



BAS Pollen-8™

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Part A-1310

INSTRUCTION MANUAL

Online Injector

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West Lafayette
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MANUFACTURER'S NOTE

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Section 1. Introduction

The Pollen-8 is an electrically-actuated injection valve designed to collect samples from a microdialysis probe and inject them onto a liquid chromatograph (LC). This process provides information at a faster rate than conventional collection of microdialysates into vials. It places the microdialysis probe “online” with the LC, providing analytical data about the dialysis stream within minutes after diffusion of analytes from the tissue being studied. It also maintains the sample order correctly since there is no opportunity to mislabel or rearrange collected samples.

Online injection of samples is appropriate whenever targeted analytes can be directly monitored by detectors on the LC such as electrochemical, UV-Vis or fluorescence devices. It is also more suitable to fast chromatographic separations which can be completed before a sample loop is completely filled with microdialysate. If these two criteria can be met, then online injection offers the advantages of convenience (no sample handling, storage or processing), improved economy (no vials or seals to purchase, no autosamplers required) and speed (direct from dialysis probe to data printout in a matter of minutes).

The Pollen-8 is part of the BAS Bee™ family of products for microdialysis. It does not require any other BAS instrument to perform standard operations. However, it was also designed to be used with the BAS Queen Bee™ Syringe Pump when variable timing between injections is required. When the Pollen-8 is used with the BAS HoneyComb™ Refrigerated Fraction Collector, a dialysis stream can be divided between alternate injections into the LC and collection of a backup sample into a vial.

System Components

The Pollen-8 is shipped in a single cardboard box which includes the following:

- Pollen-8 Controller
- 10-port Microbore (MD-1249) or Conventional (MD-1248) Injection Valve
- Valve Actuator
- Mounting Bracket
- MR-4076 Nuts and MR-4077 Ferrules (10 of each)
- MD-1255 Microdialysis Probe Port
- MD-1259 Port Connector
- Waste Port
- Power Cable
- A-1310 User's Guide

Note: The Pollen-8 **does not** include sample loops. These must be ordered separately. If ordered with the Pollen-8, they may be shipped with it in the same box. If ordered with other accessories, they may be shipped in a separate box. Loops are sold as *matched and calibrated pairs* with a nominal value of 2, 5, 10, 20 or 50 μL . The actual volume may be $\pm 50\%$ of the nominal value of the loop, but will be matched with a loop of identical volume.

The injection valve will be mounted on the black Valve Actuator box.

Shipping Damage

If there is damage to the shipping box or its contents upon arrival, please contact BAS immediately and in writing. There is a limit of 30 days after our shipping date to report any shipping damage, loss or omissions. It is important that you advise us, in writing, about these types of problems. Include your name, address, telephone/FAX/E-mail, and the model and serial number of the instruments. You can send this report to:

E-Mail: bas@bioanalytical.com
FAX: 765-497-1102
Phone: 765-463-4527

Normally, BAS shipments are fully insured and an insurance fee is listed on the invoice unless you or your organization have specifically refused to accept insurance on the shipment. If you have ordered BAS shipping insurance, the damaged item will be promptly repaired or replaced at our option. If you have not ordered BAS shipping insurance, you will have to contact your own insurance company to negotiate damage compensation.

Section 2. Installation

Environment

The Pollen-8 Controller should be placed on a hard, level surface with clearance not less than four (4) inches on all sides. It may also be stacked on top of other BAS instruments as part of a BAS 480 LC System. The valve and actuator are connected to the controller via the 4 ft. (1.2 meter) control cable. This permits placement of the valve in a variety of locations ranging from a point close to the microdialysis probe to the inside of a temperature-controlled housing such as a BAS 200 LC system. In a microdialysis experiment, the needs of the sampling system have to be weighed against the possible effects on the subject. For example, it is desirable to place the valve close to the probe since this reduces the length of outlet tubing from the probe to the valve - and therefore reduces potential problems with back pressure and loss of sample due to ultrafiltration. On the other hand, the valve makes a distinct noise as it actuates which may be disturbing to the subject. A white noise generator may be needed to help mask the sound if this is a problem.

Power

This instrument uses a fused, self-sensing power supply and can be plugged into a standard wall outlet using the style of power cord appropriate to your region. Fuse sizes appropriate to 120V or 220V operation are printed on the back panel of the Pollen-8 Controller.

Cable Connection

Turn off the power to the Pollen-8 controller. Attach the control cable to the back panel connection labeled "Valve". Attach the other end to the same type of connector on the black valve actuator. Both ends of the cable are interchangeable but one offers a straight connection while the other is angled. Use whichever style is most convenient.

Once the power is turned on, there will be a slight delay before the front panel LED lights up. As the display appears, you will hear the valve actuate several times as the controller determines which type of valve is attached. The valve will then stop at a resting position. Turn off the Pollen-8 controller at this point and proceed to the section of this manual entitled "Plumbing".

Plumbing

The Pollen-8 can be used in a variety of different ways, some of which are illustrated in this manual. A 10-port valve offers plenty of flexibility. Once you understand the basic concepts of rotary valve operation, the rest is just a matter of plumbing. A 10-port valve can inject sample into two different LC systems, or alternate injections from two different microdialysis probes into a single LC system. It can be coupled with a fraction collector to alternately inject and collect sample. Small trapping columns can be attached for column switching and backflushing operations. Refer to the illustrations and attach components to the numbered ports as illustrated.

If you are unfamiliar with LC plumbing, please refer to the section entitled "Making a Good Connection" before attempting to attach fittings and tubing. Please do not use loops and

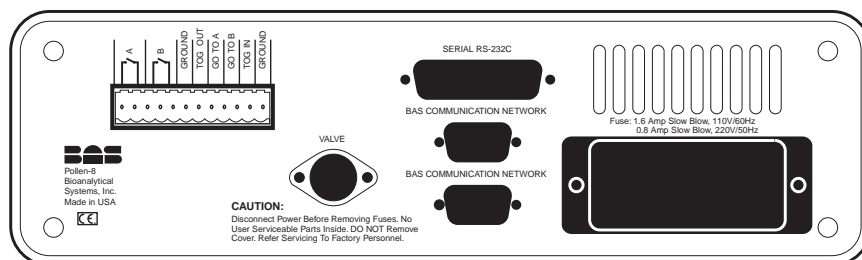
fittings other than the type provided when making attachments to the valve. Such fittings are likely to seat incorrectly and create unswept volume within the port.

