HPLC-EC D Stand Alone System
(HTEC-500)
User's Guide

Eicom Corporation
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1. Important Checklist Before Operation

- The separation column and the enzyme column must be held firmly by the column clip. The stainless steel surfaces should make good contact with each other in order to ground static electricity. This may be the cause of noise in the ECD.

- Place the mobile phase container and the waste container on the same level as the HTEC-500.

- Use glass bottles as mobile phase and ultrapure water containers. Do not use any plastic containers.

- Rinse behind the pump pistons with ultrapure water before and after use through the pump piston washing port. Do not use any organic solvents in the rinse solution. If precipitation or something blocks the line, do not push out with high pressure or force. First, disconnect the line and then remove the source of the blocking.

- Before opening the detector cell for maintenance, remove the outlet screw and aspirate all the remaining mobile phase in the detector cell with a syringe.

- Pay attention to prevent any tubing from becoming blocked. Salt solvents must be rinsed out by ultrapure water before an organic solvent of a high concentration is used. Drying out of the salt solvent should also be avoided.

- Pay attention not to allow any leakage from any connection points of the tubing. Leakage is not only harmful for the equipment but also generates pump pulse noise.

- When air is thought to be present in the tubing or the pump, please purge the air (see chapter 8, page 11). Then you can connect a column.

- Pulse free mode must be switched on during the analysis.

Fig. 1 Separation Column Installation

A separation column must be held firmly by the column clip. When an enzyme column is used, the enzyme column must also be held firmly by the column clip. The respective stainless steel surfaces should make good contact in order to ground static electricity. This can be the cause of noise in the ECD.
2. Terminology

Fig. 2 HTEC-500 Front View (Door open)

Fig. 3 HTEC-500 Front View (Door closed)
3. A List of Fitting Parts and Directions for Use

**Tough Connector**
Low pressure part. For column connections.

**Hex Nut**
High pressure part (pumps)

**Flat Seal Nut**

**Flat Seal for Degasser**
The wide side is for the degasser and narrow side is for the nut.

**Two-way Joint**
Connect the tubing and keep its tip sticking out of the ferrule (as seen above), except for the connection with the degasser which uses a flat seal. First, put the tubing through the ferrule and put its tip into the holder. Then connect it by tightening the screw. The connecting part should be sealed by a cross section of the tubing and the holder of the tubing. For the injector connection, use the nuts and ferrules for its connection only. The slit side is for the nut (see figure).

**Rheodyne PEEK nut**
**Pheodyne PEEK Ferrule**
(For Injector)

**Nut for Inlet of Cell**

**Ferrule for Inlet of Cell**
The narrow side is for the cell and the wide side is for the nut.

Fig. 4  Tubing Connection Parts and Directions for Use
4. Mobile Phase Preparation

Prepare the mobile phase according to the Eicom technical information. The mobile phase preparation depends on what you are measuring with the HTEC-500. Please read below to find a general method for mobile phase preparation. Also, the following points should be noted because the ECD has a higher sensitivity compared to other detectors:

- Use ultrapure water (the resistance of the water needs to be at least 18.2 mega-ohm/cm). Any solvent, such as mobile phase, or water applied to this system should be stored in a glass bottle.

- Use glass instruments for the preparation of mobile phase. Before use, these instruments should be rinsed out by water and then by ultrapure water.

- Use reagents of super grade or HPLC grade quality. The following are our recommendations: for sodium 1-Octanesulfonate, use Nacalai (code 3179-62, available at Eicom); for sodium 1-Decanesulfonate, use TCI (code I-0348 available at VWR); and for EDTA-2Na use Dojindo (code 372.24). Separation patterns or ECD can be influenced by the use of other reagents.

- Methanol and buffer solution/water must be measured separately and then mixed. Otherwise the methanol concentration will be higher than the designated level due to the solubility of methanol in water.

- Do not use a sonicator for dissolving salts in the mobile phase. This may raise the background current of the ECD.

- When all reagents are added, plug the bottle and shake and mix it thoroughly. Check if all reagents are dissolved.

- Filtration is good for removing unwanted particles but can be a source of chemical contamination. We do NOT recommend you to filter the mobile phase but rather encourage you to avoid any contamination during the preparation of the mobile phase. Using less glassware and always putting a cap or cover on the top of the containers helps to reduce chances of contamination. If you must filter the mobile phase, use a chemically clean apparatus and do not store the mobile phase in plastic bottles after the filtration.

- A filter at the end of the inlet tubing placed in the mobile phase can also be a cause of chemical contamination or bacterial growth. Eicom does not recommend you to use these type of filters. If you need to use one, please replace the filter frequently and keep it clean. Instead of these filters, we recommend you to set a precolumn before the separation column. (If using the PP-ODS column, please use in-line filter #PP-LF, before the precolumn and put a precolumn before the injectors.) The precolumn filter after the HPLC pump does not prevent contamination of the HPLC pump.

- Mobile phase can only be used for one week from the date of opening/preparation. Bottles should be well sealed while it is being used for HPLC. Do not add new mobile phase to the bottle of old mobile phase. This is to avoid the breeding of various germs in the system.
5. System Installation

5-1. Wiring

Connect the terminal adapter (orange color) to the terminal at the right side of the system. Then connect the AC cable to the main power supply and turn on the system. “Initializing” appears on the display, followed by the ECD controller screen. Now you can turn off the system for the next steps described below.

5-2. Tubing and Degasser

Pull the tubing’s for the pump rinse, the mobile phase suction and the cell drain out of the HTEC-500 system. Put all the equipment on a level place. Mobile phase and waste bottles should be placed on the same level as the HTEC-500 (See Fig. 5).

5-3. Connecting to the Autoinjector and the Autosampler (Plumbing)

Injectors of the autoinjector (online autoinjector, EAS-20) and/or the autosampler should be set up downstream to the manual injector. For the autoinjector, connect no. 3 port of the manual injector with no. 2 port of the autoinjector using 0.125 mm PEEK tubing (red). Then connect no. 3 port of the autoinjector with the separation column using the same kind of tubing. For the autosampler (model Alcott 719D or 719AL), connect no. 3 port of the manual injector with no. 2 port of the autoinjector and no. 3 port of the autoinjector with the separation column.
A trigger signal needs to be sent from the online autoinjector or the autosampler to the data acquisition unit to inform it of the injection timing. It also needs to be sent to the HTEC-500 in order to give the auto zero signal. The auto zero works to shift the baseline to the zero level. Please connect the trigger signal output on the autoinjector/autosampler with “signal in” of the terminal adapter located on the left side of the HTEC-500. Please also refer to Fig. 6. There is no polarity on “signal in”. To find where the output of the trigger signal is, please look at the user’s manual of your autoinjector/autosampler. If your autosampler has an “emergency stop input”, please connect this terminal of the autosampler to the pump error out on the HTEC-500 signal terminal. A non-polar contact closure signal will occur when the pump on the HTEC-500 gives error readings (see chapter 8, page 12 for the errors). This way you can prevent loosing samples when the HPLC pump HTEC-500 accidentally stops.

