User's Manual

PCX 5100
Post-column
Derivatization Instrument

For Carbamate and Glyphosate Determination

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Limited Warranty

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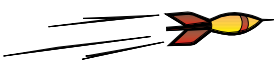
Before You Start
Please Read

Please Read

- Page 3-1 for the HPLC System Requirements, especially for ***glyphosate analysis!*** The HPLC components must be compatible with ***high pH regenerant*****.**
- Page 2-11 for the safety requirement of ***coated-bottles;*** they must not be substituted!
- Page 4-13 for the calcium hypochlorite caution! Instead of ***calcium hypochlorite,*** use ***sodium hypochlorite*****.**

Symbols

A note supplies supplementary information which may be helpful or necessary for better understanding of the material.



The caution calls attention to an operating procedure, practice, or the like, which if not correctly done or adhered to, could result in loss of information, or damage to, or destruction of part or all of the equipment. Do not proceed beyond a caution sign until the indicated conditions are fully understood and met.

The following symbols appear on the PCX5100 or its accessories.

This warning sign denotes a hazard. It calls attention to a procedure, practice, or the like, which if not correctly done or adhered to, could result in injury or loss of life. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

Ce symbole est un signal de danger. Il indique qu'une manipulation, si elle n'est pas respectée ou effectuée correctement, risque d'entrainer des blessures, voir la mort.

Dieses Warnsymbol kennzeichnet eine Gefahr. Es macht aufmerksam auf einen Vorgang, eine Handhabung oder ein Vorhaben, die bei unkorrektem Befolgen der Vorschriften zu einer Verletzung oder einer lebensgefährlichen Situation führen können.

El signo de atención indica un riesgo. Requiere atención sobre un procedimiento, práctica, o similar, que, si no se ejecuta correctamente o se sigue minuciosamente, prodía producir heridas o muerte. No continúe a partir de un signo de atención hasta que no se hayan entendido y alcanzado completamente las condiciones indicadas.

Questo avvettimento informa del pericolo. Molto attenzione riguardante il moto di usare questa macchina é molto inportante altrimenti risulterá danni e anche morte. Non continuare di piu affinché le condizioni e instruzioni sono completamente chiare.

This warning sign denotes a hot surface, a high temperature hazard. It calls attention to a column heating block hotter than 70°C. For your safety, wear insulating gloves when the column oven is warm.

Ce symbole indique une surface brûlante. Il signifie que la résistance chauffante de la colonne a atteint une température supérieure à 70°C. Pour votre sécurité, prière de porter des gants isolants.

Dieses Warnsymbol kennzeichnet eine heiße Oberfläche oder eine Gefahr durch hohe Temperaturen. Es macht aufmerksam auf den Heizblock des Saulenofens, der heißer als 70°C sein kann. Zu Ihrer Sicherheit sollten Sie isolierende Handschuhe tragen, wenn der Saulenofen warm ist.

Este signo de atención indica una superficie caliente, un riesgo de alta temperatura. Pide atención sobre un bloque calefactor de columnas por encima de 70°C. Para su seguridad use guantes aislantes cuando el horno de columnas esté caliente.

Questo avvettimento informa della temperatura molto alta che possibilmente potrebbe bruciare. Molto attenzione é necesaria specialmente al blocco caldo della colonna che é superiore ai 70°C. Per essere protetti é necesario usare guanti insulanti per questa applicazione.

Power On

En marche

An

Escendido

Acceso

Power Off

Éteint

Aus

Apagado

Spento

Fuse Specification

Spécification du fusible

Spezifikation der Sicherung

Fusible

Valvole Specificazioni

Protective Ground

Prise de terre

Erdung

Masa de protección

Protezioni a terra

Chapter 1
Introduction

High-performance liquid chromatography (HPLC) with post-column derivatization is a technique for rendering analytes more detectable than they would otherwise be in their native forms. Post-column derivatization can give improved sensitivity or better selectivity (reduction of interference) leading to lower detection limits. The Pickering Laboratories PCX5100 was developed to facilitate the determination of carbamate insecticides (5µm C_{18} column), meeting or exceeding performance requirements for precision and accuracy of U.S. Environmental Protection Agency (USEPA) Method 531.1, and the AOAC International Protocol 29.A05; and the herbicide glyphosate (5µm sulfonated cation-exchange column), meeting or exceeding performance requirements for precision and accuracy of USEPA Draft Method 547.

