



Classical 3100

USER'S MANUAL

Operation Manual

for P3500 High Pressure

Constant Flow Pump

V1.0.3

Statement

The manual is intended to help users to understand, use and maintain the instrument of P3500 pump. Our company does not assume the responsibility caused by business or special purpose use of the manual. It is subject to change without notice.

The manual has been published after careful review. It is believed to be accurate and complete, if there are still any errors, Our company shall not be liable for incidental or renewal damage in connection with, arising from or the use of it.

In any case, Our company is not responsible for the impact caused by the use of this manual and instrument.

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Please read the document carefully before using the P3500 pump.

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Precautions

Thank you for your continue patronage. To ensure correct and safe use of the instrument, please read it carefully before using.

The details of the equipment's composition, installation, method of using, maintenance, parts selection and other points are described in the documents, it will be a useful tool for you. After finish reading, please keep it well for a rainy day. When the instrument is lended or transfered, please delivery the manual by.

For safe operation, please read the following **Safety Precautions** before using the instrument.

Safety Precautions

According to the level of danger and harm, safety signs here are divided into the following three categories:



[Warning] Failure to properly follow the instructions and precautions indicated by this sign can result in serious injury or damage to health and property. The property damage include the environment around and the instrument.



[Caution] Failure to properly follow the instructions and precautions indicated by this sign can result in slight injury or damage to health and property. Slight injury means no hospitalization is needed to the wounded. Slight property damage means the instrument can be recovery through simple maintenance.



[Note] The sign is used wherever information is given to ensure optimal performance of the instrument.

1. Precaution for usage



[Warning] P3500 pump should only be used as a part of liquid chromatography. Do not use it for any other purpose. Except for special instructions, this instrument does not have explosion-proof function.

2. Ambient Conditions



[Warning] When we use organic solvent it is recommended that interior must be well ventilated and the firework should be prohibited. Also, a sink or equipment for washing eyes should be installed nearby in case of the organic solvent coming into contact with the eyes or skin.



[Note] In order to ensure good efficiency, keep the instrument away from caustic gas, dusty environment or strong magnetic. The worktable should be wide and strong enough. Ambient should be between 10°C to 30°C with a small fluctuation, and humidity should be between 20% to 80%. Avoid it from cold or hot source as well as direct sunshine. The air conditioners and other equipment should not blow directly into the instrument.

3. Precaution for installation



[Warning] The instrument should be installed following the instructions strictly by professionals, make sure that the voltage of the power socket is the same as the power supply voltage indicated on the instrument. Using the wrong power voltage could result in danger and fire.

The accessory power cable should be used to connect the pump to the power socket. Other cable should not be used.

Make sure the line cord is connected to a properly grounded power receptacle to prevent static and electric leakage.



[Caution] The instrument is so heavy that you should move it carefully and watch your hands in the same time.



[Note] The instrument should be connected following the instructions strictly. Wrong connection could cause communication error.

4. Precaution for use



[Warning] Do not use the instrument in places where heat resource, fire seat, magnetic resource, strong vibration exist or may exist. It is prohibited to put flammable nearby.

The bottle for storing the mobile phase should have a pore in cap to prevent the danger caused by negative pressure in the bottle.

A gap between the waste tube and the cork of the waste bottle is necessary to prevent the waste bottle bursting when it is overfilled. But the gap should be small to prevent evaporate of hazardous solvents. Even though, the waste needs to be clean up promptly.



[Caution] When using organic solvents, please wear safety goggles, special lab coats, gloves mask etc. If your body contact with toxic solvent accidentally, wash it immediately, and then go to hospital for specialized treatment.



[Note] When preparing mobile phase, please use HPLC grade solvents or equivalent ones. You'd better filtrate the eluent with a membrane filter (0.45 μ m), and an online filter is also necessary to prevent small particles from scratching plunger rod, seal ring or blocking pipeline. What's more, please degas all mobile phase before using, degassing is an effective method to prevent chromatogram noise and wrong indicator.

Before first use, rinse the entire piping system according to the requirements of the manual. Direct use is likely to block pipeline.

Before sample test, ensure that the pipeline in the system is filled with mobile phase without any bubbles, otherwise it will affect the reliability of test results.

If an eluent is replaced with another eluent which is insoluble, such as positive mobile phase (hexane) and reverse phase (methanol), be sure to operate according to the specified method in the manual, otherwise it will cause serious pipeline jam, and even system paralysis.

Halogen ions is harmful for stainless steel, if there is stainless steel pipe and fitting in your system, please avoid the use of a mobile phase containing halogen ions. If you can't avoid it, please minimize the content and clean the system with water as soon as finishing the analysis.

If there is peek pipe in your system, it is important to note that:

Do not use the following solvent: concentrated sulfuric acid, nitric acid, dichloroacetic acid, dichloromethane, trichloromethane, chloroform, dimethyl sulfoxide, acetone, tetrahydrofuran, etc. Such solvents can reduce the strength of the PEEK material, make it's become fragile and broken. But the impact of short-term use of aqueous solution of acetone (lower than 0.5%) in gradient performance is acceptable.

When using PEEK pipes, the pressure of the system should be lower than the tolerance pressure of peek material, otherwise it may burst.

The bending radius of peek pipe should be more than 10mm, make the peek pipe natural relaxation during installation.

The PEEK pipe should be intercepted with professional tube cutter in order to make the pipe more smoothly. Pay attention to that there should be no cutting debris left in the pipe.

5. Repair, maintenance and parts replacement



[Warning] Before repair, maintenance and parts replacement, please turn off the power in case of leakage and electric shock.

There is no need to open the host cover while daily maintenance and repair. If the repair needs to open the host cover please entrust agents or communicate with us.

You should clean the dust on the power cord plug regularly to reduce the electrostatic. Then, dry it before using, otherwise electric shock may occur.

Use dry cloth to wipe the instrument. Do not use thinner or alcohol to avoid erasing characters or color on the panel.

Do not replace components (e.g., fuses, deuterium lamp, etc.) from other company or other type, all accessories are required to be specified to prevent danger.

6. Precaution for static electricity



[Warning]As the instrument may use a lot of flammable, explosive organic reagents which may contaminate laboratory air, when the reagent concentration is too high, any spark or flame could cause fire or explosion accidents. Do not use the pump near any fire resource or hot resource, and keep reducing the electrostatic in mind. To reduce static electricity, please take the following measures:

- 1) Make the instrument grounded. It is very important, please pay attention to it.
- 2) Maintain proper indoor humidity (humidity is greater than 65% can prevent static electricity effectively) and keep the environment clean.
- 3) Metal waste bottles (external conductive) should be grounded (no ground insulation). When using other materials container, you can insert one end of the wire into liquid in the bottle and make the other end earthed.
- 4) Replace a larger I.D. pipe when the flow of mobile phase is higher than usual.
- 5) Wipe the instrument regularly.
- 6) Staffs should wear anti-static clothing. An anti-static pad is needed on the floor.
- 7) People and objects with static electricity is prohibited to touch the instruments.

7. Warning label instructions

To ensure the safety of staffs, we attach warning labels on the equipment where are dangerous. If the label is missing, please request new ones from our company, and attach to the correct position.

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1. Chapter One: Introduction

1.1 Overview

P3500 high-pressure constant flow pump is based on the experience in the research and production of P230p pumps. We have the independent intellectual property rights of it.

A HPLC system include not only constant flow pumps, but also a UV-visible detector, a high pressure injection valve, workstations and etc. In addition to P3500 high-pressure constant flow pumps, Dalian Elite Analytical Instruments Co., Ltd could supply a full range of products and parts of high performance liquid chromatography. Know more about the rest of them, please refer to the corresponding user manual.

P3500 high pressure constant flow pump is driven by a small CAM. There are two tandem plungers whose stroke is short and the infusion pulse is low. Subdivision stepping motor control technology is adopted to make the motor run smoothly at low speed. With the installation of floating guide plunger, high quality piston rod and other key components, the P3500 pump could run stably and durably in a long time. Mobile phase compression coefficient correction and flow rate correction result in high accuracy. You can easily control the instruments by workstation. P3500 high pressure constant flow pump is very suitable for pharmaceutical, biochemical, environmental protection, quality control and other areas of HPLC applications. Also, in some special areas such as high-precision feed pump, it also has a unique advantage.