Please follow this diagram to figure out the electrical connections. The ports not showing +/- have no polarity. AUTO ZERO and SIGNAL OUT 0 sec are connected before installation. With this connection, M.INJECTOR SIGNAL IN receives a signal when the manual injector is set to the inject position from the load position and then SIGNAL OUT 0 sec sends a signal to AUTO ZERO IN. Through this signal, AUTO ZERO is complete. SIGNAL OUT 3 sec sends a signal to the trigger port of the EPC-300 (not included in this system). Through this signal, a chromatograph starts to be recorded. PUMP ERROR OUT is a signal port to stop the auto sample injection from the auto sampler when the pump flow is not good. For more details, please refer to the instruction manual for the auto sampler. PUMP PRESSURE and TEMPERATURE MONITOR are ports to record operation condition of this system.
6. Online Elution Degassing System

The degassing component works to remove dissolved air from the mobile phase. The dissolved air occasionally interferes with the HPLC pumping or electrochemical detection. To prevent unexpected problems with the analyses, always use the degassing system. Mobile phase goes through the system when the pump is running and it is automatically degassed. Please note that the degassing system does not work to remove an air segment(s) from the inlet tubing.

The degassing component consists of a bunch of polymer capillary tubings located in a vacuum chamber. The dissolved gases in the solvent are pulled through the tubing walls by the differential pressure. To reduce the pressure in the chamber, a vacuum pump runs intermittently and a valve opens and closes the connection between the low pressure chamber and the vacuum pump. This valve generates a clicking noise.

Two Channels, 300 μl and 7.5 ml
Two channels (upper and lower) are installed in the online degassing system. Either the 300 μl channel or the 7.5 ml channel can be used. The 300 μl channel is more efficient at exchanging the liquid in the degassing component. At a flow rate of 500 μl/min of mobile phase, the degassing efficiency is the same between the two channels. There is no independent switch to turn on and off the degassing system. The main power switch regulates the power of the degassing system.

6-1. If you think the degassing efficiency is reduced, you may diagnose the problem by the following procedure:

• Replace the mobile phase at the end of the inlet tubing of the system with ultrapure water.

• Turn off the pump and use a syringe to aspirate 5 ml of mobile phase from the pump drain valve and then close the valve. Leave the system off for 10 min. It is not necessary to remove the columns.

• Turn on the main power and listen to the degassing pump noise (not the HPLC pump). The pump is supposed to stop in a few minutes. The pump has a function to create a vacuum chamber inside of the degassing system to allow dissolved air to go through the tubing inside.

• If the degassing pump stops within 4.5 min, the degassing system is functional.

• If the pump works for 4.5 min continuously, the degassing system is likely to have a mechanical problem. Please time precisely for 4 min. If the degassing pump runs for 5 min, the pump will stop automatically to prevent overrunning regardless of whether the degassing component is working properly or not.
Pay attention to the following points about the pump operation:

- Rinse behind the pump pistons with ultrapure water before and after use. Do not use any organic solvents in the rinse solution. It is common that an air bubble or air segment may appear in the piston wash line but it does not affect any functions.
- The mobile phase container should be a clean glass bottle. Do not use a plastic container.
- Put the mobile phase container on the same level as the HTEC-500.

Fig. 7  Pump Parts and Drain
The drain valve and the drain port are integrated. Connect a syringe to the drain port (as in the figure above) and turn the drain valve a half circle. Use the syringe to suck out some mobile phase.

Fig. 8  Pump Details
7-1. **Run/Stop**

Use the pump key to turn the pump on and off. When the pump is running, the pump light should be on at the front of the system. Also, *PUMP* on the liquid crystal display (LCD) indicates the pump is running, while -PUMP- indicates the pump is off.

7-2. **Flow Rate Setting**

- Select the pump monitor by pushing the display key (the display key switches between the ECD and the PUMP screen).
- Push the edit key once. ‘ ’ appears beside the flow rate (μl/min).
- Enter the adequate number from 1 to 750 using the 10 number keys.
- Push the edit key once more. ‘ ’ should now be beside pulse free mode.

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**Fig. 9 Display**

- These bars indicate that the ECD is turned off.
- These bars indicate the pump is running.
- Indicates the selected setting. Moves and makes changes using the edit key.
- Display can be switched between these two screens using the display key.
- Indicates the thermocontroller is ON.
- Actual temperature in the cabinet
- Current status
- Setting applied voltage (use the 10 keys for setting)
- Most sensitive at 1.0 sec.
- Noise is reduced at 3.0 sec. Select using the select key
- Indicates the thermocontroller is ON
- Use the 10 keys for setting
- Setting flow rate. (Use the 10 keys for setting)
- ON/OFF using the select key
- Cabinet setting
- Setting flow rate. (Use the 10 keys for setting)
- Use the 10 keys for setting
### 7-3. Pulse Free Mode Setting

- After setting the flow rate, turn the pulse free mode on or off by pressing the select key.

- By pressing the edit key once more, the pulse free mode is set on or off and the cursor (‘■’) is moved to the next line.

### 7-4. Function of Pulse Free Mode

A. Pulsation Quenching

The HTEC-500 is equipped with a pulse free mode. This is a pulse quenching system. A computer detects pulsatile flow as the pressure changes (this information is provided by a pressure sensor) and controls the pistons movements during each stroke but does not influence the stroke cycle or the flow rate. This feedback system works continuously when the mode is on. The pulse noise level and the pistons movements converge in a constant period. When the pressure changes slightly due to changes in the mobile phase conditions, temperature and so on, this pulse free mode improves the pistons movements. The latency of this convergence is shorter at higher flow rates.

B. Flow Rate Compensation

Always turn the pulse free mode off before starting maintenance, exchange of mobile phase, purging and so on, because the flow rate is different when the pulse free mode is on and when it is off. Generally, the flow rate decreases under high pressure in a flow system. In order to compensate for this speed loss, the pistons cycles are faster under high pressure when the pulse free mode is on rather than when it is off. To avoid inconsistent retention times between pulse free mode on and off, always have the pulse free mode on when you run the pump for sample analyses.

When the pulse free mode is on and a rapid pressure change occurs, the pump stops automatically and signals to the connected autosampler to stop its operation. On the other hand, when this mode is off, the piston always moves at a constant speed and passage. Even if the pressure changes, the pump does not stop. When the pulse free mode is off, Error 1 may occur but Error 3 and 4 do not occur. For details about pump errors, please see the next chapter.
8. How to Fix Pump Errors and Problems

8-1. Less Flow Rate, Less Pump Pressure or Unstable Pump Pressure

There are a few possibilities to resolve these problems. Please try A to C as follows:

A. Remove Air from Pump Heads and Check Valves

If the pressure is unstable or lower than normal for the set flow rate, a small trapped air bubble in one of the pump heads or the check valves could be the cause of this problem. To remove the air bubble, please follow these steps:

1) Stop the pump.
2) Disconnect the pump inlet tubing at the outlet of the degasser.
3) Run the pump.
4) Open the pump drain valve and aspirate mobile phase slowly by using a syringe from the drain port so that the pump inlet tubing gets a half an inch of an air segment in the pump inlet tubing.
5) Connect the pump inlet tubing back to the degasser. (see Fig. 11)
6) Aspirate the air you have inserted into the tubing along with mobile phase into the syringe. (see Fig. 12)
7) Close the drain valve.
8) Wait for a few minutes and see if the pump pressure goes back to the previous level and is stable.