In addition, there are a number of carbamate pesticide compounds employed worldwide which are not included in the 10 compounds mandated by USEPA Method 531.1 and AOAC Protocol 29.A05. The Pickering Laboratories PCX5150 (5µm C_8 column) can separate as many as 23 compounds. The C_8 column can also be used as a confirmation column when using a water/acetonitrile gradient instead of a water/methanol gradient.

Post-column Analysis

A complete Post-column Analysis system for carbamates or glyphosate consists of the following components:

- HPLC binary gradient pump
- Manual injector or autosampler
- Pickering Laboratories columns
- Pickering Laboratories PCX5100 Post-Column Derivatization Instrument
- Pickering Laboratories eluants, reagents, and standards
- Fluorescence detector
- Chart recorder, integrator, or data system

Carbamates

Carbamates, a class of highly effective commercial insecticides, are used worldwide to protect crops from insect pests. Applied directly to food crops such as grains, fruit, and vegetables, carbamates may seep into drinking water sources through agricultural runoff. In addition, if food crops are harvested too soon after application, residues of carbamates and their byproducts may remain in the produce. The use of carbamate insecticides has created a requirement for a simple, reliable, and sensitive method of residue analysis for these compounds found in vegetable matter, drinking water, and industrial waste-water.

The USEPA Methods 5 and 531.1, and the AOAC International protocol 29.A05, describe a direct-inject method which employs gradient liquid chromatography with fluorescence detection, accomplished by post-column hydrolysis and derivatization of the eluted carbamates.

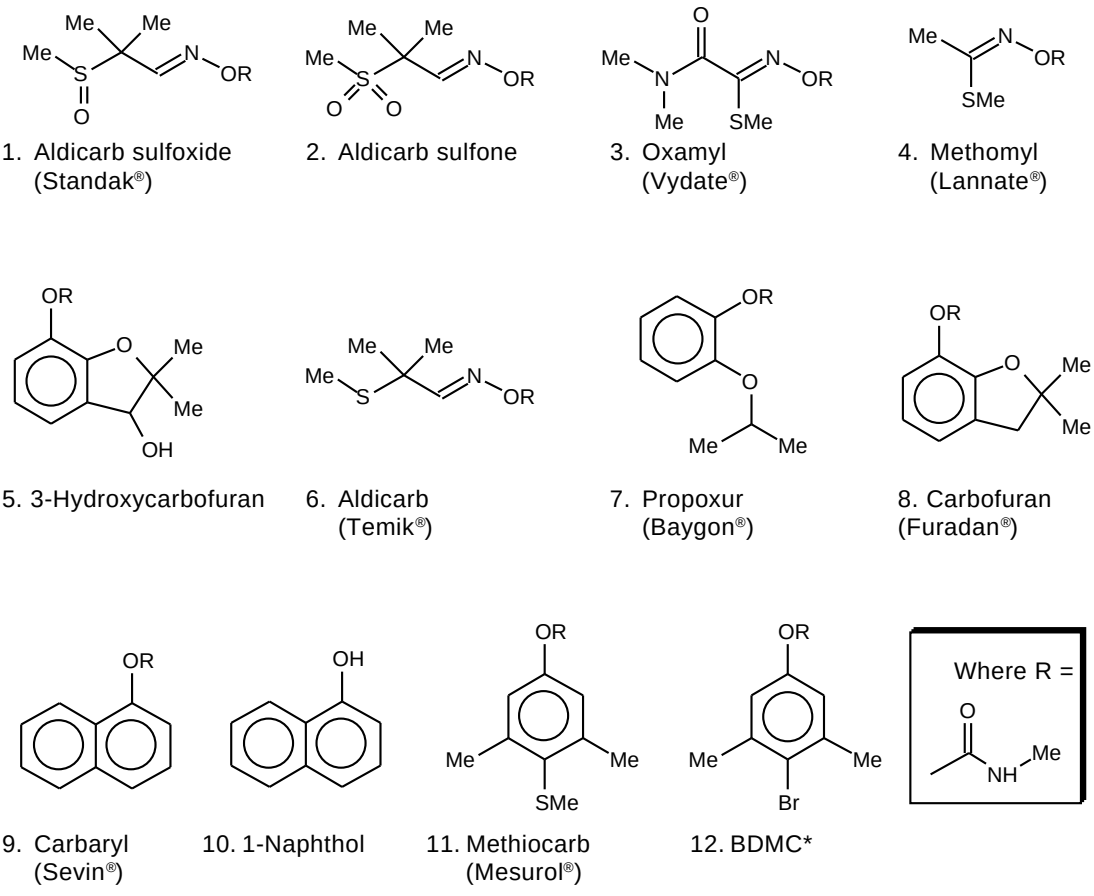
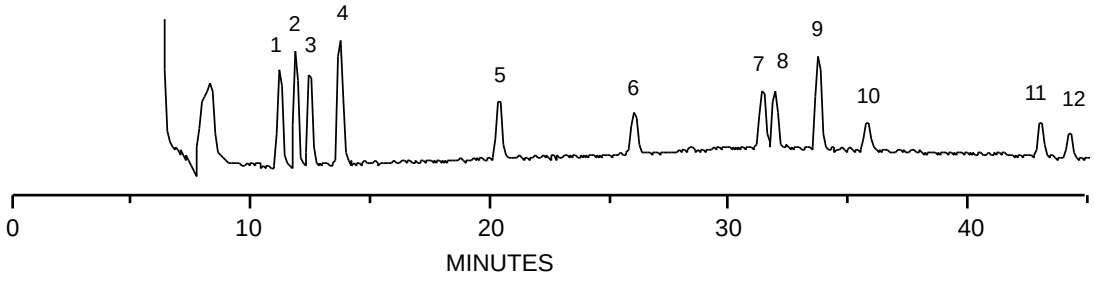