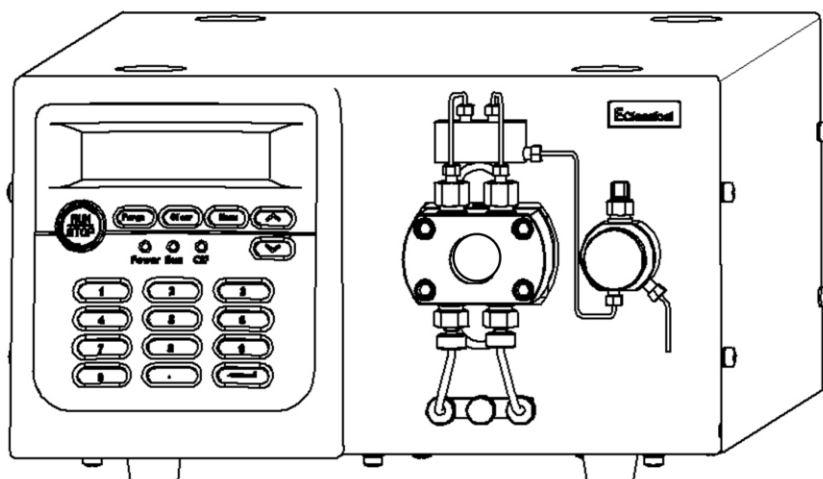


Figure 1-1: P3500 pump

1.2 Features and Functions

Dalian Elite company independently developed the P3500 high pressure constant flow pump with independent intellectual property. Its main characteristics are as following:

- Driven by a small CAM, the infusion pulse of P3500 is low. The traditional liquid chromatographic bumper is canceled, which reduces the system volume.
- Subdivision stepping motor control technology ensures that P3500 pump is accurate and precise, even at a low flow rate or binary gradient system. This greatly increases the repeatability of the results.
- Floating guide plunger installation, high quality piston rod and other key components ensure running stability and durability of the pump in long period.
- The advanced operation menu allows user to amend the flow compressibility and flow correction values. User can adjust the flow rate within $\pm 15\%$ using these two functions. Hence accurate and precise flow rate is obtained for different solvent systems.
- P3500 pump has RS-485 connector which can be used to control the instrument with workstation. Furthermore, through RS-485 connector, two pumps can communicate with each other to achieve accurate and consistent binary gradient flow.
- Real-time pressure display, maximum and minimum pressure warning ensure the security of instrument operation.
- The software used can display pressure in 3 different units: MPa, psi and bar.
- The pump has purged function to instantly increase the flow rate of mobile phase for flushing purpose (P3500 25mL/min).
- Using the integrated circuit controller AT89C55 and SPI bus technology, P3500 is compact and reliable.
- The VFD display and keypad are user-friendly and easy to operate.

1.3 Performance Specification

Table 1-3: Performance Specification of P3500 pump

| Items | Specifications |
|--------------------|---|
| Flow Rate | 0.01-50.00mL/min (Step:0.01mL/min) |
| Accuracy | ±1.0% (10.00mL/min, 8.5MPa, water, ambient temperature) |
| Precision | ≤0.3% (10.00mL/min, 8.5MPa, water, ambient temperature) |
| Max Pressure | 30MPa (0.10-20.00mL/min) 20MPa (20.01-50.00mL/min) |
| Pressure accuracy | ±3% or less than 0.5MPa |
| Pressure pulsation | ≤0.4MPa (10.00mL/min, 8.5±1.5MPa, water, ambient temperature) |
| Leak proofness | Pressure drop ≤1.5MPa(30MPa, 10min) |
| Dimension/Weight | 420mm×300mm×175mm/12kg |
| Communication mode | RS-485 |
| Power Supply | AC 220V±10%,50Hz±1Hz |
| Power | 200W |

2. Chapter Two: Installation and transport

2.1 Unpacking inspection and standard accessories

P3500 high pressure constant flow pump is packaged with corrugated boxes and foam lined structure. When you receive the instrument, please check the packaging first. If the package is damaged, please contact with Our company or local dealer.



[Warning] If there is any damage to the instruments when you receive it, please don't try to install it. You can ask Dalian Elite Analytical Instruments CO., Ltd to inspect and assess it.

2.1.1 Demolition of the packing

Put the pump on horizontal ground with the front of the packing box up. Cut the tape on the top, take out the pump and accessories package, and place them on the table. Then remove the foam and open the instrument protective film.



[Warning] The pump is very heavy, to prevent sliding, at least two people is suggested for installation.

2.1.2 Deliver checklist

Before installation, please check the deliver list carefully. If one or several of them omissions, please communicate with Our company Or local distributors as soon as possible.

Table 2-1: Deliver list of P3500 pump

| NO. | Items | Quantity |
|-----|-----------------------|----------|
| 1 | P3500 pump | 1 pc. |
| 2 | Certificate | 1 pc. |
| 3 | Service Card | 1 pc. |
| 4 | Start Package | 1 pc. |
| 5 | P3500 User manual(CD) | 1 pc. |



[Note] If there is discrepancies between the packing list in the box and in the specification, please refer to the packing list in the box. It is subject to change without prior notice.

2.2 Stack Order

In order to guarantee the best working state of the instrument, it is recommend that the instruments should stacked as shown in figure 2-1.

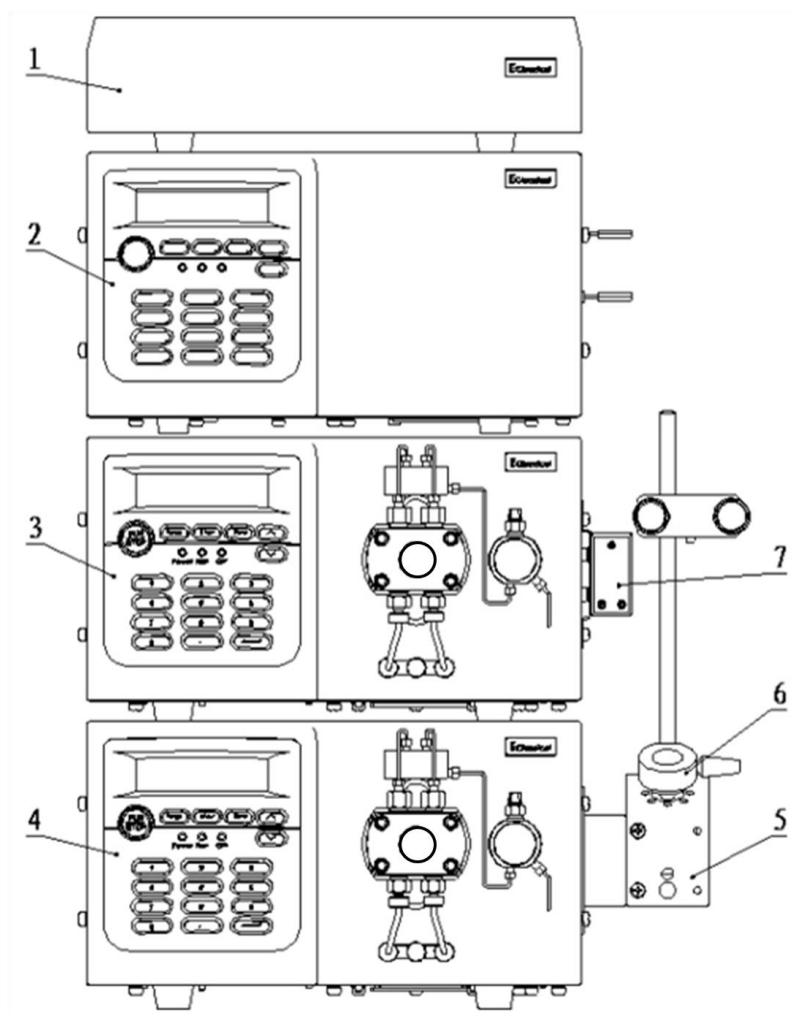


Figure 2-1: EClassical3500 stack order

- 1.Solvent tray, 2.UV-visible detector, 3.Pump A, 4.Pump B,
5.Valve bracket, 6.Injection valve, 7.Mixer

2.3 Installation Requirements

2.3.1 Site Requirements

Environment

Pump P3500 need to work under ambient conditions in Table 2-2 below:

Table 2-2: Environment requirements

| Items | Specifications | Requirements |
|-------|-------------------------|---|
| 1 | Work environment | Room should be free of dust, inflammable and explosive materials, also, good ventilation is important |
| 2 | Electromagnetic field | No electromagnetic noise nearby |
| 3 | Operating temperature | 4~40°C(39~104°F) |
| 4 | Humidity | 20%~80%, non-condensing |
| 5 | Temperature fluctuation | < ± 2°C /hr |



[Caution] Do not use the pump under the conditions of temperature fluctuations. If the ambient temperature is too low, make the room temperature increase slowly to avoid condensation inside caused by rapid heating.

Bench space

The P3500 pump can be put on almost any laboratory bench. If you want to display the complete 3500 system on the bench, make sure that the table can bear the weight of all components. It needs an additional 50mm on the left, 150mm on the right, 150mm on the back to facilitate the circulation of air and electrical connections.



[Warning] The instruments should be placed on a horizontal position, otherwise there is a risk of falling!

2.3.2 Power and power line

To keep the instrument working normally and safety, please use a dedicated power line within the specified voltage range.

Grounding, AC power to 220V ±10%, 50 Hz,

Please choose T1.25 A (250V) fuse.



[Warning] Only the accessory power cable can be used to connect the pump with the power socket. Other cable should not be used in case of danger or damage to the instrument.

If the instrument is connected to a grid above the scope of application, it may cause electrical shock or damage to the equipment and staff.

Please unplug the power cord before replacing the fuse to avoid electric shock. The external fuse is installed in the back of instrument.

2.3.3 Computer requirements

Hardware requirements

- The lowest hardware requirement: Intel Core 2 CPU, 2G internal storage, more than 1G hard-disk space;(Refer to the manual of workstation)
- The lowest resolution of displayer: 1024×800, 64K (16bit image);
- Others: USB interface for communication, CD-ROM driver for software installation.