If the problem does not get resolved, repeat these steps a few times and the pressure should become normal.
B. Remove Air from Tubing Going to the Column
   If these above steps don’t work, another cause of this problem could be that an air bubble(s) may be trapped in the tubing between the pump and the (pre)column. To solve this, disconnect the tubing upstream of the precolumn and pump mobile phase through until the air bubble(s) comes out.

C. Wash the Pump
   If the problem still persists, methanol is also effective in solving this problem. A contaminated inner surface will trap air easier than a clean surface. This problem may happen when you do not use any organic solution in the mobile phase. In some cases, a methanol wash may not work. If you use more than a few percent of methanol in the mobile phase, please skip to the section on Error 3 in chapter 8-3, page 13.

1) First, disconnect the columns from the line to prevent methanol from flowing through them. When you remove any column, plug the inlet and outlet of the column as soon as possible. Also plug the free end of the tubing which was connected to the precolumn or column to prevent air coming in when you aspirate the solution from the pump drain valve.

2) Turn off the pump and replace the mobile phase with ultrapure water. Then open the purge valve and aspirate the ultrapure water as it comes into the pump.

3) Run the pump and then close the valve. The plug on the free end of the tubing will pop out. The pump needs to run for a few minutes to wash out the mobile phase from the tubing after the pump. When aspirating from the purge drain using a syringe, the disconnected tubing, which was connected to the column, must be plugged or air will be introduced through the aspiration of solution from the drain valve by the syringe.

4) Now, you can stop the pump, replace the ultrapure water with methanol and repeat the above procedure. Following this, replace the methanol with ultrapure water and then replace the ultrapure water with mobile phase.

If these three treatments do not work, (1. Removing air from pump head, 2. Removing air form the tubing, 3. Methanol wash) there is another possibility that some small dust or lint is trapped in one of the check valves. Normally, this type of problem happens in the inlet check valve.

8-2. Types of Pump Errors

When the fluid flow system has some problems, an error message will appear on the LCD screen and the pump will automatically stop. The types of errors and the main causes are described in chapter 8-3, next page. When a pump error occurs, a contact closure signal is generated at the pump error out signal terminal which is located on the right side of the system. If the ‘pump error out’ and ‘a signal in for the emergency stop’ on your autosampler are connected by using signal cables, the upcoming run to be processed by the autosampler will be stopped and you can avoid losing samples after the pump stops.

Table 1. Types of errors and their cause(s).

<table>
<thead>
<tr>
<th>Error</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Current pressure is over the pressure limit setting.</td>
</tr>
<tr>
<td>3</td>
<td>Pressure decreases suddenly or there might be a leak.</td>
</tr>
<tr>
<td>4</td>
<td>Motor torque error</td>
</tr>
</tbody>
</table>

Error 3 and 4 only occur when the pulse free mode is on. Error 2 does not exist.
8-3. How to Fix these Errors

A push of the pump key clears the errors. If the same error occurs repeatedly, the problem can be resolved by referring to the following:

**Error 1**
The pressure limit value may be set too low. If so, raise the limit value. The optimum pressure limit is different for each column (see Table 2).

One reason for this error message is that there may be a blockage in the line. Check the precolumn first by disconnecting the precolumn from the line. If the difference in pressure with the precolumn and without the precolumn is more than 3 MPa, it is time to exchange the precolumn packing gel and the upstream filter.

If the error is still not cleared, check the pressure differences of each part as you did for the precolumn above by going from downstream to upstream of the part. This way you should identify the part which is blocked. Cut the inlet about a few centimeters if the PEEK tubing is blocked. Use a cutter knife and take care not to break the inside hole. As the tubing has a constant inner diameter, the blockage factor cannot go deeper if it comes from upstream. If an autosampler is present, sometimes dregs of vial cup can cause a blockage at the inlet tubing of the high pressure valve. Please disconnect the tubing at the first port on the autosampler immediately after the injection port and cut the inlet of the tubing.

<table>
<thead>
<tr>
<th>Column</th>
<th>Pressure limit setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-GEL, E-GEL</td>
<td>8.0</td>
</tr>
<tr>
<td>SC-3ODS, CA-5ODS, SC-5ODS (Amino Acids)</td>
<td>15.0</td>
</tr>
<tr>
<td>PP-ODS</td>
<td>10.0</td>
</tr>
<tr>
<td>SC-5ODS</td>
<td>12.0</td>
</tr>
</tbody>
</table>
HTEC-500

Error 3

The computer on the HPLC pump unit is programmed to display error 3 when one of the check valves does not work properly. If the pressure sensor detects a rapid pressure decrease and cannot return the pressure to the previous level within 12 rotations of the motor, error 3 is displayed. If you are running the pump for maintenance, please switch off the pulse free mode.

Error 3 may occur for the following reasons (Treatments are also described below):

A. The tubing connection is not good. There is a leak somewhere in the high pressure zone between the pump piston and the separation column. Check if there is any leak in the flow line. Typically, the connection at an injector valve or at the column inlet may become disconnected. If you find it has been disconnected, please re-connect it. A pressure reading of more than 150 MPa will lead to the disconnection of fittings. Please check that your column is not too old and if the precolumn is clogged.

B. Air needs to be removed from the pump. See chapter 8, page 11.

C. If air gets trapped in the pump frequently, make sure the connections are tight enough, the tubings are not damaged and the elution degassing system is working properly (see also chapter 6, page 7). The cause of resistance in the pumping system can be either that the mobile phase bottle is at a lower position compared to the HTEC-500 or an inline filter is present upstream of the pump (typically a tubing anchor filter in the mobile phase bottle is used). Check that the mobile phase bottle is on the same level as the HTEC-500. Please remove the filter if you have one.

D. One of the check valves has lint, dust or something inside. This problem happens more frequently in the inlet check valves located at the bottom of the pump head. Replace only the inlet check valve having the problem by the method described below. If the problem persists even after this exchange, the outlet check valve may be the problem.

To find out which side of the pump has the problem perform the following steps:

- Keep the mobile phase level in the bottle at the same level or a few inches lower than the inlet check valve.
- Release both the left and the right side of the hex nut that holds the tubing at the outlet check valves (see fig. 13) and see if the mobile phase comes up and at what speed it comes up at. When one of the inlet check valves has failed, the mobile phase on that side should come up significantly slower than the other side or may never come up. You can also run the pump with the pulse free mode off to check if the solution flows or not.