Figure 1-1. Analytes in the Pickering carbamate test mixture. (*4-Bromo-3,5-dimethylphenyl-N-methylcarbamate; an internal standard)

HPLC Separation

The general structure of the carbamate insecticides is an *N*-methyl substituted urethane with the variation in the ester moiety. The structural formulas for the ten analytes specified in USEPA Method 531.1 are shown in Figure 1-1 (including 1-naphthol and BDMC). They are listed in the order in which they elute from the Pickering carbamates column. All but 1-naphthol (10) contain the *N*-methylcarbamoyl moiety (indicated by –OR). The hydrolysis of carbaryl (9) in the post-column reactor also produces 1-naphthol. Note that 1-naphthol hydrolyzed from carbaryl and 1-naphthol in the calibration standard are at different retention time. This observation is useful for troubleshooting, see page 5-13.

Each unique R– group represents a different commercial product or its metabolite. The separation of the carbamates is achieved with the Pickering 5µm, C_{18} or C_8 column maintained at 42°C and 37°C, respectively. The chromatographic method recommended for this column is a simple linear water/methanol binary gradient that resolves the twelve carbamate products provided in the test standard (Figure 1-2). The carbamates elute principally in relation to their relative hydrophobicity. Aldicarb sulfone, which is minimally hydrophobic, elutes early while methiocarb, which is more hydrophobic, elutes towards the end of the gradient.



1. Aldicarb sulfoxide (Standak)
2. Aldicarb sulfone
3. Oxamyl (Vydate)
4. Methomyl (Lannate)
5. 3-Hydroxy carbofuran
6. Aldicarb (Temik)
7. Propoxur (Baygon)
8. Carbofuran (Furadan)
9. Carbaryl (Sevin)
10. 1-Naphthol
11. Methiocarb (Mesurol)
12. BDMC internal standard

Figure 1-2. 2.1 ng in 150 µL water (14 ppb); 25 cm C_{18} column

Post-column Derivatization

The separated carbamates are first saponified by sodium hydroxide (NaOH) at 100˚C to release an alcohol, carbonate, and methylamine. In the second post-column reaction, methylamine reacts with *o*-phthalaldehyde (OPA) and the nucleophilic Thiofluor™ (or 2-mercaptoethanol) to form a highly fluorescent 1-methyl-2-alkylthioisoindole derivative (Figure 1-3). This fluorescent derivative provides detection ≤ 3ng per component on-column, which meets the method requirements of the EPA. Depending on the type of fluorescence detector used, detection limits ten times better than the EPA requirements may be obtained.