Operation system requirements

- Windows XP Professional (SP3), Windows 7 or higher version (Refer to the manual of workstation).

2.4 P3500 Front

The front panel of P3500 is shown as follows. The keys and display screen are on the left of it. The pump head and release valve are on the right of it.

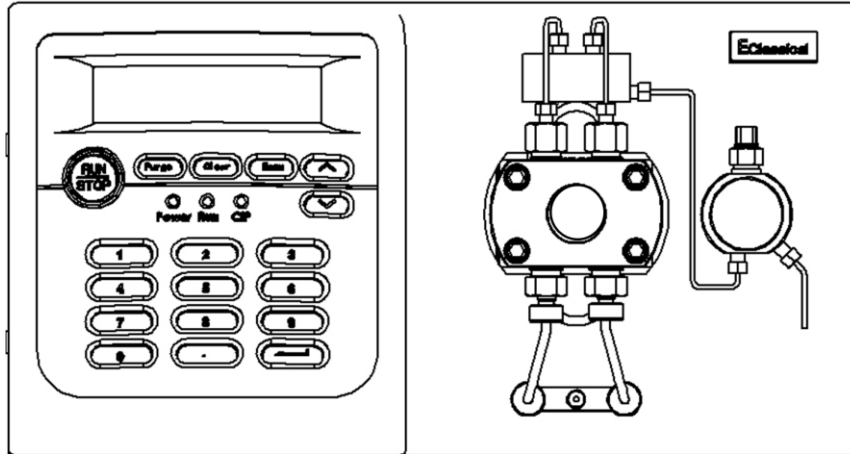


Figure 2-2: Front panel of P3500

The meaning of the keys on P3500 front panel are shown as follows.

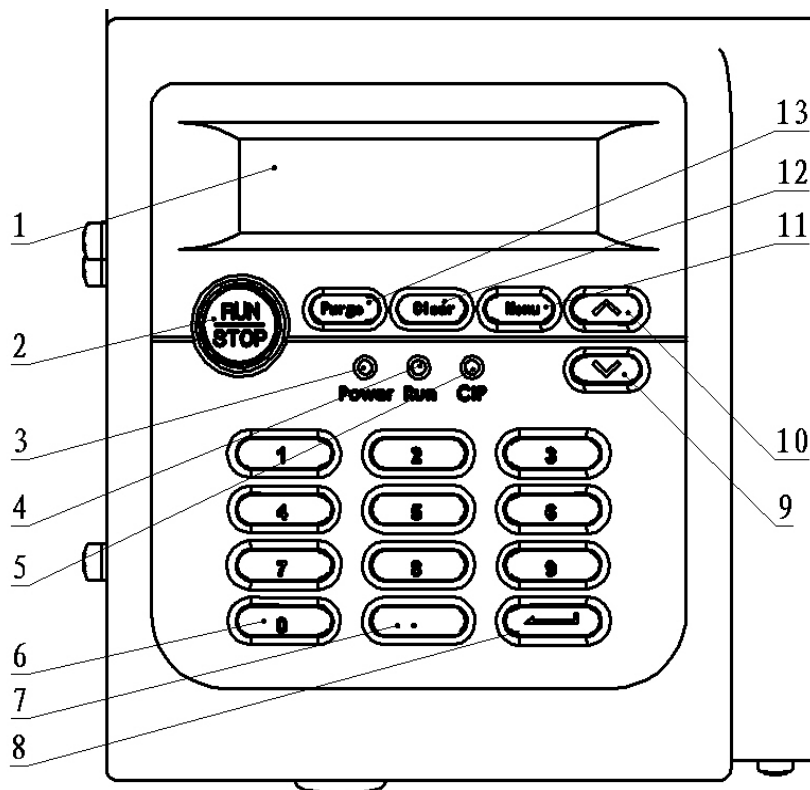


Figure 2-3: Front keys of P3500

Table 2-3: Keypad Function

| No. | Key | Function |
|-----|---------------|---|
| 1 | VFD – display | A display window, to provides the current setting velocity (unit is mL/min), current system pressure (the unit can be MPa, bar or psi) and the limit maximum pressure (the unit can be MPa, bar or psi). The flow rate and the setting maximum pressure can be modified in the state of the main interface. After entering the corresponding data, press the Enter key to confirm. |
| 2 | RUN/STOP | To start and stop the pump. Press once, the pump indicator light will be on and the pump will operate according to a pre-set flow rate. Under external control, press the key to pause the pump operation. |
| 3 | POWER | Power is on when the indicator is red. |
| 4 | RUN | When the run indicator is green, the pump is in run as the setting flow rate. When the pump is purged in a greater flow rate, the indicator is red. |
| 5 | CIP | This section is reserved functions. Light is out and the plunger rod will not be cleaned. |
| 6 | 0 – 9 | Numerical keys |
| 7 | . | Decimal point |
| 8 | ENTER | To confirm values and selections. Upon pressing ENTER key, the cursor will automatically move to the next parameter. |
| 9 | ↓ | Press ↓ key to move between different menu and sub-menu. |
| 10 | ↑ | Press ↑ key to move between different menu and sub-menu. |
| 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 pump system at a large flow for P3500. It can be used to purge out trapped air bubbles or change the mobile phase. |

The infusion part of P3500 is shown in figure 2-4. The devices and functions are shown in table 2-4.

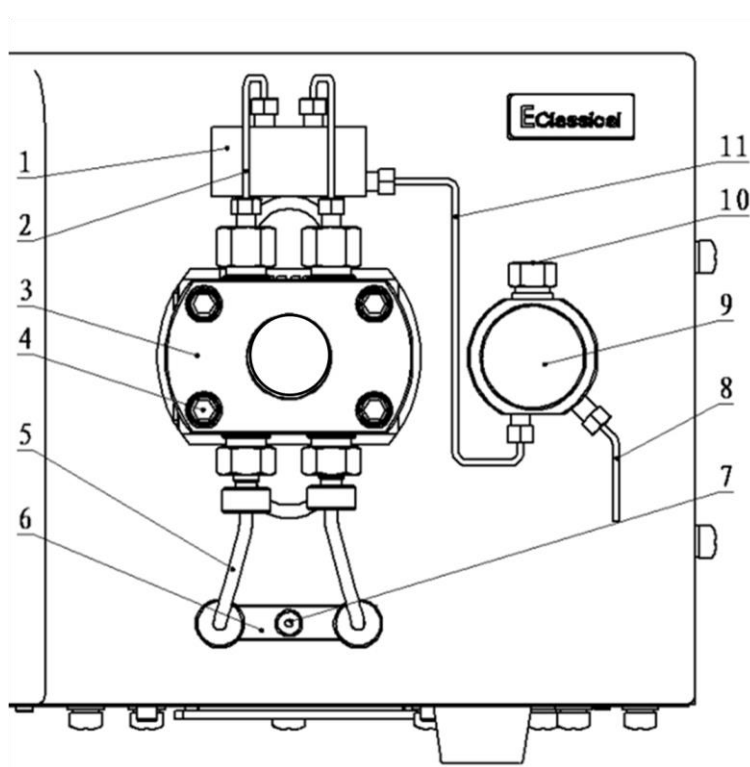


Figure 2-4: The infusion part of P3500

Table 2-4: The infusion part components

| No. | Components | Function |
|-----|--------------------|--|
| 1 | Outlet tee | To connect connecting tube1 with connecting tube3 |
| 2 | Connecting tube 1 | The outlet tube of pump head |
| 3 | Pump head | To draw and deliver mobile phase |
| 4 | Mounting screws | To fasten and lock the pump head |
| 5 | Connecting tube 2 | To connect pump head with inlet tee |
| 6 | Inlet tee | To connect connecting tube2 with mobile phase tube |
| 7 | Inlet of the pump | Connect with the mobile phase pipe |
| 8 | Relief tube | release air and waste liquid from the pump head |
| 9 | Relief valve | Loosen the relief valve knob to release pressure from the pump head. It can be used for purging and priming purpose. |
| 10 | Outlet of the pump | Mobile phase come out from here |
| 11 | Connecting tube 3 | To connect pump head with relief valve |

2.5 P3500 Rear

The rear panel diagram of P3500 is shown in figure 2-6. The devices and corresponding functions are shown in table 2-5.