- Replace the failed inlet check valve with a new one (Inlet check valve, catalog #HT-IV)
- Alternatively, the failed check valve can be cleaned but very detailed work is required to clean it well. If you are not good at detailed work, simply exchange the valve with a new one. The check valve structure is shown in Fig. 15. Please prepare 50 ml acetone in a 100 ml beaker. Then screw in a stop-fit (a plug type fitting) into the valve and push out the inside parts into the acetone (see Fig. 14). Clean all the parts with acetone and assemble it back to its original structure. Always use clean forceps to pick up each part and do not touch them with your fingers.
How to clean the check valve

1. Remove the check valve assembly from the pump head.

2. Put some acetone in a small beaker. Over the beaker of acetone, fasten a column end plug to the assembly. The end plug will help to push out the inside parts into the beaker of acetone. **DO NOT TOUCH** any of the inside parts (parts #4–#7, shown in Fig. 15) with your fingers. Use tweezers to handle them.

3. Assembling. Assemble as the smooth face of the seat ring faces to the ball (#8). The parts #7 is reversible. Put the #7 on the table and push the #3 on #7.

**Error 4**

Error 4 occurs when the pump motor needs to generate more torque. This situation happens when the pulse free program needs to quench difficult pulsations that have unusual pressure changes and cause the pump motor to generate too much torque.

If this problem happens after a few rotations of the pump motors and it does not stop immediately after starting the pump, it may be because the pump system has the same problem as described for error 3 and the computer generated too much torque on the pump motor (once you cancel the error 4 by pressing the pump key, try to regenerate the error by running the pump). In this case, the treatment is the same as described for error 3. Please refer to the section on error 3.

If this problem happens without the motor rotation after running the pump and if the pump stops immediately after pressing the pump key, it is because of the failure of the power train or the pressure sensor of the pump. Please contact Eicom to fix the power train.
9. Manual Sample Injector and Autoinjector

9-1. Structure

At the back of the manual injector, the sample loop is connected to ports No. 1 and No. 4. The tubing from the carrier pumps is joined to no. 2 port. No. 3 port outputs to the column. No. 5 and 6 are drain ports. Whenever injecting into the manual injector, use the accessory syringe. If you need to inject more than 25 μl, please order the 50 μl syringe (Eicom model number 705SNR). Do not connect any other tubings to the drain port. This is to avoid any potential problems.

9-2. Operation of Manual Injector

Caution!

Do not use a syringe with a sharp needle. Always use a blunt needle.

The manual sample injector switches the flow by changing the lever knob position from LOAD to INJECT. When introducing the sample into the manual sample injector, only the Hamilton Microsyringe type 702SNR or 705SNR is recommended.

A. Aspirate the sample into the microsyringe. If an air bubble generates inside of the syringe, please flush out the solution with the air bubble and slowly re-aspirate. Generally the knob is set at INJECT before and after the injection.

B. Insert the syringe needle until you feel a strong resistance (3 mm further than the point where you feel a weak resistance).

C. Turn the knob to LOAD and push the plunger to inject the sample into the manual injector. Through this step, the sample is injected into the loop.

D. Turn the knob from LOAD to INJECT immediately and smoothly. By moving the knob from LOAD to INJECT, an electrical signal is also generated.

E. After completing these steps, remove the syringe and wash the syringe with ultrapure water. Please wash the entire syringe each time between injections to avoid contamination. After each injection, the needle port of the manual injector must be washed with ultrapure water using the white port cleaner and a syringe.

When using an external injector, please refer to its user’s manual.

When comparing the concentration of different samples, inject the same volume every time. Wash the syringe with hydrochloride periodically to keep it clean.
9-3. Autosampler and Autoinjector Setup

When you hook up an autoinjector or autosampler to the HTEC-500, do not remove the manual injector from the system. If you ever have problems with the HTEC-500+Autosampler/Autoinjector, the manual injector will be needed to diagnose which part is the problem.

It is recommended that the manual injector is to be located upstream of the autosampler/autoinjector. Leave the manual injector at the inject position while you are using the other injectors so that the sample loop on the manual injector can be kept clean by the mobile phase.

If the sample needs to be injected from the manual injector located upstream of the external injectors (autoinjector or autosampler), please keep these external injectors at the load position. Then the injected sample will not go through the sample loops on the external injectors. The obtained peaks will have a good shape. Otherwise, the peak will be broad and the retention times will become longer.
10. Precolumn

A precolumn is applied to the system to avoid contamination of the separation column by protein etc. The lifetime of a separation column depends on the state of the precolumn. Generally speaking, a precolumn can be used for approximately 100 injections of 10 μl of blood or tissue denatured protein samples or a few months with microdialysis samples. These lifetime approximations can change dramatically depending on the method used to denature the proteins during sample preparation or the precolumn condition. Please refer to the points below at all times.

10-1. Types of Precolumn Housing and Line Filters

There are two types of precolumn housings. One is 4 mm × 3 mm ID (code PC-03) and the other one is 5 mm × 4 mm ID (code PC-04). The PC-03 has number 3 engraved on it. The PC-04 has no engraved number. The PC-03 is used with small inner diameter columns such as the SC-5ODS 2.1 mm ID, the AC-GEL 2.0 mm ID and the CA-5ODS 2.1 mm ID. The PC-04 is used with the SC-5ODS 3.0 mm ID, the AC-GEL 4.6 mm ID and the GU-GEL 4.6 mm ID. The PC-04 is also used with the PP-ODS 4.6 mm ID and the precolumn is positioned before the injector but not just before the separation column. This is because there are no compatible packing materials that can be used with the PP-ODS. To protect the PP-ODS column, it is recommended that an inline filter (code PP-LF) should be set upstream to the PP-ODS and downstream to the injectors.

Microdialysis

Fig. 16 Precolumn Position

The precolumn position depends on the kind of samples being run. For microdialysis samples, it is set between the pumps and the injector. In this case, the sample doesn’t go through the precolumn so any of our packing material can be used. For other samples, such as plasma or tissue homogenate, a precolumn should be set upstream of the separation column. In this case, the packing material should be chosen according to the kind of separation column being used. If not, the shape of the peaks may be influenced. For more information, please refer to Eicom reports.
10-2. Identification of Precolumn Quality

If the shape of the chromatogram becomes asymmetric, repacking of the precolumn may be required. This is especially true if the latter half of the peak gets dull (tailing) or if the top of the peak is divided into two small peaks.

To check if the precolumn needs to be repacked, read the following:

• Inject a standard sample and confirm that the shape of the peak is asymmetric. If the shape is normal, then there must be another cause. See Troubleshooting.
• Disconnect the precolumn and replace it with a two-way joint. Then inject a standard sample and check the shape of the peak. If it is symmetric when the sample is loaded directly onto the column without a precolumn, it indicates that the precolumn must be repacked. If not, the separation column may be damaged.

Blocking of the precolumn filter raises the outlet pressure of the pump. If the precolumn generates more than 2 MPa (please compare with and without precolumn), the upstream filter must be exchanged and the precolumn must be repacked. Repacking of a precolumn requires the precolumn packing tool set. Select the precolumn packing material according to Eicom technical notes. See also Table 3.