The valve will arrive with a protective seal over the valve ports. Keep this in place until you are ready to actually install the valve. The valve as shipped is clean and free of particulate contaminants. Open ports invite the risk of particles entering the valve and blocking ports or scratching the rotor seal - the most frequent cause of premature valve failure. Rotor seals, as consumable items, are not covered by warranty. It is therefore in your best interest to be fussy about the cleanliness of materials entering the valve. Use tubing that is clean-cut, with no burrs and cut square with the tube axis. Make sure that all steel tubing has been chemically and mechanically cleaned. Flush plastic tubing thoroughly with water and methanol. Perfusion fluids for in vivo microdialysis are normally filtered with a 0.2 μm membrane filter prior to loading into clean syringes. This practice protects the dialysis probe as well as the injector.

Rear Panel

The rear panel of the Pollen-8 controller has a terminal strip and several D type cable connectors. The D connectors are reserved for future use by the BAS Communications and the RS-232 connector is also reserved for future implementation. Remote control or output from the Pollen-8 is currently restricted to the terminal strip. Please see Section 6 for more details about the functions of these back panel connections. Always use stripped and tinned wires for connections to the terminal strip.

F1 Pollen-8 Back Panel



Connection to a BAS 200 Liquid Chromatograph

The BAS 200 Chromatograph has a special D connector on the back panel labeled as TIMED EVENTS. To make the connection between the BAS 200 and the Pollen-8, you'll need a cable with a D connector on one end and tinned wires on the other. If you use BAS cable EW-8162, you may need to cut off two of the spade lugs: pin 1 is a green wire and pin 25 is a black/white striped wire. Then strip and tin the wire (tinning wire means coating it with a small amount of solder to prevent fraying of the wires). The wire corresponding to pin 1 on the TIMED EVENTS connection of the BAS 200 represents START 200. The wire corresponding to pin 25 on the TIMED EVENTS connection of the BAS 200 represents GROUND.

Connect the START 200 wire on the BAS 200 Timed Events cable to the TOG OUT terminal on the back panel of the Pollen-8. Connect ground to ground on each back panel.

Note: The longest chromatographic run time, plus the time required to save data must equal a time less than the collection time set on the front panel of the Pollen-8. The BAS 200 must be in the EQUIL mode and waiting for the next injection before the Pollen-8 switches. If a START 200 input is received during a chromatographic run, it will be ignored. The result would be that half of the programmed injections would be ignored. If this happens, check the chromatographic run time vs. the collection time set on the Pollen-8 front panel.

Connection to a ChromGraph Data Station

The heart of a ChromGraph Data Acquisition and Analysis station is the DA-5 interface. Detectors and the computer connect to the DA-5. There are two approaches which may be used to make a connection between the DA-5 and the Pollen-8.

The Pollen-8 Controls the DA-5

Connect the TOG OUT terminal on the Pollen-8 back panel to the START IN (+) terminal on the DA-5. Connect GROUND terminal on the Pollen-8 to the START IN (-) terminal on the DA-5. Put the DA-5 into EXTERNAL START trigger mode and make the Pollen-8 cyclic interval longer than the chromatography run time. When the START button is pushed on the front panel of the Pollen-8, the DA-5 will start acquiring data from the chromatographic run. The next chromatographic run will begin when the cycle ends and the valve is toggled again.

The DA-5 controls the Pollen-8

Connect the DA-5 START OUT (+) terminal to TOG IN on the Pollen-8. Connect the DA-5 START OUT (-) terminal to GROUND on the Pollen-8 as well. When the DA-5 starts, the Pollen-8 valve is toggled to a new position and stays there until the DA-5 issues a new start command.

Connection to a Strip Chart Recorder

If the recorder has a CHART MARK input, then the TOG OUT and GROUND terminals on the Pollen-8 can be used to mark injections.

Connection to a Queen Bee Intelligent Syringe Pump

Locate the Remote Control Interface for the Queen Bee pump. This is a small box with two terminal strips and an illustration of a bee tethered to a control box. Connect the Remote Control Interface to the Queen Bee Controller via the ribbon cable. Locate the terminal strip on the Remote Control Interface and identify the output section for the pump which will be managing the perfusion. You have a choice of 4 pumps and your choice should match the Configuration Screen on the Queen Bee software display. Connect the TOG IN terminal on the Pollen-8 to the Pump OUT positive (+) terminal on the Remote Control Interface. Then connect GROUND to the negative (-) terminal.

Special Connections

Please see section 4 of this manual for descriptions of the various methods which can be performed using the Pollen-8. Two of these require special consideration when making connections:

Inject and Collect

There are two terminals on the Pollen-8 back panel labeled as (RELAY) A. Connect one of these to the HoneyComb NEXT input terminal and the other one to the HoneyComb GROUND input terminal.

There are two terminals on the Pollen-8 back panel labeled as (RELAY) B. Connect one of these to the START RUN input on the chromatograph and the other one to the GROUND input terminal on the chromatograph. The labels for these inputs on the chromatograph will vary according to each manufacturer's preferences, so read the manuals to determine which inputs have a START RUN or GROUND function.

Relay A and B are both contact closures that are active (closed) when the valve actuates to their corresponding positions. For example, Relay A is closed when the valve is in position A and open when the valve is in position B.

Split Dialysate

There are two terminals on the Pollen-8 back panel labeled as (RELAY) A. Connect one of these to the START RUN input on the first chromatograph and the other one to the GROUND input terminal on the first chromatograph. The labels for these inputs on the chromatograph will vary according to each manufacturer's preferences, so read the manuals to determine which inputs have a START RUN or GROUND function.

There are two terminals on the Pollen-8 back panel labeled as (RELAY) B. Connect one of these to the START RUN input on the second chromatograph and the other one to the GROUND input terminal on the second chromatograph. The labels for these inputs on the chromatograph will vary according to each manufacturer's preferences, so read the manuals to determine which inputs have a START RUN or GROUND function.

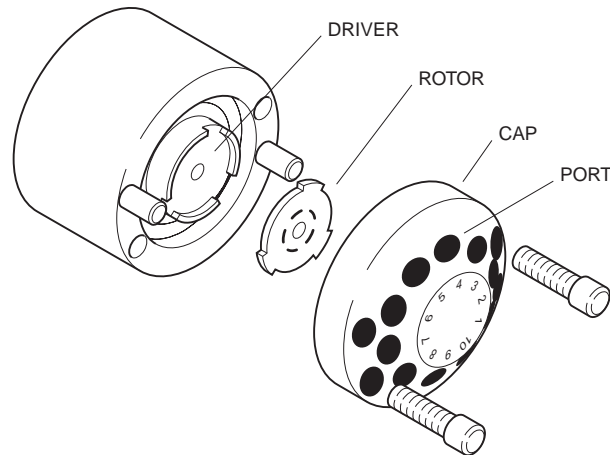
Relay A and B are both contact closures that are active (closed) when the valve actuates to their corresponding positions. For example, Relay A is closed when the valve is in position A and open when the valve is in position B.