The Pickering carbamate post-column derivatization instrument, when used with an HPLC binary gradient pump, fluorescence detector, and recorder or integrator, will meet or exceed EPA requirements:

- High sensitivity: detection limits of 0.1–0.5ng (or 0.2–1ppb levels for drinking water) can be routinely achieved.
- Selectivity (specificity): only *N*-methylcarbamates and *N*-methyl carbamoyloximes plus components reactive to OPA under the specified operating conditions are detected.
- Minimum sample preparation: drinking water can be directly injected into the HPLC after filtration. No pre-extraction or sample cleanup is required.
- The analysis is easily automated for unattended analyses with the addition of an autosampler.

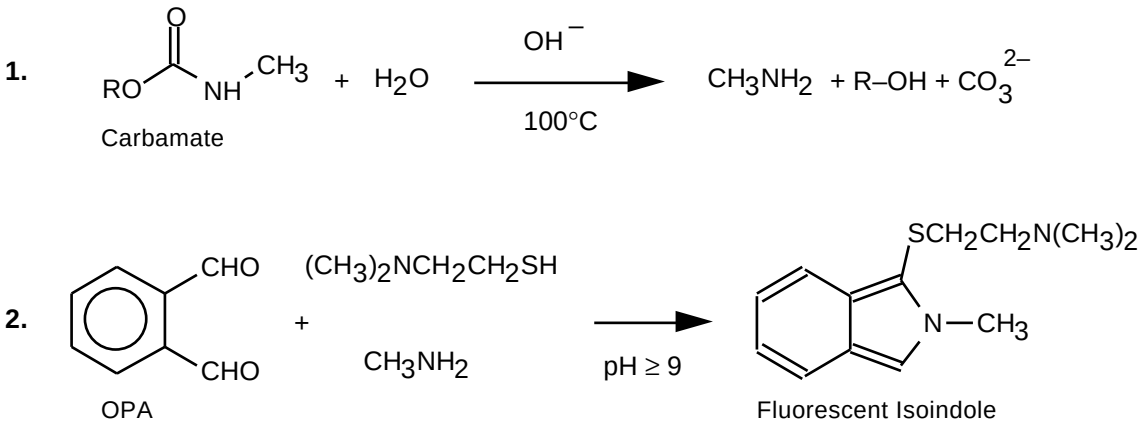


Figure 1-3

Glyphosate

Glyphosate (*N*-Phosphonomethylglycine, Rodeo™, Roundup™) is a broad-spectrum herbicide. Its wide use in agriculture can result in its presence in ground water. A sensitive analytical technique has been developed to monitor levels of glyphosate and its principal metabolite, aminomethylphosphonic acid (AMPA). This method is an improved version of USEPA Draft Method 547.

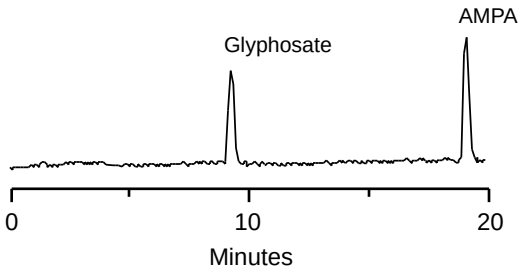


Figure 1-4

Glyphosate and AMPA are separated on a strong cation-exchange column (fully sulfonated, cross-linked polystyrene, mixed K^+/H^+ form). The eluant is a phosphate buffer with $[K^+] = 0.05M$, and $[H^+] = 0.10M$. After isocratic separation, the column is regenerated with dilute KOH, then re-equilibrated with eluant (Figure 1-4).