If Using the PP-ODS Column

It is difficult to know if the precolumn should be repacked because the sample is not introduced into the precolumn in the case of microdialysis. For this reason, repack it every two or three months. Blocking of the precolumn filter raises the outlet pressure of the pump. If this pressure rises more than 1.5 MPa more than usual, the upstream filter must be exchanged and the precolumn must be repacked.

Table 3. Precolumn packing material

<table>
<thead>
<tr>
<th>Separation column</th>
<th>Pre-column packing material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns for microdialysis</td>
<td>Any packing material can be applied. (AC-ODS is the cheapest.) Set it upstream of the injector.</td>
</tr>
<tr>
<td>AC-GEL</td>
<td>CH-GEL</td>
</tr>
<tr>
<td>CA-5ODS</td>
<td>CA-ODS</td>
</tr>
<tr>
<td>E-GEL</td>
<td>CH-GEL</td>
</tr>
<tr>
<td>SC-3ODS, SC-5ODS</td>
<td>AC-ODS</td>
</tr>
<tr>
<td></td>
<td>CA-ODS (for mobile phase whose pH is around 6)</td>
</tr>
</tbody>
</table>
10-3. How to Repack

Disconnect the precolumn as shown below and take the packing material away. Then wash the inside of the precolumn with running water and then rinse it with ultrapure water. For every two times you repack, please exchange the upstream filter once as described in chapter 10-4, page 21.

Put the precolumn packing material in methanol (it will become like a slurry type solution). Add methanol to the bottle containing the packing material, put the cap on and shake well. Connect a syringe union to a disposable syringe filled with 0.5 ml methanol as shown below. Fill up the precolumn with the packing material in the methanol using a pipette. Then slowly suck methanol out of the precolumn using the disposable syringe.

Repeat these steps until the precolumn is full of the packing material (the packing material must rise up from the inside of the precolumn). Then tighten the two parts using a wrench.

**Important**

Aspirate the methanol using the syringe slowly. Fill up the precolumn with the packing material before the inside of the precolumn becomes dry. Avoid any gap in the precolumn.

Fill up the syringe with fresh methanol after disposing of the methanol collected during the packing procedure. Connect the syringe to the inlet of the precolumn and wash the precolumn using this syringe (see Fig. 19). Repeat this step twice. Then wash the packed precolumn with ultrapure water in the same way as described above.

After these steps, connect the precolumn to the line again. First, connect the tube of the upstream side only and wash inside by pumping mobile phase through. After several minutes, connect the tube of the downstream side. Except in the case where the precolumn is located before the injector, if the repacking of the precolumn was not successful, the shapes of the peaks will be abnormal.

If the shape of the peak of a standard sample with the repacked precolumn doesn’t become better and the shape of the peak is good without a precolumn, the repacked precolumn is not good. Try to repack the precolumn again. If the specified packing material was not used for repacking, then the shape of the peak will be asymmetric.
10-4. Filter Exchange

If you try to suck methanol through the precolumn with a syringe and it does not do so smoothly, this indicates that the upstream filter (PF-04 for 4 mm ID, PF-03 for 3 mm ID) is blocked and it should be exchanged. The filter can be disconnected from the precolumn using an exchange tool (see below).

Fig. 20
11. ECD Operation

Attention
- The tip of the reference electrode should be soaked in mobile phase or lithium chloride solution.
- The reference electrode needs to be replaced to a new one once a year, even if it is not used.
- The graphite working electrode (WE-3G) cannot be used and stored at the condition of 30% methanol.

11-1. Basics

The ECD cell consists of three types of electrodes. These include the working electrode, the reference electrode and the counter electrode. The electrochemically active substances are eluted from the separation column and then electrolyzed by the working electrode in the electrode system. A current is produced by electrolysis and this is in proportion to the concentration of the substances to be measured. This current is converted to a voltage in the amplifier and an output is given from the integrator. 0.1 nA generated in the cell equals to 1 mV on the output of the integrator.

\[ 0.1 \text{ nA} = 1 \text{ mV} \]

Fig. 21 (a) ECD Cell

Fig. 22 (b) ECD Cell
11-2. Working Electrode

There are six types of working electrodes. Please select the working electrode based on the Eicom technical information. Each application/technical note provides a guide for the working electrode selection. The gasket (GS-25) has a 25 micron meter thickness and this allows for higher sensitivity. However, coupling this with other electrodes, other than the WE-3G, induces higher noise.

<table>
<thead>
<tr>
<th>Working Electrode</th>
<th>Code</th>
<th>Gasket</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphite</td>
<td>WE-3G</td>
<td>GS-25</td>
<td>Standard. Methanol in mobile phase is less than 35%.</td>
</tr>
<tr>
<td>Platinum</td>
<td>WE-PT</td>
<td>GS-25P for ø2.0 mm AC-GEL column and E-GEL. GS-50P for ø4.6 mm AC-GEL column.</td>
<td>For acetylcholine analysis or online glutamate analysis only. (for OPA-Amino Acids, use graphite)</td>
</tr>
<tr>
<td>Glassy Carbon</td>
<td>WE-GC</td>
<td>GS-50</td>
<td>OPA-GABA analysis at 50% methanol in mobile phase.</td>
</tr>
<tr>
<td>Pure Graphite</td>
<td>WE-PG</td>
<td>GS-50</td>
<td>High applied voltage analyses.</td>
</tr>
<tr>
<td>Gold</td>
<td>WE-AU</td>
<td>GS-50</td>
<td>Thiol analyses only.</td>
</tr>
<tr>
<td>Silver</td>
<td>WE-AG</td>
<td>GS-50</td>
<td>Iodine analyses.</td>
</tr>
</tbody>
</table>

11-3. Applied Potential

Please refer to the Eicom technical information to determine the best applied potential for your target substance.

Applied potential is set in the following way:
- Push the display key until the ECD screen appears.
- Push the edit key until ‘ ’ is displayed in front of applied potential.
- Input the target potential with the number keys (+/- is set with the select key).
- Push the edit key again to fix the input potential.

The potential set here is called the setting applied potential. The actual applied potential can be different from the setting potential in the following cases:
- If the inside of the reference electrode is dry and the electrical resistance increases. In this case, the reference electrode should be exchanged with a new one.
- If the surface of the reference electrode is dirty. In this case, clean its surface (See Fig. 23, next page).

Substances that are detected by an ECD (at a constant potential) are oxidized at the surface of a working electrode. The oxidation potential differs according to the substance. Most substances are detected under a higher applied potential. If the potential is higher than the appropriate value, impurities will be detected and disturb the detection of the target substance.

Oxidation-reduction potential varies with the pH of the mobile phase. When the pH of the mobile phase increases by 1, the actual applied potential decreases by 60 mV.
1. Loosen the outlet screw and insert a syringe. It’s easier if the outlet tubing is disconnected. Suck the liquid from inside the ECD cell. Without this step, the gasket will become wet by the mobile phase when the cell is disassembled, resulting in some problems.