Section 3. The Rotary Valve

Rotary Valve Operation

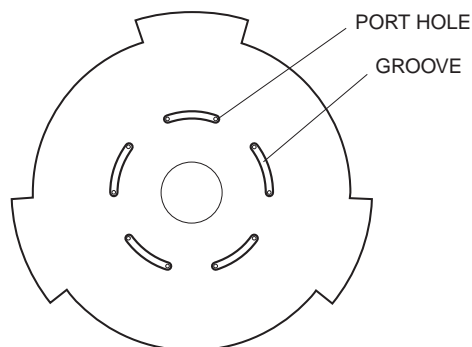
If you are unfamiliar with the operation of a rotary injection valve, step back from the frenzy of tubing in our illustrations and take a moment to consider how this device works. First, consider the term “10-port” and look at the valve as it was shipped to you. There are 10 threaded holes in the stainless steel cap of the valve. Each of these is a “port”. Other types of valves may use 6 or 7 or 8 such ports. The ports provide a point of attachment for sample loops, the outlet tubing from the microdialysis probe, tubing connected to the LC pump and other accessories such as the port connector.

F2 Exploded View of the Rotary Valve



The next layer in the valve is the rotor seal. In a 10-port valve, this rotor seal also has ten holes, but they are much smaller and they line up with the holes on the cap. The rotor also has small grooves between pairs of holes, as illustrated. These grooves route flow from one port to an adjacent port. The cap and ports remain stationary while the rotor moves behind them. The movement only advances the rotor by one position and then back to the original position. For example, if flow starts by running into port 1 and out of port 2, after the rotor is actuated the flow will go into port 1 and out to port 10.

F3 The Rotor Seal



Rotor Seal

To appreciate the function of the rotary injection valve remember the arrangement of the pairs of holes joined by grooves in the rotor. If port 1 and 2 are joined, then so are port 3 and 4, 5 and 6, 7 and 8, and 9 and 10. If the valve is actuated, the pairing skips to port 10 and 1, 2 and 3, 4 and 5, 6 and 7, and 8 and 9. Flow can't stop in a rotary valve - it always has to go somewhere because there are no dead ends. The diagrams show the numbered ports and the devices that are connected to them. In a 10-port valve, one sample loop may be in line with the microdialysis probe, while the other sample loop is in line with the LC pump. When the valve actuates, the sample loops will now reverse their roles. Follow the flow path using your finger to get a better grasp of how the fluids enter and exit the valve when the rotor is in either position.

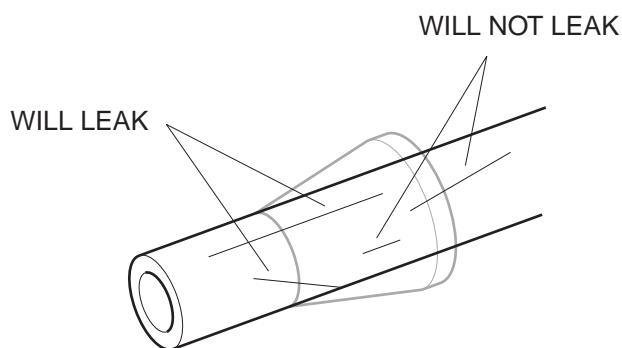
Making a Good Connection

Good LC plumbing requires the use of an appropriate nut and ferrule. Always use connectors designed for the particular type of tubing in use and suitable for your valve. If you are using a stainless steel nut and ferrule, use wrenches which match the task at hand. If you are using fingertight plastic connectors, use the appropriate plastic ferrule for that type of connector. The valve used in the Pollen-8 is a VICI model C2. BAS supplies matched and calibrated loops made from PEEK plastic tubing for this valve. The port connector and waste tube will be supplied with 1/16" Zero Dead Volume stainless steel nuts and ferrules installed.

Tubing

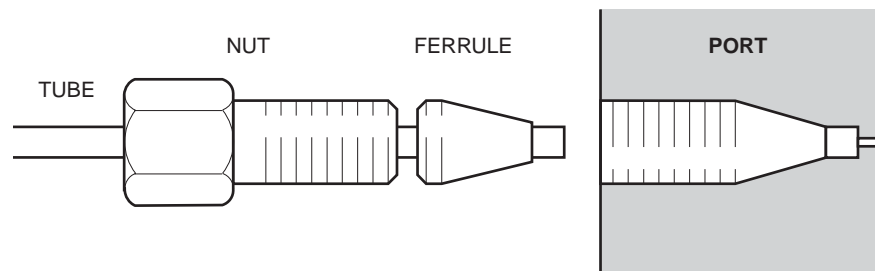
If you are preparing stainless steel tubing for connection to the valve, make certain that all tubing ends are square cut with the tube axis and that both the ID and OD are completely deburred (free from steel slivers or particles). Inspect the end of the tubing where the ferrule will seat. Visible scratches along the length of the tubing at the position where the ferrule will seat are unacceptable. Scratches behind the ferrule are not a problem. You may try to fold fine emery cloth or wet-dry sandpaper (200-400 grit) around the tubing and then roll it between your fingers. This will make concentric lines around the perimeter of the tube - not ideal but better than a longitudinal scratch. Flush methanol or isopropanol through the tubing to dislodge any particles and then dry with clean compressed air.

F4 Tubing must be free of scratches.



Fittings

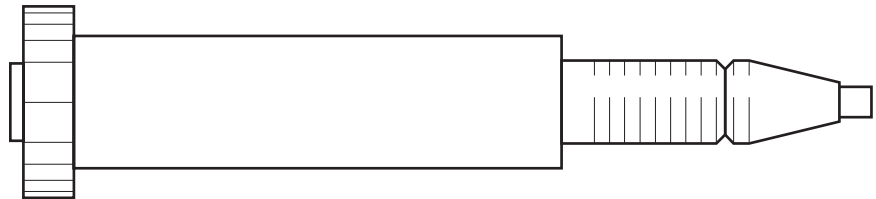
1. Slide the stainless steel nut and ferrule onto the tubing in the order shown.
2. Insert this assembly into the valve port and tighten the nut by hand for two or three turns
3. Push the tubing all the way forward into the port until it seats firmly.
4. Continue to turn the nut until it is finger tight.
5. Using an appropriate open end wrench, turn the nut 1/4 turn (90°) past the point where you can feel the ferrule start to grab the tubing.
6. Remove the fitting and examine it. If made properly, the ferrule should spin around the tubing but will not move along its length. If the ferrule does shift laterally, reinstall and tighten it another 1/8 turn past fingertight.
7. Remove and reinspect and reinstall.
8. Once a fitting is correctly made, it can be removed and reinstalled many times. It will usually take not more than a 1/8 turn (45°) with the wrench to complete the job. If more torque is needed, apply in 1/16 turn increments as needed.
9. If you are installing a piece of plastic tubing, place the tube as far into the port as possible before pushing the fingertight fitting into place. After a few turns, push the tubing in until fully seated then tighten the fitting completely. Always tighten plastic fittings by hand alone.