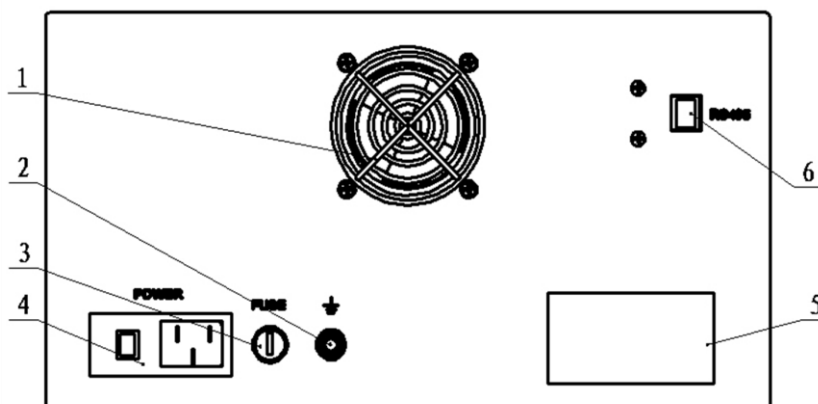


Figure 2-5: P3500 pump rear panel

Table 2-5: Rear panel

| No. | Components | Function |
|-----|----------------------------------|--|
| 1 | Ventilation duct | For ventilation. |
| 2 | Ground terminal | Ground the main body of the pump. |
| 3 | Fuse | Fuse is in it. (T 1.25A) |
| 4 | Power connector and Power switch | The power cable is connected into grounded power outlet. Turn on / turn off the power. |
| 5 | Instrument panels | Identify the information of instrument. |
| 6 | RS485 interface | You can connect the pump with detector or workstation for remote control from here. |

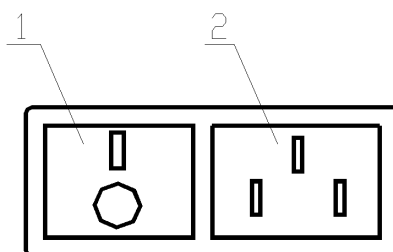


Figure 2-6: Power connector and Power switch

Table 2-6: Power connector and Power switch

| No. | Components | Function |
|-----|-----------------|--|
| 1 | Power switch | Turn on the / turn off the power. |
| 2 | Power connector | Connect the pump with grounded power outlet from here. |

2.6 Fluid Connection System

2.6.1 Solvent pipeline system diagram

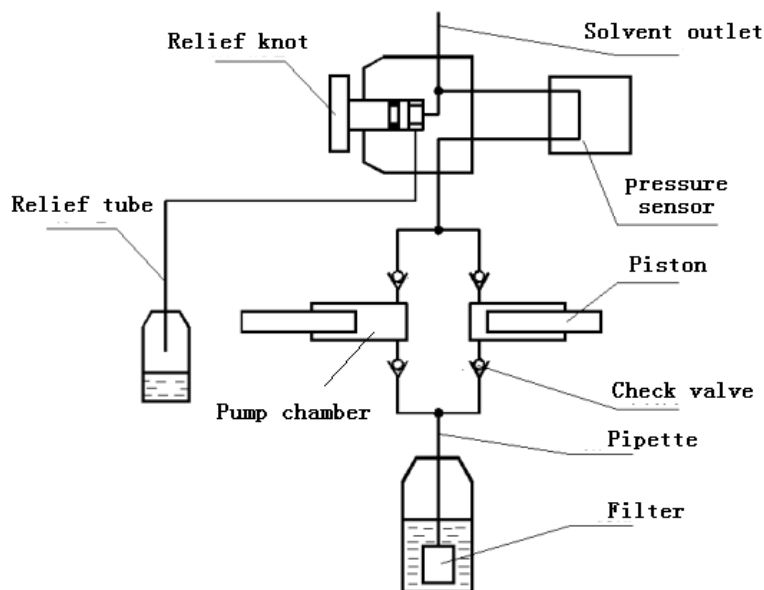


Figure 2-7: Fluid connection diagram

2.6.2 Installation Preparations

A proper solvent (500mL or larger) reservoir is needed. There should be two 3-4mm holes on the cap. Remove the seal plug of the pump outlet that is above the vent valve. Connect the stainless steel tube (O.D.1/16"× I.D.0.04") with the pump port, the other end of the tube will be connected with injection valve.

2.6.3 Connecting solvent reservoir to the pump

Attach the solvent filter to one end of the solvent tube, then put it inside the solvent reservoir. The other end of tube should pass through the hole in the cap and then connected with the inlet of the pump. You can refer to figure 2-5 and 2-4.



[Note] In addition to the holes for infusion tube, there should also be a vent hole in the cap to avoid the negative pressure in the reservoir during infusion.

Wash solvent filter frequently to prevent contamination.

To ensure a good performance, the mobile phase should be degassed before use, especially in summer.

The mobile phase should be filtered with a 0.45 μ m filter membrane.

2.6.4 Connecting injection valve to the pump

Connect the outlet of the pump to the inlet of injection valve (No. 2 hole is usually the inlet for the mobile phase) with stainless steel tube (with screw connection and sealing edge ring).

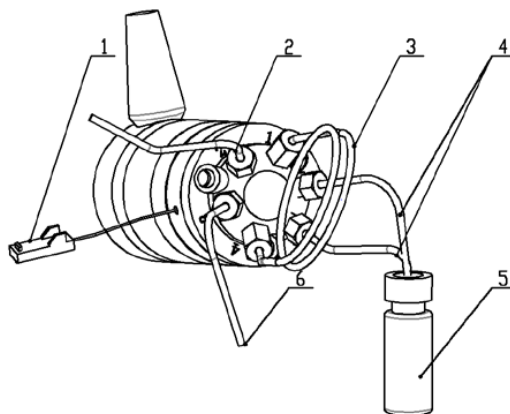


Figure 2-8: Fluid connection of manual filling valve

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



[Note] Port #2 of injection valve should be connected to the outlet of pump.

Port #3 of injection valve should be connected to the entry of column.

Do not over tighten the connecting screw, as it may damage the ferrule.

If there is leakage, please replace the ferrule.

To reduce the column efficiency loss, the pipe length between the injection valve and the column, between the column outlet and the detector inlet should be as short as possible, and the ID of connection tube should be proper.

Make sure the end of all the stainless steel tube is smooth to reduce dead volume of the system (It is recommended that you'd better choose the universal column connector to connect column inlet and the outlet, the connector is available from Dalian Elite).

2.6.5 Connecting the pump to the mixer

A mixer is required when the pump is used in gradient system. The mixer ensures the mobile phases came from different pumps be mixed well before entering HPLC systems. It is placed besides the P3500 pump. The outlet of pumps are connected to the inlets of mixer respectively, and the outlet of mixers is connected to port #2 of the injection valve.

You can choose different mixer (Mix volume: 1.5mL, 3.0mL and 4.5mL) according to the analysis. The larger volume, the better mix performance and the longer lag time in gradient system.

2.6.6 Solvent waste bottle

The solvent waste bottle should be place at a lower position with respect to the equipment.

2.7 System Composition

The following are two HPLC systems composed by P3500 pumps.

2.7.1 Isocratic system

Isocratic system is a relatively simple system, shown as figure 2-10. It can meet the requirement of analysis when the mobile phase is invariant. The configuration is listed in table 2-7.

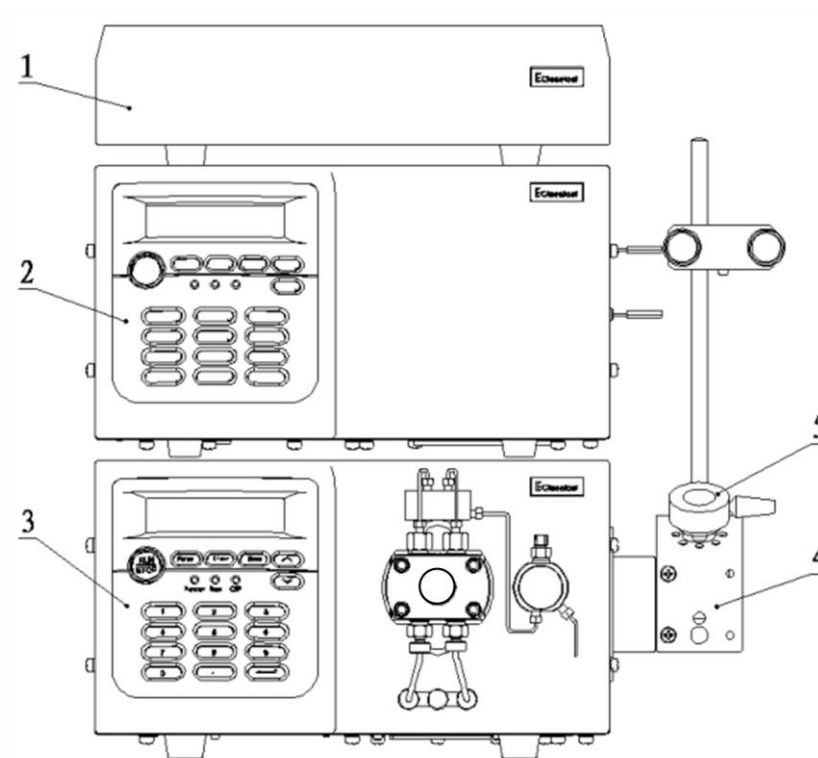


Figure 2-9: P3500 isocratic system

Table 2-7: The configuration of isocratic system

| No. | Components | Function |
|-----|----------------------------|--|
| 1 | TP3100 solvent tray | Used for placing solvent bottles. |
| 2 | UV3100 UV-visible detector | Detect the signal and control the other instruments. |
| 3 | P3500 pump | Transport the mobile phase |
| 4 | Valve bracket | Used for fixed injection valve. |
| 5 | Injection valve | Used for injecting the sample into the chromatographic system. |

2.7.2 Gradient system

The binary high-pressure gradient system is controlled by chromatography workstation, as shown in figure 2-11. It can realize that the composition of the mobile phase changing while analysis. The system can analyze complicated samples such as proteins, peptides, medicines and so on. The configuration is list in table 2-8.