Fluorometric detection follows a two-stage post-column reaction. In the first stage, glyphosate is oxidized by hypochlorite to glycine. In the second stage, glycine reacts with *o*-phthalaldehyde and Thiofluor (or 2-mercaptoethanol) at pH 9–10 to produce a highly fluorescent isoindole. AMPA does not need the initial oxidation to react with OPA (Figure 1-5); indeed oxidation reduces its fluorescent yield.

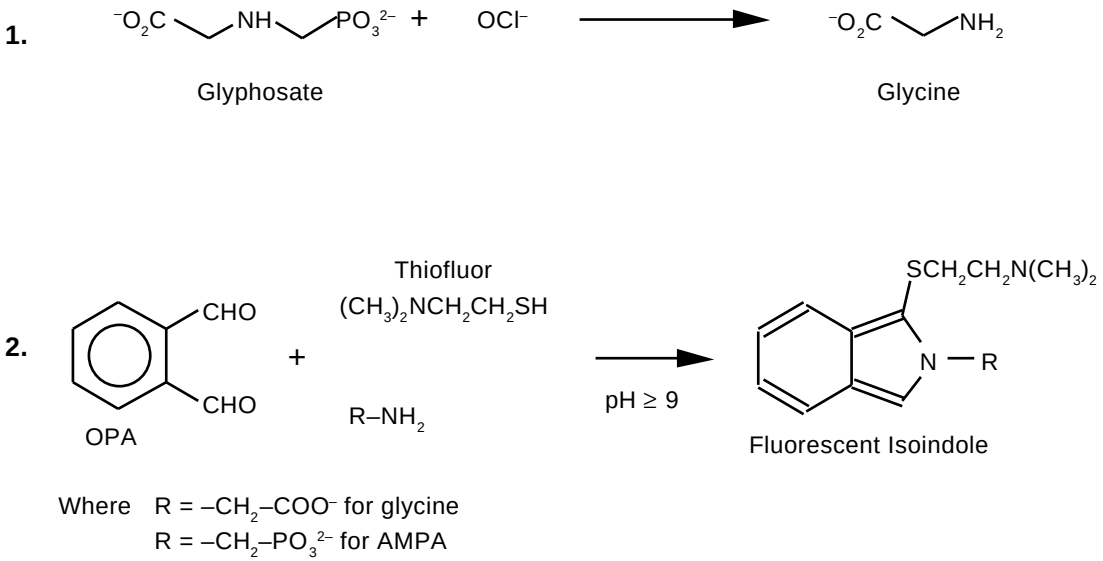


Figure 1-5

Post-column Hardware

At its minimum, a post-column reaction instrument consists of a pulse-free reagent pumping system, a mixer to combine the flows of reagent and eluate, and a continuous-flow reactor. To perform the carbamate and glyphosate procedures, you need two post-column systems in series.

The Pickering design (Figure 1-6) uses a single-piston reagent pump to deliver the reagent. Pulses are eliminated by the combination of a gauge followed by a packed-bed restrictor. The pulses are absorbed by the mechanical action of the Bourdon tube inside the gauge, and then released through the restrictor. The mixing device is simply a steel tee-fitting with a 0.010 inch bore. The continuous-flow reactor is a length of 0.011 inch ID Teflon capillary.

There are, of course, many refinements in a practical instrument. First, the reaction temperature may need to be controlled, as is the case for hydrolysis of carbamates. Elevated temperatures then require a back-pressure regulator to suppress boiling inside the heated reactor. The Pickering design also includes a gauge to monitor pressure at the first mixing tee, which is also the pressure at the first reactor. For the convenience of operation, bypass valves are provided for priming or purging the reagent pumps. Another refinement is the use of pressurized reagent reservoirs allowing the pump to operate more precisely at low flow rates, and also provides an inert atmosphere to protect air-sensitive reagents.