F5 Fitting Assembly

Probe Port

The Microdialysis Probe Port is lined with a flanged teflon tube. Insert the microdialysis outlet tubing (0.65 mm OD) into this tube before inserting the Probe Port into the valve. Slightly tighten the probe port with a few turns and then insert the microdialysis tubing as far as possible until it bottoms out. Then completely tighten the probe port to secure the connection.

F6 Probe Port

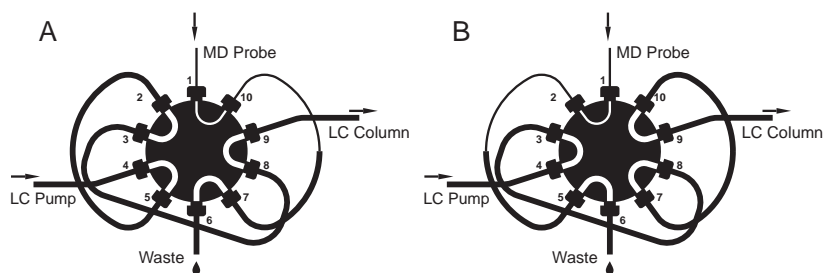


Section 4. Methods

Partial Loop Loading

When the Pollen-8 is used with a microdialysis probe, the filling of each loop is controlled by the syringe pump which perfuses the probe. The microdialysis flow rate (generally < 2.0 $\mu\text{L}/\text{min}$) is also the rate at which the loop is filled. At this slow and controlled flow rate, the sample loop can be filled to 95% of its volume for reproducible injections. If the loop was filled by a hand-actuated syringe, not more than 50% of its volume could be normally be filled for reproducible injections.

F7 Partial Loop Loading



In this first method, one loop is filled by the dialysis line, while the other loop is being injected onto the LC. Partial-filling captures all of the microdialysate. Notice that the sample is loaded in one direction and evacuated in another direction. This maintains peak integrity by minimizing diffusion within the loop, since the sample moves in and out the same end of the loop.

At a typical dialysis flow rate of 2.0 $\mu\text{L}/\text{min}$, the interval between injections onto the LC ranges from 57 sec (2 μL loops) to 23.75 min (50 μL loops). The chromatogram would have to be completed within the time limits imposed by the loop size and dialysis flow rate before the next injection was made. Notice how the time range is affected by dialysis flow rate. At a slower dialysis flow rate of 1.0 $\mu\text{L}/\text{min}$, the range would increase to 1.9 min (2 μL loops) and 47.5 min (50 μL loops), since the sample moves in and out the same end of the loop.

Overfilling a Sample Loop

If the time required to complete the chromatographic separation is longer than the interval required for partial loop loading, then it will be necessary to overfill each loop and adjust the injection interval to match the chromatography. In this case, there will be some sample loss through the waste port. This loss should be considered when plotting the final dialysis data. For example, if data is plotted as a bar graph, each chromatographic peak would be a bar as wide as the time required to fill the loop while the sample loss from overload would be represented as gaps between the bars.

Matched and Calibrated Sample Loops

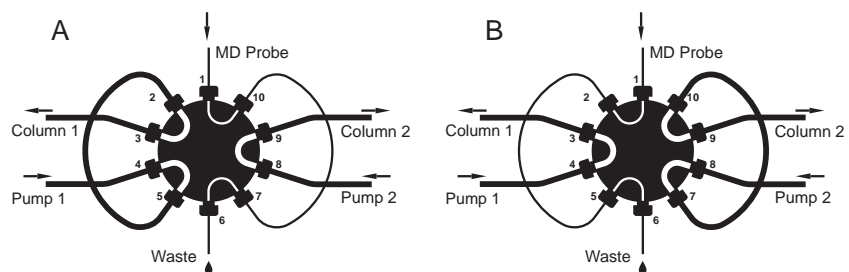
It is during the overfilling of a loop that the value of matched sample loops becomes apparent. If both loops are being injected onto the same LC, but have different volumes, you would have to adjust the data for every other chromatogram to account for the variance.

Partial filling does not require matched loops but it certainly helps to have calibrated loops on hand so you know the limit available to you. Sample loops typically vary dramatically. A loop that is nominally 5 μL in volume may actually be $\pm 50\%$ of that volume depending on the nature of the tubing. Stainless steel loops are typically the most variable because of the variation in the inner diameter.

Split Dialysate

In this approach, the output from one microdialysis probe is divided between two different liquid chromatographs. The injection interval must match the longest chromatogram. If two matched loops are used, some overfilling may be required while waiting for the longest chromatogram to be completed. It is also possible to use two calibrated loops of different values. For example, you could use a large loop for the chromatograph with the shortest duration and a small loop for the chromatograph with the longest duration. This would allow you to capture more dialysate in the large loop while waiting for the longer chromatographic separation to finish.

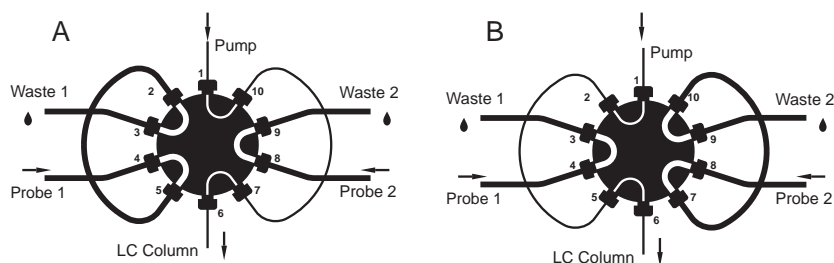
F8 Split Dialysate



Two Probes

The output from two separate microdialysis probes is routed to a single liquid chromatograph in this method. Such an approach might be valuable if you were comparing blood vs. brain levels of a specific drug. It would also be useful when comparing dialysates from two different animals. Again, the loop is loaded from one direction and backflushed when injected onto the LC system.