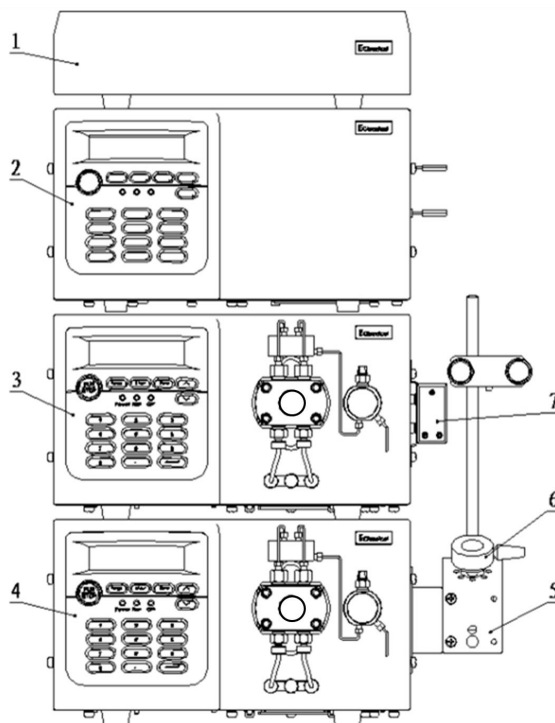


Figure 2-10: P3500 gradient system

Table 2-8: The configuration of gradient system

| No. | Components | Function |
|-----|-------------------------------|---|
| 1 | TP3100 solvent tray | Used for placing solvent bottles. |
| 2 | UV3100 UV-visible detector | Detect the signal and control the other instruments |
| 3 | P3500 pump A | Transport the mobile phase A |
| 4 | P3500 pump B | Transport the mobile phase B |
| 5 | Valve bracket | For fixed manual sampling valve and mixer (optional) |
| 6 | Mixer | Mix mobile phase A and B |
| 7 | Rheodyne7725i injection valve | Used for injecting the sample into the chromatographic system |
| 8 | Column | Separate the sample |



[Caution] Isocratic system or gradient system can be chosen according to the need.

All the instrument should grounded well.

The power socket for the pump should have grounding terminal.

In order to prevent the device charged and guarantee the stability of equipment operation, the best way is embedded outdoor ground (earth resistance is less than 2 ~ 3 Ω).

2.8 Verification

In normal instance, the instrument customers received have been tested and came with verification. the performance met our requirements in factory. Users have no need to test and verify. If you have any doubt about the performance of it, verify it refer to the following steps:

2.8.1 Check the sealing performance

- 1) Connect the chromatographic column properly, then, start the pump and observe whether the pressure displayed is stable. If not, please clean check valve or relief bubble in pump head.
- 2) Set the max alarm pressure to 30MPa. Close the outlet of the injection valve. For the valves that the infusion pipeline cannot be closed, you can connect the outlet of it to a two-way, then seal the other end of the two-way.
- 3) When start the pump, the pressure will raise slowly to 30MPa, and it will stop automatically. Observe the pressure drop on the screen.
- 4) If the pressure drops less than 1.5MPa in 10min, sealing of the pump is qualified.
- 5) If the pressure drops more than 1.5MPa in 10min, many factors should be considered, such as seals lax of check valve, sampling valve or piping joint, or air bubbles in the pump head.

Isocratic system

- 1) Take a chromatographic column. For the positive phase system SiO_2 column can be selected, while C18 column can be used for inverse system.
- 2) Prepare mobile phase and samples according to evaluation report provided by the column manufacturer.
- 3) Empty air bubbles in system. When the system is stable, detect the signal according to the testing requirement.
- 4) If the result and column efficiency is confirmed to the information provide by column manufacturer within the error range, that means the HPLC is qualified.

Gradient system

In addition to the sealing performance of each pump and the repeatability of the sample analysis, the gradient system should also run the gradient curve to test the gradient performance of the system.

- 1.The method for testing sealing performance of each pump is the same as 2.8.1.
- 2.Testing the gradient performance.

- 1) Take two 500mL solvent bottles labeled A and B.
 - 2) Bottle A is filled with purity water, ultrasonic degassing is needed.
 - 3) Add 1mL acetone in bottle B, ultrasonic degassing is also needed.
 - 4) Connect the outlet of injection valve and inlet of detector with a stainless steel tube(OD1/16 " ~ID0.04 " ,250mm in length).
 - 5) Set the detected wavelength to 254nm.
 - 6) Set the total flow rate to 10.0mL/min, 50% for each pump, flush until the baseline is stable.
 - 7) Set gradient parameters according to table 2-9.
- 3.Start to collect analysis data
- 4.Check whether the curve of each step is approximately perpendicular to the change, otherwise the gradient mixing is not ideal.

Table 2-9 Setting the gradient curve in workstation

| NO. | Time(min) | Flow rate (mL) | Pump A% | Pump B% | Gradient curve |
|-----|-----------|----------------|---------|---------|----------------|
| 1 | 0 | 10.00 | 100 | 0 | 0 |
| 2 | 2 | 10.00 | 80 | 20 | 0 |
| 3 | 4 | 10.00 | 60 | 40 | 0 |
| 4 | 6 | 10.00 | 40 | 60 | 0 |
| 5 | 8 | 10.00 | 20 | 80 | 0 |
| 6 | 10 | 10.00 | 0 | 100 | 0 |
| 7 | 12 | 10.00 | 100 | 0 | 0 |



[Warning] Before operating gradient curve, running pump A and B (50% of the total flow rate each) for 10 minutes, and then running A pump 100% of the total flow rate, B pump 0% for 10 minutes to ensure that the mobile phase in pump A and B has been fully replaced. For high pressure gradient control parameters, please refer to the operation manual of workstation. Because the P3500 high pressure constant flow pump is designed for semi-preparation, the analysis result may be slightly different from that of the special analysis pump.

2.9 Transportation

High pressure constant flow pump is a precision instrument, please be careful while long-distance transportation. Severe vibration and drops are likely to cause damage to the internal parts of the instrument. The original packaging can effectively protect the instrument. When the instrument is required to move or returned for service, please follow these steps for packaging.

Turn off the power.

Unplug the power cord and 485 line.

Remove the connecting pipe and other elements between components.

Remove the pump from chromatography system. Put it on a large platform, then put it into a special sealed bag.

Put the pump into the original packaging foam and fix it.

Place the fixed pump and other accessories into original packaging carefully.

Tape the box sealed to prevent liquid from entering. Covering the packaging box with plastic wrap is recommended.

Transport packaged instrument.



[Warning] Before packing, please check the box. If the original packaging has been damaged, do not use it. You should consult your local dealer or Our company customer service staff to solve!

3. Chapter Three: Basic Operation

3.1 Power On

- Plug the power cord into the power outlet. The power switch should be turned off at this time (“I” means power on, “O” means power off).
- Turn on the power switch.
- Power indicator lights, display screen is bright and P3500 pump begins to self-test. The VFD display sequence is as follows:

```

E C l a s s i c a l   P 3 5 0 0
V e r   1 . 0 0
    
```

After passing self-inspection, the following information will occur on the display screen, you can set or modify the parameters in this state, and then start analysis.

The following is working interface of P3500(the main interface).

```

F l o w       1 0 . 0 0       m L / m i n
P   1 0 . 0   M P a   P m a x   3 0 . 0
    
```



[Caution] If this is the first time you use the pump, the parameters displayed on the main interface are default ones. Otherwise, all parameters of the pump are maintained the previous parameters before last shutdown.



[Caution] The default parameters of P3500pump are list as follows:

Flow: 1.000mL/min;

Pmax: 10.0MPa;

Pmin: 0MPa;

Pressure unit: MPa.

3.2 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 which going to be modified. After typing the new parameter value, press "←" button to confirm. Then, press the "Run / Stop" button directly, the pump will run according to the new 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 come back to the value before the modification. Press the "Menu" button once to enter "MENU-1 Basic Operation" functional state. You can move into the setting interface of the flow rate, the maximum limit of the pump pressure, the minimum pressure by "↑, ↓" key and modify them in the corresponding interface. Then, you can type a new value directly when the cursor is flashing on the parameter you want to modify. Press "←" button to confirm.

F l o w : 1 0 . 0 0 m L / m i n



[Caution] Flow rate range: 0.01-50.00mL/min

P m a x : 2 0 . 0 M P a



[Caution] Pmax: ≤30MPa (flow rate: 0.01-20.00mL/min) Pmax: ≤20MPa (flow rate: 20.01-50.00mL/min)

P m i n : 0 . 0 M P a



[Caution] The work pressure of the pump is real-time detection. If the pressure less than Pmin in one minute from it starts or reaches Pmax, the pump will send an alarm signal and stop working. For all parameters, if the setting parameters beyond the scope, P3500 pump will not accept it and continue to maintain 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.

| | | | | | | | | | | | | | | |
|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| S | e | a | l | W | a | s | h | i | n | g | : | O | f | f |
| 1 | . | O | f | 2 | . | O | n | 1 | 3 | . | O | n | 2 | ? |



[Caution] The input number of the plunger rod washing menu indicate:

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

2(Light is green): clean the plunger rod continually,

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

There is no plunger rod washing function in P3500 pump, this part is reserved.