Safety systems have also been incorporated into the design. Two greatest hazards to post-column systems are rupture of the reactor because of the excessive pressure and the back-flow of caustic reagent onto the analytical column. The first hazard is managed by providing a relief valve that opens at about 525 psi (36 bar) and diverts flow away from the reactor. Two devices protect against reagent back-flow. To ensure flow through the column during operation, a pressure switch upstream of the analytical column must detect at least 500 psi (34 bar) or else the entire system turns itself off. Second, check-valves in the reagent delivery system prevent reagents from siphoning when the pump is off.

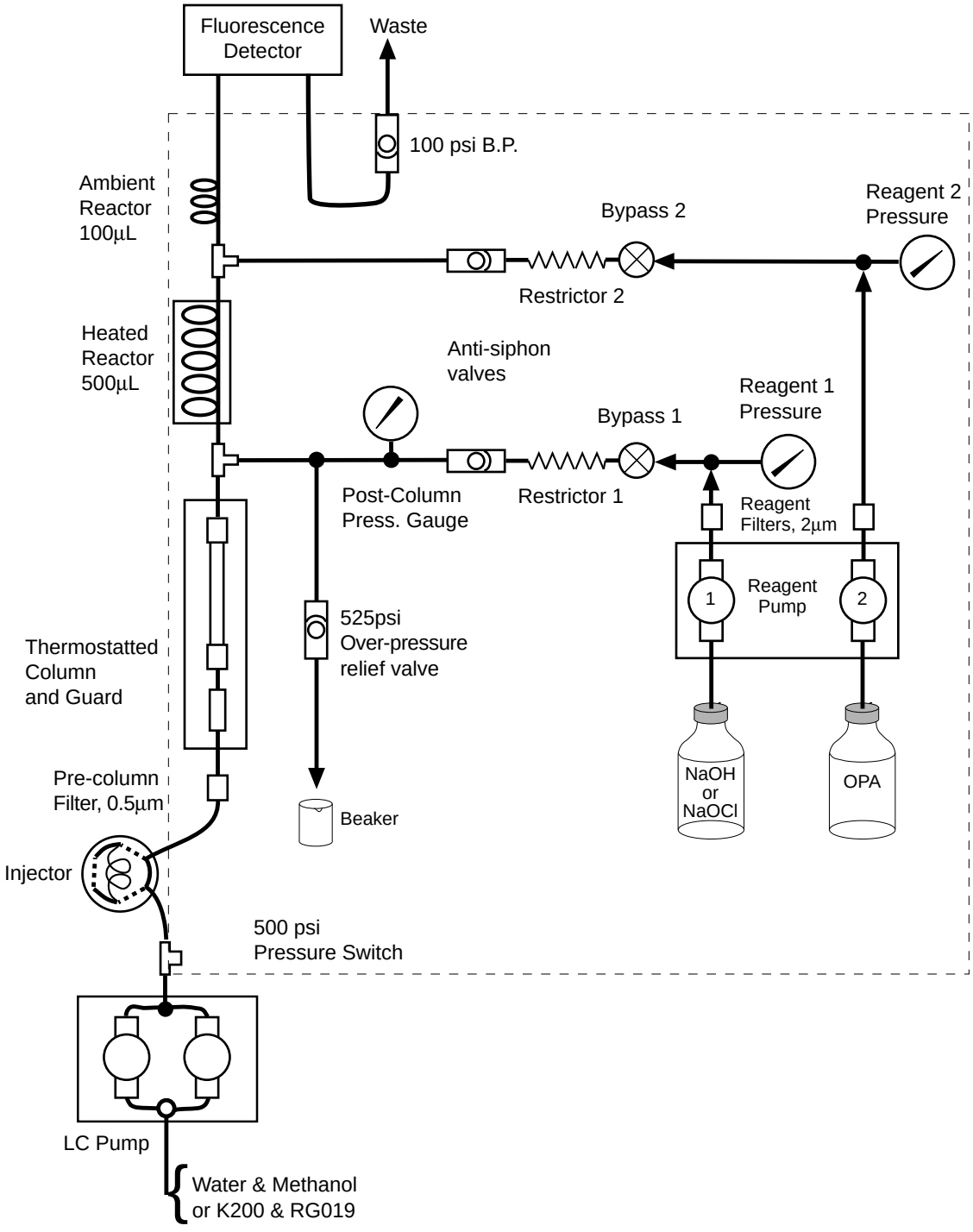
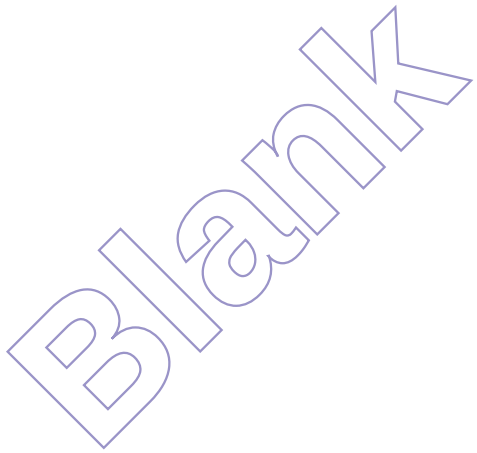