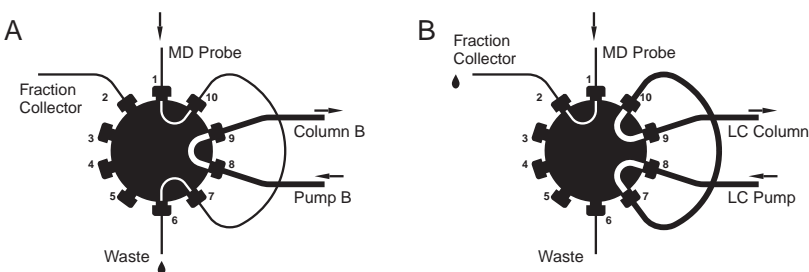
F9 Two Probes



Inject and Collect

This method requires the use of the BAS HoneyComb Refrigerated Fraction Collector. Using this approach, it is possible to divide the dialysate between alternately injecting sample onto the LC and collecting it in a vial. This is particularly useful if an analyte can not be directly detected by the LC such as compounds which required pre-column derivatization, *e.g.*, amino acids. If you wanted to examine both amino acids and biogenic amines in the same dialysate, you would use this approach to inject samples onto an LC with EC detection to directly monitor the biogenic amines, and also collect samples in vials for later derivatization and analysis of the amino acids. Notice that the collected fractions are never contaminated or diluted by the mobile phase which flows through the valve. Only dialysate passes through this port.

F10 Inject and Collect

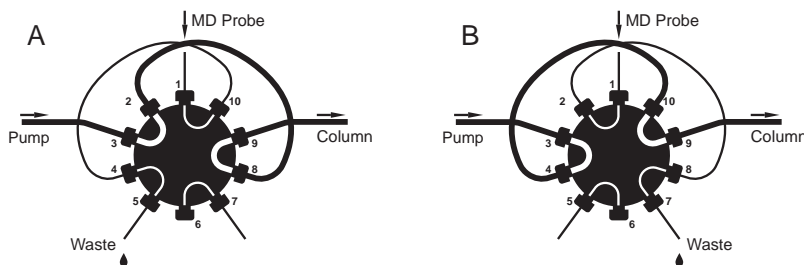


The Inject/Collect method is also useful when you need “insurance” during a long experiment. Automated instruments are often run overnight or unattended. If something unexpected happens to the LC system, a set of backup samples is still available in the fraction collector.

Microbore Chromatography

Microbore separations may be particularly sensitive to variations in flow paths. In the conventional method for partial-filling, the port connector is used to cross between one side of the valve and the other. This adds additional tubing between the loop and the column. With conventional columns, this will have no adverse effect. In some microbore separations, you may notice a variation between injections due to this extra tubing. If this is the case, we recommend re-plumbing the system to an 8-port injector as shown.

F11 Microbore Chromatography



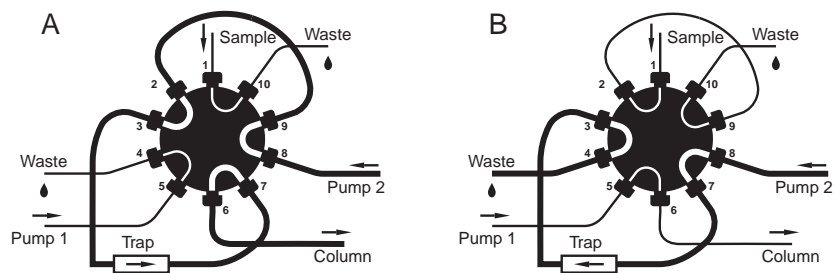
Waste Port

Please utilize the waste port provided with the injection valve. Do not substitute tubing with a smaller bore or longer length. Such changes may result in increased back pressure which can affect the reproducibility of filling the sample loop.

Trapping Column

A trapping column is used to retain components with long retention times while other analytes pass through to a separate column. A trapping column such as the BAS MF-6262 is used to retain physostigmine which would otherwise “kill” an acetylcholinesterase reactor column downstream by irreversibly binding to the enzyme. A trapping column such as the ChromTech BioTrap 500 C18 is used to trap drugs from a plasma sample and separate them from undesirable plasma proteins which would otherwise foul the analytical column. Trapping protocols are often designed using 6-port valves. A 10-port valve can also be plumbed for such applications as illustrated below.

F12 Trapping Column



Section 5. Basic Operation

Front Panel Controls

POWER

The valve control cable must be attached to the valve actuator and back panel jack labeled "Valve", and the power cord must be plugged in. A red LED lights up over the power switch when power is applied. Then the LED display panel lights all annunciators (A, B, TIME, LIMIT) and digits for 1 second followed by the software version number for 1 second.

If the SAMPLE switch is set to TIME, the previously set interval will be displayed. If the SAMPLE switch is set to LIMIT, the previously set limit to injections will be displayed. The valve will cycle to start operation in valve position A. If the CONTROL switch was set to REMOTE, 3 dashes (- - -) will be displayed.

CONTROL

Set the switch to LOCAL when setting up a method or during standard operation. Set the switch to REMOTE when control of the timing interval is being surrendered to another device connected via the back panel terminal strip.

In REMOTE, 3 dashes (- - -) will be displayed and the only annunciators visible will be valve position markers A or B. The back panel inputs TOG IN, GO TO A, and GO TO B are now enabled.

VALUE

Push buttons increase or decrease value of number displayed on LED screen. The longer each button is pushed, the faster its rate of change.

TIME

Start or Stop method using push buttons. The value on the screen represents a time (0.1 to 99.9 minutes). Once started, the decimal point flashes slowly and the number decreases in 0.1 min decrements until the injection event is reached. At this point a 1 second low-going pulse outputs from the back panel TOG OUT terminal. and a new time cycle is displayed on the screen. Pressing START will always reset the cycle time. Pressing STOP will stop the countdown process and will also reset a valve position error displayed as "Err".

SAMPLE

TOGGLE is a temporary setting which changes valve position (A ↔ B). It will return to the time setting unless the switch is held in the toggle position. A one second low-going pulse outputs from the back panel TOG OUT terminal.

TIME is selected when you want to display the time interval between injections. This switch must be in this position during normal operation or the unit will not start.

LIMIT is selected when you want to view the number of injections that will be made by the valve. There is an upper limit of 999 injections. If this value is set to zero, the Pollen-8 will

be set to continuous cycling and LIMIT will then display the word "ALL". If there is a power interrupt any preset LIMIT will return to the initial state. For example, if there is a power fluctuation during a 50 injection protocol, the unit will reset to the first injection.

Operation

1. Once the valve is correctly plumbed, start the LC pump and microdialysis syringe pump to make sure that all connections are secure and dry.
2. The LOCAL/REMOTE switch should be set to LOCAL before attempting to configure a method.
3. On the front panel of the Pollen-8 Controller set a limit to the number of injections by switching to the LIMIT position and entering a total number of injections up to a maximum of 999. If no limit to the number of injections is desired, set the limit to ALL initially.

NOTE: Whether setting a limit, or no limit to the number of injections, always keep in mind that there are other factors which limit the duration of the experiment, including the volume of mobile phase in the LC system and the volume of the syringe used in the dialysis experiment.