3.3 Advanced Operation

From the main interface, press MENU button to access **MENU-2 Advanced Operation**. Use ↑ and ↓ to access the setting interface of various parameters in MENU2, including the coefficient of the mobile phase, the velocity correction factor, A/B pump assigning and pressure unit. By typing a new value directly when the cursor is flashing to modify the parameters. Then press "←" button to confirm.

```

M E N U   2
A d v a n c e d   O p e r a t i o n
    
```

3.3.1 Setting pump A / B in Gradient system

P3500 pumps can be controlled from the front panel, you can also control them by a computer through our workstation software. Further gradient analysis, flow time program analysis and other functions can be found in the workstation too, it can meet the requirements of users in the field of production and research.

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

```

N u m b e r : P - A
1 . P - A . 2 . P - B . ?
    
```

3.3.2 Flow compressibility

Different solvents exhibit different compressibility characteristics and the compressibility of the same solvents may be different when the pressure change. The compressibility of the solvents in use will affect flow rate of mobile phase. In order to minimize this effect, the P3500 provides a compressibility compensation feature which optimizes the flow stability according to the solvent type.

The flow compressibility is set to a default value of 100% (using degassed distilled water as mobile phase) and can be changed through the user interface.

Measurement of the flow compressibility

Equipment: A measured 100mL volumetric flask, stopwatch, degassed solvent.

Procedure:

- (a) Disconnect the column and eliminate the backpressure of the system to zero.
- (b) Select a proper flow rate according to the analysis method. Press the “RUN/STOP” key to start the pump.
- (c) Start the stopwatch and begin to collect the mobile phase from the pump in the 100mL volumetric flask.
- (d) Record the time it takes to reach 100mL, t.
- (e) Repeat step (b) to (d) three times to get the average, t1.
- (f) Stop the pump and reconnect the column. Then repeat step (b) to (e). Calculate the time with column attached to the system, t2.

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

If the relative error is allowable, you have no need to change the compression coefficient. If the error is obvious, adjust the compression coefficient following the steps blow.

Adjusting the compressibility parameter

Enter the “MENU2 Advanced Operation” to adjust the compressibility parameter, input a new compressibility parameter and press "←" to confirm.

C o m p . F l o w : 1 0 0 %



[Caution] The range of the flow compressibility is 85 - 115%. If the entered flow compressibility value exceeds the range, the cursor will blink and the value will return to 100%.

3.3.3 Flow correction

Several factors will affect the accuracy of flow rate: the state of block filter and on-line filter, dirty check valve, the combination of plunger rod and seal ring etc. P3500 pumps are designed with the function of flow correction.

The method of the flow accuracy correction is the same as the flow compressibility measuring. If the flow accuracy is beyond the permitted standard, you should adjust the calibration parameter as below:

Enter the “MENU2 Advanced Operation”, press “↑、↓”button to get into the flow correction interface. Then input a calibrated parameter and press “←” to conform.

C a l i b r a t e : 1 0 0 %



[Caution] The range of the flow correction is 85 - 115%. If the entered flow correction value exceeds the range, the cursor will blink and the value will return to the previous one.

The accuracy of flow rate may be affected by the block of solvent filtration head, on-line filter, pipe and so on, please check them carefully before adjusting the flow correction coefficient.

It is necessary to ensure that the accuracy test method is reliable, or the modification of flow correction coefficient may cause a large deviation of the analysis result.

3.3.4 Pressure unit

P3500 pump can display pressure in three different units: MPa, bar and psi.

$$1 \text{ MPa} = 10^6 \text{ Pa} = 10 \text{ bar} = 145.04 \text{ psi}$$

Enter the “MENU2 Advanced Operation”, press “↑、↓”button to get into unit interface. Then chose the unit you need and press “←” to conform.

P r e s s u r e U n i t : M P a
1 . M P a 2 . b a r 3 . p s i ?



[Caution] Select “1” for MPa, “2” for bar and “3” for psi.

3.4 Mobile phase replacement

There are various liquids used in HPLC as mobile phase, including organic solvents, water, buffers, etc. Intermiscibility and buffer salting out are important problems should be considered. Please take care when you need to replace mobile phase. If necessary, you can refer to the corresponding physical chemistry handbook.

3.4.1 Replacing with miscible liquid

E.g. replacing methanol with acetonitrile

- 1) Store acetonitrile in a clean solvent bottle.
- 2) Take the filter components out from methanol reservoir, wash it with acetonitrile, then, put it into acetonitrile.
- 3) Open the relieve valve. Flush the pump with approximately 20mL of the new mobile phase.
- 4) Close the relieve valve, disconnect the column from the system, then, put a bottle at the outlet of the inject valve.
- 5) Turn on the pump and pump the new mobile phase out about 10 mL.
- 6) Reconnect the column. Turn on the pump at a suitable flow rate. The system need to equilibrate for at least 30 minutes.

3.4.2 Replacing with immiscible liquid

E.g. replacing methanol with n-hexane

Choose an intermediate washing liquid about 500 mL, which must be miscible with both the new and old mobile phase. In the above example, iso-propanol can be used as the intermediate washing liquid, because iso-propanol is miscible with both methanol and n-hexane.

Follow the procedures as described in section 3.4.1 using the intermediate washing liquid to replace old one.

Repeat the procedures as described in section 3.4.1 using the new mobile phase to replace intermediate washing liquid.

3.4.3 Replacing organic solvent with salt buffer

Crystalline salt may appear when buffer salts mix with an organic solvent. Crystalline salt is harmful to the seals, the plunger rod, the injection valve and the internal rotor and stator, moreover, it may block the tube. So, please transition the system with a certain proportion of water-containing organic solvent before analysis. After the experiment is finished, do transition the system again with the same method above, and save the column with organic solvent.

4. Chapter Four: Maintenance and Repair

4.1 Troubleshooting

Troubleshooting a pump can be frustrating and sometimes a mysterious process. This section outlines a common sense process for troubleshooting a pump. If troubles can not be solved when referring to this section, please contact Dalian Elite post-sales service or local vendor.

4.1.1 Elimination of Air Bubbles

Air bubbles are likely to be trapped in pumps under the following circumstances: the initial use of pump, the pump has been left idle for some time, improperly degassed mobile phase etc. It is crucial to eliminate these trapped air bubbles. Otherwise, they affect system operation in a number of ways, such as: pressure fluctuation, unstable flow rate, and damage the column.

How to eliminate trapped air bubbles

Open the relief valve and press “PURGE” key, flush with high flow rate to remove air bubbles in pump head and inlet lines.

If the pump is unable to draw mobile phase after PURGE key was pressed, use a syringe to withdraw mobile phase from outlet of relief valve until the liquid flow out.

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

4.1.2 leakage of pump head

Pump head leakage is a common problem. Seal damage in the pump head is the main cause of leakage. Therefore, when the pump discharge occurs, replace worn parts in time.

4.1.3 Overpressure

In the process of operation, real-time pressure monitoring system works. When the pressure exceeds the set maximum pressure, P3500 pump will automatically stop and alarm. Open the vent valve to release the system pressure is a good choice at this time. Then reset the pressure cap, or check the entire flow path to look for the reason of the surplus pressure.



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

4.1.4 Pump not delivering solvent

Pump not delivering solvent is usually caused by air bubbles in pump head or blocking in solvent filter. In general, the problem can be solved by eliminating air bubbles or cleaning solvent filtering cup. But a dirty check valve can also cause the problem. So if the pump does not suck liquid after filtering and cleaning solvent cup, please clean the check valve. Cleaning check valve refers to the related contents in the user manual. Common failures and solutions are shown in table 4-1.