2. Pull the handle slowly and then draw the shaft out. The cell is now open.

3. Lift and remove the setting plate, working electrode and gasket in turn.

4. Carefully wipe the surface of the electrode (smoother side) with acetone using a Kimwipe. Always wipe in the same direction. Do not rub it back and forth. Please be extremely careful not to damage the surface.

In this photo, the ECD cell is disconnected from the ECD but it’s not necessary for normal use.

Fig. 23 How to Open the ECD Cell
11-4. Time Constant

There are three settings for the time constant. These are 1.0, 1.5 and 3.0 sec. These influence the peak height and the noise level. The time constant determines if the signal response speed will reach 60% of the real signal. For example, when the time constant is set at 1.5 sec and 1 nA real signal is generated in the cell, the ECD integrator output generates 0.06 mV (0.1 nA = 1 mV) in 1.5 second.

1 nA \times 0.1 \text{ mV/nA} \times 60\% = 0.06 \text{ mV}

The optimal setting for the time constant should be based on the peak shape, the signal height and the flow rate. Generally, when the lower theoretical platelet column is selected, select a longer time constant. When using Eicom applications, please follow the application notes. 1.0 sec is rarely used. Please select 1.5 sec or 3.0 sec for our applications.

11-5. Background Current

When the columns are set up and mobile phase is allowed to flow at a certain condition before injecting samples, the ECD monitors the current, which is called background current. Generally speaking, lower background current allows higher sensitivity. This is because there is less background noise and more stability.

11-6. If Background Current is High

- The surface of the working electrode could be dirty. In this case, disassemble the cell (see Fig. 23) and wipe the surface with acetone using a Kimwipe.
- Resistance of the working electrode could be increased. In this case, change it for a new one.

11-7. Caution for the Platinum Working Electrode

Alcohol or hydrogen peroxide can be detected with the platinum working electrode.
- If hydrogen peroxide is to be detected, do not use alcohol in the mobile phase.
- If the mobile phase contains little or no alcohol, bacteria can generate. To avoid this, do not use mobile phase for more than one week. Make fresh mobile phase every week.
- A new platinum electrode should be conditioned with mobile phase under applied potential for one day and analysis is then possible. This conditioning is required in order to form a thin oxidized layer on the surface of the electrode.
- Background current is sometimes very low with the platinum electrode. This situation could be the cause of low sensitivity. This is caused by the surface of the platinum electrode becoming covered with non-electrolyte contamination mainly from the mobile phase or the columns. Please open the cell and wipe the surface of the working electrode (see fig. 23)
12. Storage of the System

The method of storage depends on how long the storage is for.

12-1. Short-term (A few days to a few weeks)

Just switch off the system and leave it running in mobile phase. Wash behind the pumps with ultrapure water. In the case of ACh analysis, please skip to section 12-2 even if storage is for a few weeks.

12-2. Long-term (more than a few days or a few weeks)

A. Remove the precolumn from the system and replace it with a two-way joint. Repack the precolumn before the start of the next analysis. If using a GU-GEL column, remove the column from the system and plug each end. If using another type of column, leave the separation column connected to the system for now. All enzyme immobilized columns (reactors) need to be removed and stored in an appropriate buffer and kept in a refrigerator.

B. Mobile phase in the whole system and in the separation column should be substituted with 30% methanol in water. Methanol will prevent bacterial growth. Replace the mobile phase with 30% methanol and aspirate some solution from the drain valve of the pump using a syringe so that the mobile phase in the degasser is substituted with 30% methanol. Then set the position of the injector to INJECT and let the 30% methanol flow for 1 hour through the line at 70% or 80% of the set flow rate value. This value is specific to the type of separation column being used. During this process, switch off the ECD. Take the drain just after the column and put a plug on the inlet tubing of the ECD to prevent the ECD from drying out. Wash out the ECD content with the flow from the column after washing the column for 1 hour. Connect the column back to the ECD and run the pump continuously for another 10 min.

C. After washing the ECD, close the bottle of mobile phase with lab film surrounding the inlet tubing going into the bottle. Disconnect the tubing from the waste bottle. Wash the piston using ultrapure water from the pump wash port. Wash the ports of the manual injector using ultrapure water.

D. Unscrew the fixing nut at the reference electrode on the ECD cell and put the reference electrode at 4°C keeping it’s tip in 1 M LiCl/0.1 M CH₃COOH. This solvent is present in it’s cap. Other parts of the ECD will become dry.

E. Disconnect the separation column from the line and plug it at each end. Plug the free end of the tubing with a tubing plug.

F. After these steps, the degasser, the pump, the injector and all the tubing are filled with 30% methanol.

G. At the end of these steps, disconnect the power cable.
13. Storage of a Standard Sample

Substances that are active to the ECD are easily oxidized and should be stored carefully. Check the conditions where they are stable.

The methods to store a standard sample for some typical substances are described below:

**Catecholamine:** Put the standard in cold storage with 0.1 M HCl containing 1 mg/mL EDTA-2Na at a concentration of 1 ng/μL. Dilute using 20 mM phosphate buffer (pH3.5) containing 20 mM EDTA-2Na for every analysis. It dissolves easily when the concentration is less than pg/μL.

**Indoleamine:** Put the standard in cold storage with 0.1 M CH3COOH containing 1 mg/mL EDTA-2Na at a concentration of 1 ng/μL. Dilute using 20 mM phosphate buffer (pH3.5) containing 20 mM EDTA-2Na for every analysis. It decomposes easily when the concentration is less than pg/μL.

**ACH:** The standard is relatively stable but is easily metabolized by unwanted bacteria. To avoid breeding of bacteria, the standard should be diluted using 20 mM phosphate buffer (pH3.5) containing 20 mM EDTA-2Na.

**Glutathione:** Put the standard in cold storage with 0.1 M HCl containing 1 mg/mL EDTA-2Na at a concentration of 1 ng/μL. This can be used for analysis for approximately one week. Dilute with the mobile phase for a glutamate enzymatic assay. The mobile phase contains HDTMA which prevents bacterial growth.
14. Regular Maintenance Schedule

**Daily Maintenance**

**Washing the Port of the HPLC Pump**
Each time the system is shut down or before running the system, flush the pump piston washing port with 2-3 ml of ultrapure water through the attached syringe. This is required in order to flush out, and prevent crystallization of, any mobile phase present. Do not force water in with the syringe if any back pressure is felt. This may lead to a leakage in the washing line.

**Occasional Maintenance (typically required once or twice per month)**

**Working Electrode**
If the ECD background current is unusually high or low (ideal range is between 5 to 15 nA), please wipe the smooth surface of the working electrode with 100% acetone using a Kimwipe. This will remove pollution that is considered to be hydrophobic substances on the surface. The source of this pollution can be the mobile phase, samples and/or columns. If you need to wipe it frequently (once a week or more), please check if the mobile phase ingredients and your sample preparation protocol match with the requirements shown in the Eicom user’s manuals. If you find that the installed gasket is dusty or the edge of it is not clearly cut, please replace it immediately. If you are using a graphite electrode and it has been exposed to more than 35% methanol or 15% acetonitrile, please change it.