4. Return the sample switch to TIME. Set an interval between injections. This interval is the same for both loops and represents a time in minutes. To determine what this interval should be you will need to consider the time required to complete the chromatographic separation, and the time required to fill the sample loop:

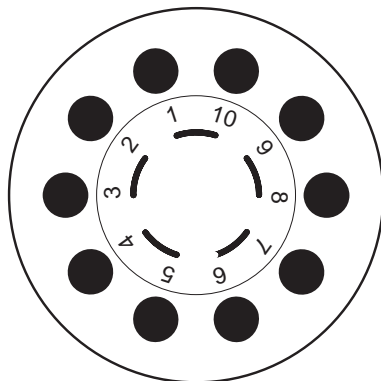
$$\text{Loop Volume } (\mu\text{L}) \div \text{Syringe Pump Flow Rate } (\mu\text{L}/\text{min}) = \text{Time to Fill Loop}$$

$$\text{Loop Volume } (\mu\text{L}) \div \text{Syringe Pump Flow Rate } (\mu\text{L}/\text{min}) = \text{Time to Fill Loop}$$

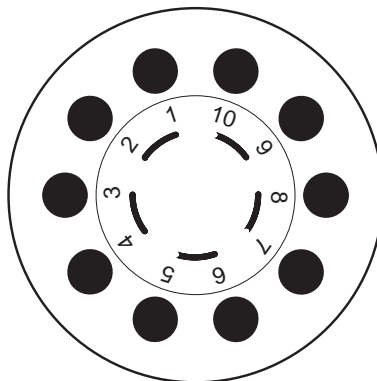
5. Set a starting position for the valve (A or B). This refers to the valve position and therefore also defines which loop is online with the LC system. Use TOGGLE to change position.

F12 Valve Position

VALVE POSITION A



VALVE POSITION B



6. Once the parameters are set, press the START button to begin the countdown to the first injection. The decimal point will flash and the interval will decrease in 0.1 min. decrements until it reaches zero and the injection is made.
7. The valve will then change position (to A or B) and the interval will again be displayed for the next countdown.
8. Injections will continue this manner until the limit is reached, or the STOP button is pressed.

Standards

Injection of standards can be accomplished in one of two ways. The microdialysis probe port may also be used as a syringe port for direct loading by a handheld syringe. Be sure to either overload the sample loop or load less than 50% of the loop volume. If additional standards will be injected at a later time, an alternate approach would be to change over to a standard solution using a liquid switch between the microdialysis outlet line and the probe port. You would have to calculate the amount of additional volume between the liquid switch and the valve and add this to the amount injected so that any remaining dialysate in the line would be flushed out.

Section 6. Remote Control Operation

Rear Panel Terminal Strip

Communication with the Pollen-8 is accomplished via a rear panel terminal strip. There are also D connectors labeled for "BAS Communication Network" and "Serial RS-232C". These D connectors are reserved for future options: do not attempt to control the instrument through them.

The rear panel terminal strip provides 3 inputs (Go To A, Go To B, TOGIN), 4 outputs (RELAY A, COMMON, RELAY B, TOG OUT) and 3 ground connections. All inputs are TTL and outputs are relay contacts. Voltages below 0.5 VDC are considered ground. Relay contacts are rated at 24 VDC at 35 mA.

GROUND

Reference. A digital ground reference level. There are 3 ground terminals.

RELAY A

Two terminals. Closed when the valve is in position A.

RELAY B

Two terminals. Closed when the valve is in position B.

TOG OUT

Produces a one second output TTL pulse +5 VDC to ground whenever the valve changes position.

GO TO A

When this input is grounded momentarily, the valve goes to position A.

GO TO B

When this input is grounded momentarily, the valve goes to position B. Note that GO TO A and GO TO B are mutually exclusive - only one can be grounded at a time.

TOG IN

When this input is grounded momentarily, the valve changes position from A→B or B→A.

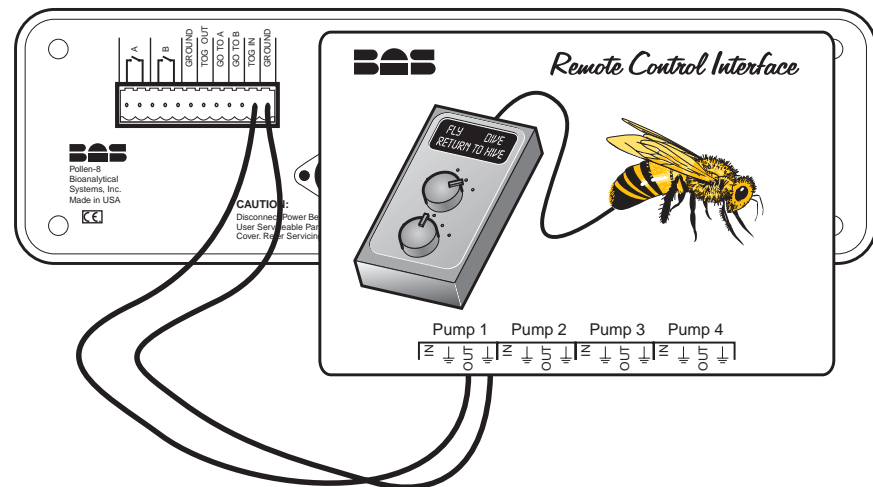
Variable Timing

During basic operation of the Pollen-8, it is possible to set only one sampling interval. If variable timing is required, it will be necessary to set the Pollen-8 into REMOTE status and use the back panel connections. The Pollen-8 can then be controlled by the BAS Queen Bee Syringe Pump, or an external timer of your choice.

Queen Bee Syringe Pump

To create collection times other than the interval set on the Pollen-8 front panel, use the Queen Bee Syringe Pump. It will be necessary to make two connections to the terminal strip, using good quality wire. First, turn off the power to the Pollen-8. Strip the plastic coating from about 1 cm on both ends of two pieces of wire. The wire should be sufficiently long to reach from the back panel of the Pollen-8 controller to the Remote Control Interface on the Queen Bee. Using a screwdriver, open up two connectors on the strip labeled TOG IN and GROUND. Insert one piece of wire in each position and tighten the connector with the screwdriver. Examine the Remote Control Interface of the Queen Bee. There are input and output sides to this interface arranged in sets to match each of the four pumps controlled by the Queen Bee. Select connections for the pump (1, 2, 3, or 4) that will be delivering fluid to the Pollen-8 (via the microdialysis probe). Attach the other end of the TOG IN wire into the (+) positive connector on the Remote Control Interface. Attach the other end of the GROUND wire to the (-) negative connector on the Remote Control Interface. Use a screwdriver to tighten the screws around the wires on the Interface.

F14 Connecting the Pollen-8 to the Queen Bee Remote Control Interface



Before setting up the interval method on the Queen Bee, turn on the power to the Pollen-8 and set the LOCAL/REMOTE switch to REMOTE.