Table 4-1: A summary of most common problems affecting system operation

| Symptoms | Cause | Solutions |
|---|--|--|
| Infusion instability and the pressure fluctuation | Bubbles in pump head | 1.Eliminating air bubbles through the relieve valve. 2.Spare bubbles with a syringe through the relieve valve. |
| | The old solvent remained in the pump cavity | Increase the flow rate to replace old solvent from the relieve valve. |
| | bubbles in the solvent filter and the inter line | 1.Vibration the solvent filter to eliminate air bubbles. 2.If the solvent filters are dirty, clean them with ultrasonic cleaner or replace solvent filters. 3. Degas the mobile phase. |
| | Something wrong with the check valves | Clean or change the check valve. |
| | Piston rod or seal leakage | 1.Replace piston rod and seal. 2.Change the faulted part. |
| | Pipeline leakage | 1.Tighten the screw where leakage occurs 2.Replace the failure parts. |
| | Pipeline jam | Clean or replace the tube. |
| Pump is running, but no liquid out | Air bubbles in the pump cavity | 1.Eliminating air bubbles through the relieve valve. 2.Spare bubbles with a syringe through the relieve 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 pump head, open the relieve 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 exchange the check valve. |
| | No solvent in the reservoir | Fill up the reservoir. |

| Symptoms | Cause | Solutions |
|---|--|--|
| The actual flow rate is lower than the set value | Abnormal check valve | Clean or exchange the check valve. |
| | Dirty filter | Clean or exchange 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 power supply. |
| Flow rate decreases when the pump is running | Air in the pump is gathering | Open the relieve valve, let the pump run under high velocity to eliminate air bubbles. |
| | Solvent filters plugged | Open the cap of the pump head, if there is no liquid out from infusion tube quickly, it means solvent filter is plugged. Please clean or replace the filter. |
| | Immiscible solutions in the pump | Use transition solvent to dissolve Immiscible ones. |
| | Plunger seal leakage | Inspect and replace the piston rod and seal. |
| | Compression compensation failure | Check or replace the pressure board or pressure transmitter. |
| Flow rate is too high | The velocity compensation failure | Inspect or replace it (see instructions). |
| | P.C. board failure | Replace the P.C. board. |
| | Compression compensation failure | Inspect or replace it. |
| High pressure | Tubing blockage | Screening each section of pipe to find blocking part. Replace or cut off the block tube. |
| | Improper pipe diameter | Replace it with proper tube. |
| | Online filter blocking | Clean or replace filter sieve. |
| | Chromatographic column plugged | Replace the chromatographic column. |
| No/Low pressure | No solvent in reservoir | Fill up the reservoir. |
| | Vent valve is not tight | Tighten the relieve valve. |
| | Pump pressure sensor fault | Check or replace the pressure sensor. |
| | Pipe joint leakage | 1.Tighten the screw or ferrule where is leaking. 2.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 relieve valve and let the pump run under high velocity to eliminate air bubbles. |
| The pump has a humming sound and can not start properly | The motor failure | Stop the pump and check it. |
| | Phase voltage is too low | Phase voltage is too low. |

| Symptoms | Cause | Solutions |
|--|---|---|
| Flow rate is zero | Air bubbles in pump head | Eliminate the air (refer to the above item). |
| | The entrance check valve plugged | Check and replace it. |
| | The export check valve plugged | Check and replace it. |
| | The direction of check valve is reverse | Install the check valve in the right direction. |
| The pump stops in operation. | Pressure is higher than limit pressure | a) To reset the highest limit pressure. b) Replace the column. c) Replace tubes with proper ones. |
| | Power cut | a) Check the power supply. b) Shut off the main power supply, check the fuse. |
| The pump is on, the pressure is common, but no liquid comes out. | Serious leakage in the system | Repair injection valve or pipeline and fasteners between the pump and detector. |
| | Fluid passage blockage | Clean injection valve or the connection between the column and detector or particles in flow cell. |
| | The entrance of column is plugged with particles | Clean or replace the column inlet filter, or replace the column. Degas mobile phase and samples properly. |
| The column pressure increases, but the flow rate decreases. | Column or protect 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. |

4.2 Maintenance and repair

In order to ensure the normal operation of the high pressure constant flow pump, maintenance and repair is important. Maintenance means simple repair. Such repairs can be done from the front panel of the pump. There is no need to take out the pump from the stacked configuration. And repair is mainly referred to those which need to change the internal parts and remove the pump body from the stacked configuration and disassemble.

4.2.1 Maintenance of solvent filters

Dirty reservoir and mobile phase contamination is likely to cause blockage to solvent filter. Also, using buffer for a long time may lead to filter pollution and flow impassability. The blockage, pollution and impassability will affect pump operation. Recommended practices can extend the service life of the filter and guarantee the normal work of the pump. Replacing colorless reservoir with brown reservoir is an effective way to prevent fungi biological from growing.

- Filter all solvents through a 0.45 micron (or smaller) filter.
- The aqueous mobile phase needs to be replaced or filtered often.
- If it is allowed, you can add 0.0001 to 0.001 mole of sodium azide in the mobile phase to prevent the growth of fungi

When the solvent filter is blocked seriously, air bubbles will appear in infusion tube even if the mobile phase was degassed. Please clean the solvent filter at this time. Cleaning steps are as follows:

- 1) Remove the solvent filter from the bottle-head assembly and immerse it in a beaker with 30% nitric acid (v/v). Sonicate for 15 minutes.
- 2) Thoroughly rinse the solvent filter with distilled water. Sonicate the solvent filter in distilled water for 10 minutes.
- 3) Blow-dries the filter.
- 4) Sonicate the solvent filter again in distilled water for 10 minutes.
- 5) Blow-dries the filter again.
- 6) Reassemble the solvent filter, and put it into mobile phase bottle.

4.2.2 Cleaning the check valve

Pump outlet and inlet are equipped with check valves. The components are the same, so a check valve component can be used for any end of the pump. The appearance of the check valve is shown in figure 4-1.



Figure 4-1: Appearance of the check valve

Usually, check valves have no need to clean. If sediment occurs in valve or valve ball and seat is adhesive, cleaning should be carried out.

Overall cleaning:

When the check valve has been assembled completely, overall cleaning can improve the adhesion condition.

- 1) Take out the check valve at the pump entrance: remove infusion tube from the entrance pressure cap, unload entrance pressure cap using a wrench and take the check valve components out.
- 2) Take out the check valve at the pump exit: remove infusion tube above pump head with a wrench, then, unload the pressure cap and take the component out.
- 3) Put them into ethanol and clean with ultrasonic twice (15 min each time) Then blow off the solvent inside.
- 4) Assemble the cleaned check valve properly while pay attention to the direction of the check valve.



[Caution] The entrance of the check valve has ring mark near by.

If overall cleaning can not solve the problem, deep cleaning becomes necessary. Cleaning steps are as follows:

Deep cleaning:

- 1) Unload the pressure cap with a wrench. Take the check valve out.

- 2) Make wallpaper knife into the gap between the coat and the gasket of the valve. Take the valve ball, seat and seal components out carefully. Put them into ethanol and clean with ultrasonic.
- 3) Observe the valve ball and seat with magnifying glass. If the valve ball or seat is damaged, please change a new one.
- 4) Assemble the cleaned check valve referring to figure 4-2. Roughness of different sides of the valve seat is not the same I Identify them carefully before installation (the gem ball seats the smooth side).
- 5) Blowing is a good method to check the correction of the installation. Using an aurlave to blow the check valve from the inlet where have a ring mark near by. You can feel airflow at the other side. Otherwise, air can not pass through.



[Caution] It is suggested using a small container during cleaning to avoid the loss of the valve components.

Each group of the valve ball and seat is grinded in pairs, therefore the components cannot mix between different valves.

The system should be washed with methanol and deionized water successively if no one uses the pump for more than one month, or troubles may occur.

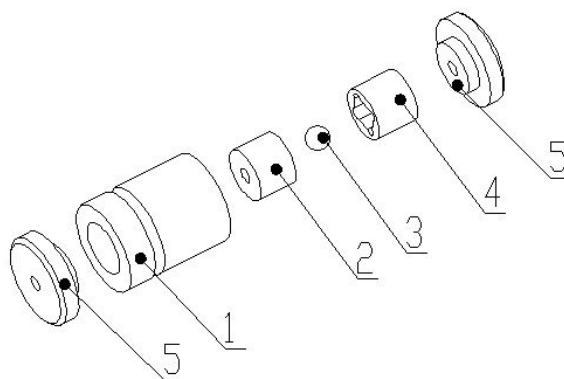


Figure 4-2: Assembly of the check valve

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

4.2.3 Cleaning the online solvent filter

In order to prevent the particles in mobile phase coming into chromatographic system, the pump is installed an online filter in the vent valve to filter the mobile phase coming out from the pump outlet. It is recommended that users should clean the online filter in time. Use a wrench to unload the pressure cap, remove the sealing ring and the sintered stainless steel filter, and then clean them.

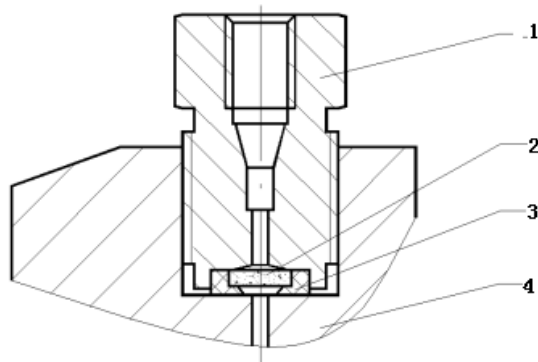


Figure 4-3: Assembly of the online filter

1.Pressing cap, 2.Filter disc, 3.Sealing ring, 4.Release valve

- 1) Remove the filter disc. Immerse it in a beaker with 25% nitric acid (v/v). Sonicate for 15 minutes.
- 2) Thoroughly rinse the solvent filter with distilled water. Sonicate the solvent filter in distilled water for 10 minutes.
- 3) Blow-dries the filter.
- 4) Sonicate the solvent filter again in distilled water for 10 minutes.
- 5) Blow-dries the filter again.

4.2.4 Cleaning the pump head

Crystalline salt may appear when using buffer salts for a long time. Crystalline salt is harmful to the seals and plunger rod. In order to avoid this situation, cleaning should be carried out frequently on the flow path and sealing ring. Customers can prepare a suitable cleaning fluid (such as distilled water and 10% methanol, 20% ethanol aqueous solution, etc.).

