Diameter 1/16 inch × Inner Diameter 0.007 inch)	1 Pc
5	Rheodyne 7725i Six-Way Injection Valve	1 Pc
6	Valve Bracket	1 Pc
7	Solvent Bottle Tray	1 Pc
8	Kromstation Workstation (Basic)	1 Set
9	High Performance Liquid Chromatography System Toolkits	1 Set

Optional Accessories

S. No	Accessory Name	Quantity
1	Autosampler	1 Pc
2	Column Oven	1 Pc
3	500 ml Solvent Bottle (With Cap And Stopper)	2 Pcs

12. Replacement

12.1 Mobile phase replacement

Water, buffers, organic solvents, and other liquids are among the many liquids that are utilized as mobile phases in HPLC. Consideration should be given to buffer salt precipitation and miscibility. When replacing the mobile phase, proceed with caution.

12.1.1 Replacing with a miscible liquid

E.g., replacing methanol with acetonitrile

1) Store acetonitrile in a clean solvent bottle.
2) Following their removal from the methanol reservoir, the filter components should be cleaned with acetonitrile and then placed in acetonitrile.
3) The relief valve should be opened. Use around 20 mL of the new mobile phase to flush the pump.
4) After disconnecting the column from the system and closing the relief valve, place a bottle at the inject valve's outlet.
5) Turn on the pump and pump the new mobile phase out about 10 mL.
6) Reattach the column. Set the pump to a reasonable flow rate, such as 1 milliliter per minute. At least half an hour is required for the system to equilibrate.

12.1.2 Replacing with an immiscible liquid

E.g., Replacing methanol with n-hexane

Select a washing solution that is insoluble in both the old and new mobile phases. Since isopropanol is miscible with both methanol and n-hexane, it can be utilized as the intermediate washing liquid in the example above.

Follow the procedures as described. Use the intermediate washing liquid to replace the old one.

Repeat the procedures as described. Using the new mobile phase to replace the intermediate washing liquid.

12.1.3 Replacing organic solvent with salt buffer

When buffer salts combine with an organic solvent, a crystalline salt may form. In addition to potentially blocking the tube, crystalline salt damages the internal rotor and stator, the injection valve, the plunger rod, and the seals. Therefore, before analysis, introduce a specific amount of organic solvent that contains water into the system. Once the experiment is complete, save the column with organic solvent and switch the system again using the previously described procedure.

12.2 Plunger seal replacement

Long-term use will cause wear on the plunger and plunger seal. It primarily depends on the type of mobile phase, operating pressure, and flow rate. It is challenging to estimate the extent of wear on certain parts because the influence of these elements is unknown. When changing the piston ring, the plunger rod's surface must be examined. Replace the plunger rod if there is even the slightest blemish on its surface. Refer to "**Pump head assembly**" above for the procedure for disassembling and reassembling the plunger.

12.3 Fuse replacement

The fuse replacement process is as follows:
1) Turn off the power switch.
2) Remove the power cable from the power outlet.
3) Open the fuse box with a screwdriver.

After replacing a new fuse, lift the fuse lid.

13. Appendix

Introduction to the connecting tube materials:

In HPLC systems, column systems, tubing, fittings, and extra-column volumes in the injectors and detectors are likely to cause peak broadening. Improper tube material will also lead to peak broadening and even cause the sample degeneration, which affects the reliability of analysis results directly.

A strong connection can maximize the instrument's performance and increase productivity. Depending on the system pressure as well as the characteristics of the mobile phase and samples, different pipeline materials are required. Teflon, polytetrafluoroethylene, vinylidene fluoride, polyethylene, polypropylene, stainless steel, and polyether ketone (PEEK) are all frequently used pipe materials. The most widely used type of pipe is made of stainless steel.

Outer diameter of HPLC system connecting pipe is 1/16"(1.59mm). Inside diameter can be chosen according to your need. Commonly used inside diameter includes 0.007"(0.175mm), 0.01"(0.25mm), 0.02"(0.5mm), 0.03"(0.75mm) and 0.04"(1.0mm) etc. Stainless steel tube is generally used in high-pressure parts. In HPLC systems, the part from the pump to the column inlet is high high-pressure section. Stainless steel tube is recommended.

Coaxial stainless steel tube resists corrosion well. During use, the pipe's bore should precisely match the fitting's.

Additionally, polymer tubing can be utilized in a variety of low-pressure HPLC system components, including the pump, detector output, sampler drainage port, emptying valve outlet, and liquid bottle. Teflon is the most commonly used plastic pipe that is inert to HPLC liquids.

Peek tubing is more pliable than stainless steel tubing and appropriate for biological sample examination at pressures below 20 MPa.