Pull down the Queen Bee Configuration Menu for the pump that will be used with the Pollen-8. Make sure that all other variables describing the perfusion (syringe size, flow rate, limit, and limit type: time, volume, amount) are entered correctly. Any change to these parameters will require setup of a new remote control method on the Queen Bee. Click the EVENT OUT AT box. Then click the INITIAL LEVEL box labeled HI. Then click the EVENT TYPE box labeled PULSE. An X in the box indicates that it is now active.

F15 Queen Bee Configuration Menu

QBC Configuration [QBCINI.QBC]

User Name: Buzz Syringe: 1 mL

Flow Rate: 2.0 uL/min Stop At: 482 uL

Stop after: Time Volume Amount Completed

| Concentration | Units | Name |
|---------------|----------|--------|
| 1 pmole/mL | pmole/mL | Drug_2 |

Event Out At: 482 uL

Initial Level: Hi Low

Event Type: Pulse FlipFlop

Buttons: Add, Clear All, Delete Line

Injection Sequence:

- (1) 19 uL - Pulse
- (2) 47 uL - Pulse
- (3) 19 uL - Pulse
- (4) 47 uL - Pulse
- (5) 19 uL - Pulse
- (6) 47 uL - Pulse
- (7) 19 uL - Pulse

Pump Active Alarm External (input) Control

Tabs: Pump 1, Pump 2, Pump 3, Pump 4

Buttons: ? Help, X Cancel, OK

Next, you need to determine the sampling interval that will be used. It will be necessary to enter a value for every valve actuation event. There is a limit of 100 events in the Queen Bee, which means a limit of 100 injections. The value is entered using either the (and (key in the open box next to EVENT OUT AT, or by typing in a number. Note that the units here will be shown as time, volume, or amount according to the setup of your perfusion method on the same screen. If you are trying to capture all of the dialysate into each sample loop, it may be easier to use a perfusion method based on volume in microliters (μL).

If you want a variable injection method - where the volume (or time, or amount) injected into one loop is different from the volume (time, amount) injected into the other loop - then you will need to enter a sequence which alternates between two sample volumes. This task is made easier by entering one of these volumes, *e.g.*, 10 μL , and clicking on the ADD key to enter the same value again. Keep clicking ADD until you have entered a string of the same value. The bracketed numbers represent the sequence of the injections: (2) is the second injection, (3) is the third injection, etc. Next, use the cursor to highlight the first line, *e.g.*, (1) 10 μL , in your sequence. Enter the value for the other volume in the dialog box and click ADD again. The new volume should be inserted as a new line after line (1).

NOTE: You can not enter anything prior to line (1), only after it. To insert a new line, always highlight the line preceding the position where you want to insert. For example, if you want to insert a line between lines (2) and (3), highlight line (2), enter the new value and click ADD.

Repeat the process of adding the second value throughout your sequence until you have enough lines to match the number of injections planned, or the limit of 100.

NOTE: Events always require whole numbers. Selecting volume, gives you finer control than selecting time or amount. For example, if your microdialysis flow rate was 2.0 $\mu\text{L}/\text{min}$, the smallest value you could enter for time would be 1 minute, which would represent 2 μL of fluid. The smallest value you could enter for volume would be 1 μL .

To change the value for a particular vial, highlight the line in the method by clicking on it and then use the DELETE LINE key to remove the line. Highlight the line describing the prior vial and then use ADD to insert a new value for the deleted line.

When you are satisfied with the method that you have created, click the OK button at the bottom of the configuration screen and return to the operations screen. Pull down the configuration menu and select SAVE to retain the file describing this configuration. The file name will have the extension .qbc once saved.

Return to the front panel display of the Pollen-8 and check to see whether A or B is displayed. A or B refers to the valve position and if you have two sample loops installed, it also refers to the sample loops. Return the LOCAL/REMOTE switch to LOCAL and use the TOGGLE switch to change the starting position of the valve to A or B as needed to match the variable injection method you have just set up on the Queen Bee. Return the switch to the REMOTE position before beginning your method.

Pull down the configuration screen and open the REPORT window, if desired. Begin the perfusion and the variable sampling interval by pressing the appropriate pump START button at the bottom of the screen (P1, P2, P3 or P4). The progress of the perfusion will be displayed on the bottom line of the OPERATIONS screen. The history of the protocol, including external events will be displayed on the REPORT screen.

Example:

A protocol was designed to split microdialysates between two different LC systems. The probe would be perfused at a rate of 2 $\mu\text{L}/\text{min}$ using a 1 mL syringe, for a period of 4 hours. The dialysate flowing into the Pollen-8 valve would be divided between one 20 μL loop and one 50 μL loop. A method was needed to deliver 19 μL to the 20 μL loop and 47 μL to the 50 μL loop.

Method setup would begin by entering the syringe size (1 mL) and flow rate (2 $\mu\text{L}/\text{min}$). This protocol was developed in terms of volume collected within the sample loops. To set up the external output on the basis of volume, click the STOP AFTER parameter to Volume. Then enter the STOP AT parameter in μL . Since the perfusion should last for four hours, convert this time to minutes (240 min.) and multiply by the flow rate (2 $\mu\text{L}/\text{min}$) to determine the total volume of fluid delivered by the syringe (480 μL). You could increase this to a larger number, *e.g.*, 500 μL , to allow some leeway between the timed events and the perfusion experiment. When the STOP AT limit is reached, the syringe pump will stop. When the last timed event is reached the valve will remain in its final position and no longer actuate. The following chart illustrates how the external input commands would be entered:

| Injection Number | Ext. Output At: |
|------------------|-----------------|
| 1 | 19 μ L |
| 2 | 47 μ L |
| 3 | 19 μ L |
| 4 | 47 μ L |
| 5 | 19 μ L |
| 6 | 47 μ L |
| 7 | 19 μ L |
| 8 | 47 μ L |
| 9 | 19 μ L |
| 10 | 47 μ L |
| 11 | 19 μ L |
| 12 | 47 μ L |
| 13 | 19 μ L |
| 14 | 47 μ L |
| 15 | 19 μ L |
| Total | 481 μ L |

Notes on Remote Control Methods:

1. If the total of the sampling intervals entered represents a volume larger than the volume in the syringe, the Queen Bee will still allow you to proceed. For example, if you have set up a method in which twenty (20) samples of 100 μ L each are collected and you have indicated the use of a 1.0 mL syringe, you will have exceeded the syringe capacity by 1,000 μ L. Unless you stop the pump and replace the syringe (not recommended for microdialysis!), you will run out of perfusion fluid before your protocol is halfway completed.
2. The Pollen-8 Controller must be set to REMOTE using the front panel switch.
3. The Queen Bee controls the syringe pump as well as the variable sampling interval. Once the method is executed, the pump will begin the perfusion and the external events protocol will be initiated. Make sure that you have already filled all the fluid lines in the dialysis probe and valve before beginning the method.
4. If you have set up a method involving variable sampling intervals controlled by the Queen Bee, the valve will stop actuating after the final event but the pump will continue until you physically interrupt the method by pressing the PUMP STOP button on the Queen Bee or have reached the limit set by your perfusion protocol.
5. You can monitor the progress of the external event output by opening the REPORT window which will record all external outputs as they occur. At the end of the protocol, you can save this report as a disk file which can be later printed.