4.2.5 Pump Head Assembly

There is no need to open the case cover when removing the pump head. Methods for pump head disassembly and installation are as follows:

- 1) Turn off the pump power.
- 2) Remove the infusion line connected with the pump.
- 3) Use 3# hexagonal key (Allen key) to unscrew the 4 pump head screws and remove the pump head body.
- 4) When installing the pump head, pay attention to evenly tighten the four fixed screws, referring to figure 4-3.
- 5) Connect the infusion line removed in step 2 above.

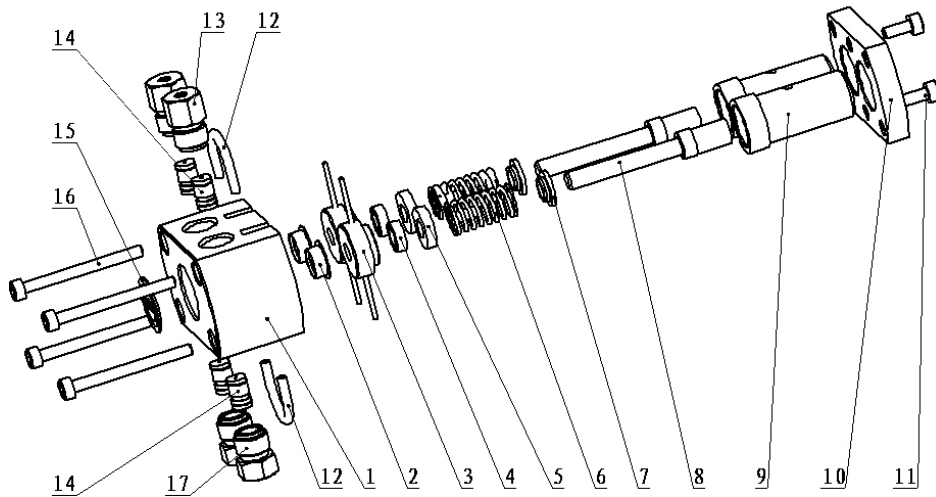


Figure 4-4: pump head assembly

1. Pump head, 2.Primary seal, 3.The positioning sleeve with flush pipe, 4.The secondary seal, 5.Spring outer positioning sleeve, 6.Plunger spring, 7.Spring inside locating sleeve, 8.Plunger rod set, 9.The sleeve , 10.Clamp, 11.Screw , 12.Washing tube, 13.The outlet clamp, 14.Check valve, 15.The badge on pump head,16.Screw, 17.Entrance pressure cap

Pump head disassembly and installation methods are as follows (Refer to figure 4-3, figure 4-4):

- 1) Place the pump head body on a flat surface. Use 2.5# hexagonal key to unscrew the 2 screws, #11 on Figure 4-4. Remove the clamp(10#).
- 2) Unload the sleeve (figure 4-4 #9), plunger rod components (figure 4-4 #8), plunger spring(figure 4-4 #6), locating sleeve(figure 4-4 #3) etc.
- 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.
- 4) Take out the primary seal carefully (figure 4-4 #2)(user should not remove it unless replace a new one is needed).
- 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 4-4 #1).

Place all parts in order according to figure 4-4. Install the pressure plate.

- 7) Put the pump head on worktable, push the pressure plate slowly, press the two pressure plunger rod into the primary sealing ring, and then tighten two screws evenly.

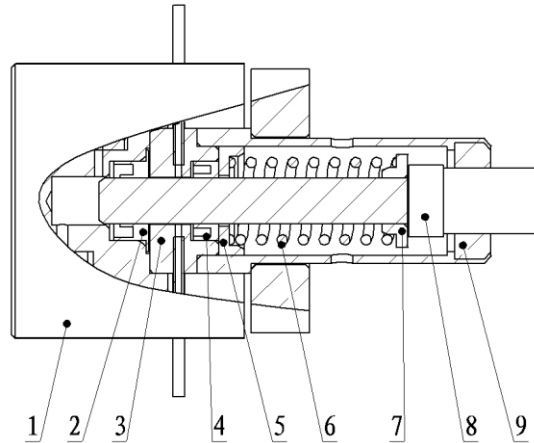


Figure 4-5: Cross-section of the pump head

1.Pump head, 2.Primary seal, 3.The positioning sleeve with flush pipe, 4.The secondary seal, 5.Spring outer positioning sleeve, 6.Plunger spring, 7.Spring inside locating sleeve, 8.Plunger rod set, 9.The sleeve



[Caution] Plunger rod is easy to break. Be careful while unloading and assembling.

4.2.6 Plunger seal replacement

Plunger and plunger seal will wear-out during long-term use. It is mainly related to the flow rate, the operating pressure and the kind of mobile phase. The impact of these factors is uncertain, so estimating the wear-out degree of those parts is difficult. It is necessary to check the surface of the plunger rod when replacing piston ring. If there is an even small scratch on the surface, please replace it. The method for plunger disassembly and installation can refer to “4.2.5 pump head assembly” above.

4.2.7 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 referring to figure 2-6.

After replacing a new fuse, lift the fuse lid.



[Caution] Please use the same model fuse. Fuse model: T 1.25A/250V.

5. Chapter Five: Components and Material list

5.1 Consumption parts

| NO. | Describe | PN |
|-----|---|------------|
| 1 | Mobile phase bottle, transparent, 500mL | 33110001 |
| 2 | Mobile phase bottle, transparent, 1000mL | 33110002 |
| 3 | Mobile phase bottle, transparent, brown, 500mL | 33130008 |
| 4 | Mobile phase bottle, transparent, brown, 1000mL | 33130009 |
| 5 | PTFE tube O.D.1/16"~I.D.0.04" | 12010009 |
| 6 | Solvent filter | 18040009 |
| 7 | Green Flangeless-Nuts | 3215P-305X |
| 8 | Yellow Flangeless-Ferrules | 3215P-300X |
| 9 | 3/16"-32 Stainless steel connection screws | 14510027 |
| 10 | 1/16"Stainless steel ferrules | 14990070 |
| 11 | PEEK Finger Tight I | 3215F-120X |
| 12 | Type A number tube | 19000072 |
| 13 | Type B number tube | 19000073 |
| 15 | Power line | 17000001 |
| 16 | RJ11-485 line | 82000020 |
| 17 | T1.25A/250V fuse | 15080015 |
| 18 | Waste tube | 13010033 |

5.2 Replacement Parts

| NO. | Describe | PN |
|-----|-------------------------------|----------|
| 1 | Grounding line column | 14010081 |
| 2 | O-rings $\Phi 3.5 \times 1.8$ | 14992280 |
| 3 | plunger rod | 14010011 |
| 4 | check valve component | 14010012 |
| 5 | primary seal | 14990084 |
| 6 | secondary seal | 14990075 |

Appendix

Introduction for tube materials

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

Good connection is an important way to fully exert the function of the instrument and improve the work efficiency. Different pipeline material is needed according to the system pressure and the properties of mobile phase and samples. There are several commonly used materials including stainless steel, polyether ether ketone (PEEK), teflon, polytetrafluoroethylene vinylidene fluoride, polyethylene or polypropylene. The stainless steel pipe is the most common one.

Outer diameter of HPLC system connecting pipe is 1/16"(1.59mm). Inside diameter can be chosen according to your need. The regularly used inside diameters includes 0.007"(0.175mm), 0.01"(0.25mm), 0.02"(0.5mm), 0.03"(0.75mm) and 0.04"(1.0mm) ect.

Stainless steel tube is generally used in high pressure part. In HPLC systems, the part from the pump to the column inlet is high pressure section. Stainless steel tube is recommended.

Stainless steel tube has good corrosion resistance and coaxiality. The bore of the pipe should match that of the fitting well while using it.

Also, polymer tubing can be used in many sections of HPLC systems, such as low-pressure parts: from the liquid bottle to the pump, the detector outlet, the sampler drainage port, the emptying valve outlet and others. Teflon is inertial to HPLC solvents and is the most commonly used plastic pipe.

When the pressure is lower than 20MPa, peek tube is lazier than stainless steel tube and it is suitable for biological sample analysis.


Safety information

General safety information

At the different stages of the instrument operation, maintenance and repair, everyone should abide the following general safety rules. Breaking these rules may cause damage to instruments or staffs. Our company does not responsible for the impact caused by non-standard operation.

Standard of security

The safety class of this equipment I (to provide protection grounding terminal), and it is manufactured and tested according to national safety standard.

| Symbols | Descriptions |
|--|---|
|  | Before using equipment marked with this symbol, please refer to the instruction manual first to avoid harm to the operator and the equipment. |
| [Warning] | Casualties may appear. Please do not operate beyond the scope of warning, unless you have fully understood and met the required conditions. |
| [Caution] | Data loss or equipment damage may appear. Please do not operate beyond the scope of caution, unless you have fully understood and met the required conditions. |
| [Note] | Unsatisfactory experimental data and instrument failure may appear. Please do not operate beyond the scope of note, unless you have fully understood and met the required conditions. |