**Precolumn**
(The following steps should be carried out after 50 homogenate sample injections or 500 microdialysis sample injections.)

If you find odd-shaped peaks, it may be time to repack the precolumn. To judge the quality of the precolumn, remove it and connect a separation column directly to the injector. Following this, inject a standard sample. If the peak now has a good shape, it indicates that the precolumn is contaminated. The sample capacity of a precolumn is dependent on the sample type and its preparation. The guideline mentioned above is only for reference. The packing materials and size of the precolumns vary depending on the application. Please see the corresponding user’s manual provided by Eicom. If you are using the PP-ODS column, the precolumn should be installed before the injectors. In this case, the quality of the precolumn is difficult to judge. Therefore, please repack the precolumn every month.

**Yearly Maintenance (For acetylcholine analysis, every 6 months)**

**Reference Electrode**
Change the reference electrode once a year regardless of its usage (if you have been keeping the reference electrode on a shelf with it’s tip dipped in LiCl solution, you can use the electrode for another year). The date of production is printed on the electrode (for example, 0805 means May 2008). In the case of acetylcholine analysis, the lifetime of the reference electrode is only 6 months. Sometimes, verifying the quality of the reference electrode is difficult. A decayed reference electrode may lead to misdiagnosis of problems with the HTEC-500. The rate of decay of the reference electrode accelerates after one year. Therefore, please change the electrode periodically even if the system works without significant problems.
**Pistons and Piston Seals (periodically, usually once a year or longer)**

If the mobile phase leaks more than a few ml over a 24 hour period, please change the pump seals. It is not required to change the seal periodically when this problem does not occur. Usually the leaking fluid drains from the pump washing port. To confirm the leaking volume, slowly push the piston of the syringe attached to the tubing from the washing port all the way to the end. Let the pump run to verify the leaking volume. Changing the pump pistons and piston seals at the same time is recommended. If the piston itself does not need to be changed, at least change the piston seals. If you find any deterioration of the piston, please replace it. Pistons and seals for maintenance are provided separately from the HTEC-500 and require special tools which are provided with the system. Please see Appendix A for a protocol for how to replace pump parts. Eicom also provides a maintenance service. Please contact us for more details.

**Injector Seals**

If the injector is used continuously for more than a year, change the stator and stator seal. Please contact Eicom or Rehodyne for details. If you use an autosampler for the majority of your injections, the lifetime of the manual injector seal may be longer but the seal on the autosampler needs to be replaced periodically. See the autosampler’s manual for details and required maintenance. Even if there is no leakage from the seal, a worn seal may be the cause of unknown peaks on a chromatogram.

**Ordering Information**

<table>
<thead>
<tr>
<th>Description</th>
<th>Code</th>
<th>Package</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gasket (Regular)</td>
<td>GS-25</td>
<td>3 pcs</td>
</tr>
<tr>
<td>Gasket for Platinum</td>
<td>GS-25P</td>
<td>3 pcs</td>
</tr>
<tr>
<td>Working Electrode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graphite Electrode</td>
<td>WE-3G</td>
<td>2 pcs</td>
</tr>
<tr>
<td>Piston</td>
<td>HT-PP</td>
<td>1 pc</td>
</tr>
<tr>
<td>Piston Seal</td>
<td>HT-PS</td>
<td>1 pc</td>
</tr>
<tr>
<td>Precolumn Filter</td>
<td>PF-03 or PF-04</td>
<td>3 pcs</td>
</tr>
<tr>
<td>Precolumn Packing Material</td>
<td>AC-ODS, CA-ODS or CH-GEL</td>
<td>1 bottle for more than 30 times repacking</td>
</tr>
<tr>
<td>Precolumn Packing Tool</td>
<td>PREPACKTOOL</td>
<td>1 set</td>
</tr>
<tr>
<td>Reference Electrode</td>
<td>RE-500</td>
<td>1 pc</td>
</tr>
</tbody>
</table>
15. **Troubleshooting** (Please refer to these points after reading chapter 1-14)

When you discover a problem, you need to find out which part is affected, the HPLC-ECD, the autosampler or the sample itself. Please use the manual injector of the HTEC-500 to inject samples and only inject standard samples to diagnose the problem at first. Do not use the autosampler to diagnose the problems listed below. Once you find that the HTEC-500 is OK, please start to use the autosampler again.

**No peak appears even though a sample was injected. Possible causes are as follows:**
- Chromatogram time setting is too short.
- The drain port of the injector is blocked and sample is not loaded into the sample loop.
- The pump doesn’t work. A potential cause is that an air bubble may be present (see chapter 8, page 11).
- The ECD is switched off.
- There is no connection between the “integrator out” of the HTEC-500 and the EPC-500 or other integrators.
- There is no signal input from the “auto zero”.
- The background current is too high.
- The precolumn is very contaminated.

**The pump is unstable or has frequent errors. Possible causes are as follows:**
- Refer to chapter 7, page 8.
- The injector works too slowly or it’s not used in the designated way.
- The precolumn is very contaminated.

**The background current increases gradually over several hours or stays high. Possible causes are as follows:**
- The separation column and/or precolumn are new. Please wash the columns with clean analytical mobile phase overnight.
- The wrong mobile phase conditioning was used. Please check the pH of the mobile phase. Do not dip a pH probe into the bottle. Take some of the mobile phase in a small vial and use the pH probe to check the pH.
- A reagent in the mobile phase is not suitable or the ultrapure water has been contaminated. Eicom recommends specific brands for specific reagents, especially for methanol, SDS and SOS.
- The mobile phase is too old.
- The precolumn/separation column is contaminated. Use Eicom brand columns.
- The reference electrode is old.
- The working electrode is covered by contamination.
- The graphite electrode was applied under a methanol-rich condition.
- The mobile phase was not shaken well before being set up on the HPLC.
- The mobile phase that was used for the previous analysis still remains in the degasser. Use a syringe to aspirate solution from the drain valve of the pump so that the old mobile phase flows out. Disconnect the column and wash the line using clean mobile phase or ultrapure water.

**A big unknown peak appears. Possible causes are as follows:**
- The sample injected before the current analysis contained a high concentration of a hydrophobic substance that was detected by the ECD. For example, sample refinement such as aluminum adsorption can be applied in the case of catecholamine analysis to help with this problem.
Unstable background current. Possible causes are as follows:
• The reference electrode is old.
• The working electrode is not clean enough.
• The gasket of the cell is wet.
• There is a leakage from the cell or from the tubing joints or there is a scratch on the cell block.
• The system or the mobile phase bottle is placed where the temperature is changing dramatically. Do not put it in direct sunshine or close to wind from an air conditioner.
• A reagent in the mobile phase is not correct.
• The mobile phase that was applied for the previous analysis still remains in the degasser. Use a syringe to aspirate solution from the drain valve of the pump so that the old mobile phase flows out. Disconnect the column and wash the line using clean mobile phase.