Section 7. Maintenance

Routine Care

If the Pollen-8 is used with in vivo microdialysis sampling devices, it will be exposed primarily to saline solutions. Use the same care with the valve that you would with any LC injector. Disconnect the LC column and microdialysis probe and then flush salts from the valve thoroughly by using copious amounts of deionized, filtered water. After the water wash, a final rinse with methanol is usually sufficient to dry the lines. Don't leave the valve ports open and exposed to dust or other particulates.

Note: If passivating your LC system with strong acids, disconnect the valve. PEEK injection loops are not resistant to strong acids and pumping nitric acid through them at high pressure is not recommended.

Replacing the Rotor Seal

The rotor seal is the heart of the valve. With proper care it will serve you long and faithfully. Never disassemble a valve unless system troubleshooting definitely points to the valve as the cause of a malfunction.

1. Use a 9/64 hex driver to remove the socket head screws which secure the cap on the valve.
2. To protect the sealing surface of the cap against damage, leave it attached to the tubing on the system so it dangles in the air instead of resting against any surfaces.
3. Examine the rotor seal as it sits in the driver and note the orientation.
4. Using your fingernails, gently pry the rotor seal away from the driver.
5. Examine the rotor seal carefully for scratches. If any are visible to the naked eye, replace the seal. If no scratches are visible, clean the seal with a thorough water wash followed with methanol, taking care not to scratch the surface.
6. It is not necessary to dry the seal.
7. Replace the seal in the driver, making sure that the side with the etched grooves faces outwards. The pattern is asymmetrical to prevent improper placement.
8. Replace the cap. Insert the two socket head screws and tighten them by hand until they are snug. Do not overtighten - these screws simply hold the assembly together and do not affect the sealing force.
9. Test the valve by pressurizing the system. If it doesn't hold pressure, return the valve to BAS for repair.

Troubleshooting

| Symptom | Possible Cause | Remedy |
|--|---|--|
| Does not power up. | Power cord unplugged or faulty. | Plug in or replace cord. |
| | Blown fuse(s) | Replace fuse(s) in power entry module: 1.6 amp slo blo (120V) or 0.8 amp slo blow (220V) Valve is silent when Pollen-8 is powered up |
| | Faulty Control Cable | Replace Cable |
| Valve is silent after power up | Control cable loose or unplugged | Check connection of cable on both ends |
| | Faulty cable | Replace cable |
| Won't toggle to new valve position | LOCAL/REMOTE switch is in remote mode | Change switch to local mode |
| Value on Interval display is not decreasing | Didn't press the START button | When START is pressed the decimal point will flash slowly and the number will decrease in 0.1 min. increments |
| | Sample switch set to LIMIT | Turn sample switch to TIME |
| Valve stopped after a few injections | Limit set too low | Press the stop button and then change the adjacent switch to limit. Increase the number of injections or change to ALL. |
| When injecting standards loaded from a syringe pump peak heights are not uniform | Not using matched loops | Use matched and calibrated loops. Make sure that the tubing is fully seated in the valve port before tightening the fitting. |
| | Not using VICI fittings | Don't use fittings from other valves. There is a possibility of unswept dead volume (and carryover) from fittings which don't conform to valve. |
| | Improper waste tube | Ideally the waste tube should be short and a large ID to minimize back pressure |
| When an input pulse is sent to rear panel of Pollen-8 nothing happens | Ground wire not connected properly | Connect ground wire from control instrument to a GROUND terminal on the Pollen-8 back panel |
| ERR (Error Message) | Power cord was wiggled or not completely seated in back panel | Turn off power and push cord firmly into back panel |
| | Valve control cable disconnected | Connect valve control cable. Push STOP to clear, then retry. |
| | Defective Valve | Contact BAS Service |
| | Switch | Check to see if any front panel push button is stuck on |
| | Valve Actuator | Check for valve body movement in the clamping ring. Tighten ring and push STOP button. If actuator sound is longer than 0.3 sec., push STOP. Disconnect power from unit and check for a defective mechanical coupling between valve and rotor. |
| ER2 or ER3 | Internal memory failure | Call BAS Service |

Replacement Parts

| | |
|---------|---|
| MD-1250 | Complete Pollen-8 OnLine Injector Package for Conventional LC |
| MD-1251 | Complete Pollen-8 OnLine Injector Package for Microbore LC |
| MD-1253 | Conventional 10-port injection valve |
| MD-1252 | Microbore 10-port injection valve |
| MD-1255 | Probe Port |
| MD-1257 | Replacement Liner for Probe Port |
| MD-1258 | Replacement Ferrule for Probe Port |
| MD-1259 | Port Connector |
| MD-1254 | Rotor Seal for Conventional Valve |
| MD-1256 | Rotor Seal for Microbore Valve |
| MD-1260 | One pair of Matched/Calibrated Loops: 2 μ L nominal |
| MD-1261 | One pair of Matched/Calibrated Loops: 5 μ L nominal |
| MD-1262 | One pair of Matched/Calibrated Loops: 10 μ L nominal |
| MD-1263 | One pair of Matched/Calibrated Loops: 20 μ L nominal |
| MD-1264 | One pair of Matched/Calibrated Loops: 50 μ L nominal |
| MR-4076 | 1/16" Stainless Steel Nut, each |
| MR-4077 | 1/16" Stainless Steel Ferrule, each |
| MD-1265 | Waste Port |

Limited Warranty

Bioanalytical Systems Inc. (BAS) warrants that equipment manufactured by the company will be free from defects in material and workmanship for a period of one (1) year from the date of shipment, except as provided hereinafter. Needles, rotor seals, fittings, cannulae and other components exposed to repeated wear are exempt from this warranty. Damage caused by use of the incorrect fittings, seals, or contamination with particulates is exempt from warranty. Claims for shipping damage are invalid unless the company is notified within 30 days of the shipping date. When the customer has ordered and paid for shipping insurance, BAS is liable only to the extent of replacement of any items missing, or broken during shipment. BAS will not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever arising from the use of this device. The foregoing warranty is in lieu of all other warranties expressed or implied including but not limited to the implied warranties of merchantability and fitness for a particular purpose.