Occasional keen peak (spike). Possible causes are as follows:
• Salt extracts by mixing the mobile phase and the sample.
• The edge of the gasket is not flat. Change it for a new one.
• There is dust on the gasket.
• An air bubble is trapped in the cell.
• Columns are not held by column clips properly to ground static electrical current.
• Put the waste bottle on the same level as the analyzer.

The peak of a standard sample is lower than usual. Possible causes are as follows:
• The standard has decomposed (oxidized). The standard is not stable when its concentration is low and it should be prepared just before the analysis.
• The syringe is not clean. Washing using hydrochloric acid is necessary.
• The drain port of the injector is blocked.
• Some unwanted bacteria may have generated in the HTEC-500. Change the tubing and fill up the degasser with hypochlorous acid overnight and then wash away using an excess volume of ultrapure water. Take care NOT to allow hypochlorous acid through the HPLC pump.
• The graphite electrode (WE-3G) may have been used with mobile phase of a high methanol concentration.

Pulsatile flow. Possible causes are as follows:
• There is a leakage from the flow line.
• Columns are not held by column clips properly to ground static electrical current.
• The pump washing port does not contain enough ultrapure water. Please aspirate ultrapure water into the syringe.

Less reproducibility of the retention time during one day or day to day. Possible causes are as follows:
• The ion pair and the column haven’t reached a state of equilibrium yet. Continue to allow mobile phase to flow for a few to several more hours.
• The mobile phase isn’t mixed well.
• The room temperature changes too much.
• The temperature controller of the HTEC-500 has a defect.
The retention time is longer than usual. Possible causes are as follows:

- The mobile phase condition is different from previous analyses.
- A small air bubble is trapped inside the pump. Open the drain valve and tap the check valves (see chapter 8, page 11).
- The piston seal or the piston is worn. It must be changed for a new one. You may observe lower pressure than before.
- The setting temperature of the cabinet of the HTEC-500 is lower than before.
- Contamination on the precolumn. Please repack.

The separation from impurities is not good although the retention time is the same as usual. Possible causes are as follows:

- The reference electrode is old or dry.
- The working electrode is dirty.

High pump pressure or pump error 1. Possible causes are as follows:

- The precolumn is contaminated.
  Inspect the cause of the high pump pressure by disconnecting the tubing from downstream to upstream. When the pressure of the tubing is high, the inlet of the tubing is often blocked. To solve this, you can cut off several centimeters using a cutter. Downstream of the valve of the autosampler can often be blocked by a piece of vial cup.

The shape of the peak is not good. Possible causes are as follows:

- The precolumn is dirty.
- The separation column is old or damaged.
- When more than one injector is applied, the injected sample flows through the downstream injector. Set the position to LOAD.
- There was too large a volume of the sample injected.
- The pH of the sample is too different to that of the mobile phase and at the same time the mobile phase doesn’t have enough buffer capacity. Please dilute the analyte in mobile phase and inject. If this works to improve the shape, this is the reason why the peak shape was bad.
Note: Please read each step completely before you proceed. Also, please remember the order and orientation of all parts as you remove them. This helps to avoid having to disassemble the pump and risk breaking or damaging the piston.

1. Remove the PEEK tubing at the inlet and the outlet of the check valves. Unscrew the hex nut at the outlet by using a wrench (8 mm). Using your fingers, unscrew the tuff connector at the inlet.

2. Unscrew the two nuts on the pump head by using an Allen wrench.

3. Remove both nuts.

4. To remove the pump head, slowly and carefully pull the pump head directly towards you without twisting it. Vertical rotation as piston becomes axis. Other movement in other directions will break the piston that lies inside.

5. The piston will now be exposed. As you can see the piston is very fragile. Please be very careful when handling it. Please look at the surface of the piston and note if the piston is worn or not. Actually, it is difficult to see the quality without removing the piston from the body. To remove the piston, in order to replace it or to examine it more carefully, please continue to the next step. If you find the piston is in good condition, and would just like to change the piston seal, please skip to step 11.
6. Remove the pump wash port connectors. If your machine was purchased before June 2006, you do not need to use the wrench as shown in the picture. Instead, you can pull out the connectors carefully using a set of pliers. In both cases, these connectors will be used again.

7. Remove the nuts using an Allen wrench.

8. Pull the pump head mount (made of stainless steel and held by fingers in the picture below) straight out after removing all the screws, being careful not to break or scratch the piston. Do not lose the back-up ring. This is a khaki-colored part which supports the piston seal from the back side of the pump head. Please do not forget to put back this part during reassembly. Sometimes, the pump head mount holds the back-up ring.

9. When you look at the piston you will notice it has screw threads. To understand the structure of the piston and how it is mounted on, please look at your new piston first. Please insert the piston exchange tool into the hole on the stainless steel part of the piston (as shown in the picture) and turn it counterclockwise. Now you can remove the piston.
10. Remove the piston and look at it under bright light. If the reflection of the piston is different at the area around the piston seal, it needs to be changed. If the piston is covered by something, it also needs to be changed in this case.

11. The piston seal is found inside the back of the pump head. To change the seal, pour ultrapure water into the seal and pump head.

12. Next, put a plug in the outlet check valve. Insert the longer stick on the piston exchange tool into the seal. Water in the pump loosens the seal and pushes it up. Please note that the pump internal volume is sometimes too small to push the seal easily. If the seal does not come up, please try to pour in water again and insert the tool.

13. The seal should come up like in this picture. If this step proves unsuccessful, please pull the seal carefully using pliers. Be careful not to scratch the pump head.

14. Place a new pump seal on the shorter side of the tool. Please note the order of the seal on the tool. The white end of the seal goes inside of the pump first as seen above and below. Remove the plug connected to the outlet check valve.

15. Lubricate the inside of the pump with ultrapure water (no oil). Then push the tool, with the new seal attached, into the hole. Now the seal will be inserted into the pump head.
16. Now remove the tool.

17. The next step is the installation of the new piston. If the washing port diaphragm is worn, please replace it. The diaphragm is shown as the black part in this picture, but may also be a different color. Fasten the screw securely to set the piston in place. Then wipe the surface of the piston with acetone using a Kimwipe to remove oil from your fingers and dust.

18. Carefully place back the pump mount. Please pay attention to the orientation of the parts. The wider screw holes should be on the top and the bottom as seen below.

19. Do not forget to put on the back-up ring.

20. Fasten the shorter screws.
21. Reassemble the pump head carefully. Take care not to break the new piston by twisting the pump head. After insertion of the piston slowly and softly into its position at the back of the pump head, please push the center of the pump head a little bit harder, but carefully, using a finger. Any movement, in any direction other than directly straight in, will break the piston.

22. Tighten both screws. Do so alternatively and evenly (like an automotive tire). Do not completely tighten up one screw before the other one.
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