Figure 1-6. The Pickering post-column system is depicted inside the dotted-box.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Chapter 2
The PCX5100

System Components

PCX5100 Post-Column Instrument

The PCX5100 is available for 120V or 240V operation, and is shipped completely assembled, calibrated, and tested. The PCX5100 consists of a duplex reagent pump, heated and ambient reactors, column heater, backflow and over-pressure safety devices, filters and flow conditioners, reagent reservoirs, Saran® gas tubing, and other accessories.

EC5100 system for carbamate analysis

The complete system adds the following accessories for carbamate analysis:

1846150	Carbamate column, 4.6mm ID x 150mm, 5µm C_{18} with test chromatogram
1846250	Carbamate column, 4.6mm ID x 250mm, 5µm C_{18} with test chromatogram
18ECG001	Guard cartridge holder with 3 carbamate guard cartridges
O120	*o*-Phthalaldehyde, chromatographic grade crystals, 5g
CB910	OPA diluent for carbamate pesticide analysis, 4 x 950mL
3700-2000	Thiofluor, chromatographic grade crystals, 2 x 10g
CB130	Hydrolysis reagent for carbamate pesticide analysis, 4 x 950mL
1700-0063	Carbamate test mixture comprised of 12 components, 1.5mL, 2.5µg/mL, one each, included with each column
1700-0132	ChlorAC™ buffer for preservation of aqueous carbamate samples, 250mL

EC5150 carbamate expanded resolution

The EC5150 system is similar to the EC5100 except the two columns are replaced with:

0840250	Carbamate column, 4.0mm ID x 250mm, 5µm C_8, with test chromatogram

EG5100 system for glyphosate analysis

The complete system adds the following accessories for glyphosate analysis:

1954150	Glyphosate column, cation-exchange, 4mm ID x 150mm, 8µm K^+/H^+ form, with test chromatogram
1953020	Glyphosate guard column, cation-exchange, 3mm ID x 20mm, 8µm K^+/H^+ form
O120	*o*-Phthalaldehyde, chromatographic grade crystals, 5g
GA104	OPA diluent for glyphosate analysis, 4 x 950mL
GA116	Hypochlorite diluent for glyphosate analysis, 4 x 950mL
K200	Eluant for glyphosate analysis, 4 x 940mL
RG019	Column regenerant for glyphosate analysis, 1 x 950mL
3700-2000	Thiofluor, chromatographic grade crystals, 2 x 10g
1700-0080	Glyphosate test mixture containing 2.5µg/mL each glyphosate & AMPA, 1.5mL
1700-0140	RESTORE™ for removal of metal ion contamination from glyphosate ion-exchange column and guard, 250mL

Getting to Know Your PCX 5100

Front Panel

- Post-column pressure gauge measures the liquid pressure at the first mixing tee. This is effectively the pressure on the heated reactor. This gauge indicates pressure when liquid is flowing through the system.

- Reagent 1 pressure gauge measures the pressure of the reagent at the outlet of the reagent pump. This gauge is an integral part of the pulse-dampening system. When the reagent pump is on, the needle swings over a range of about 200 psi in time with the pump piston. This gauge is downstream of the reagent filter and upstream of the bypass valve and restrictor.

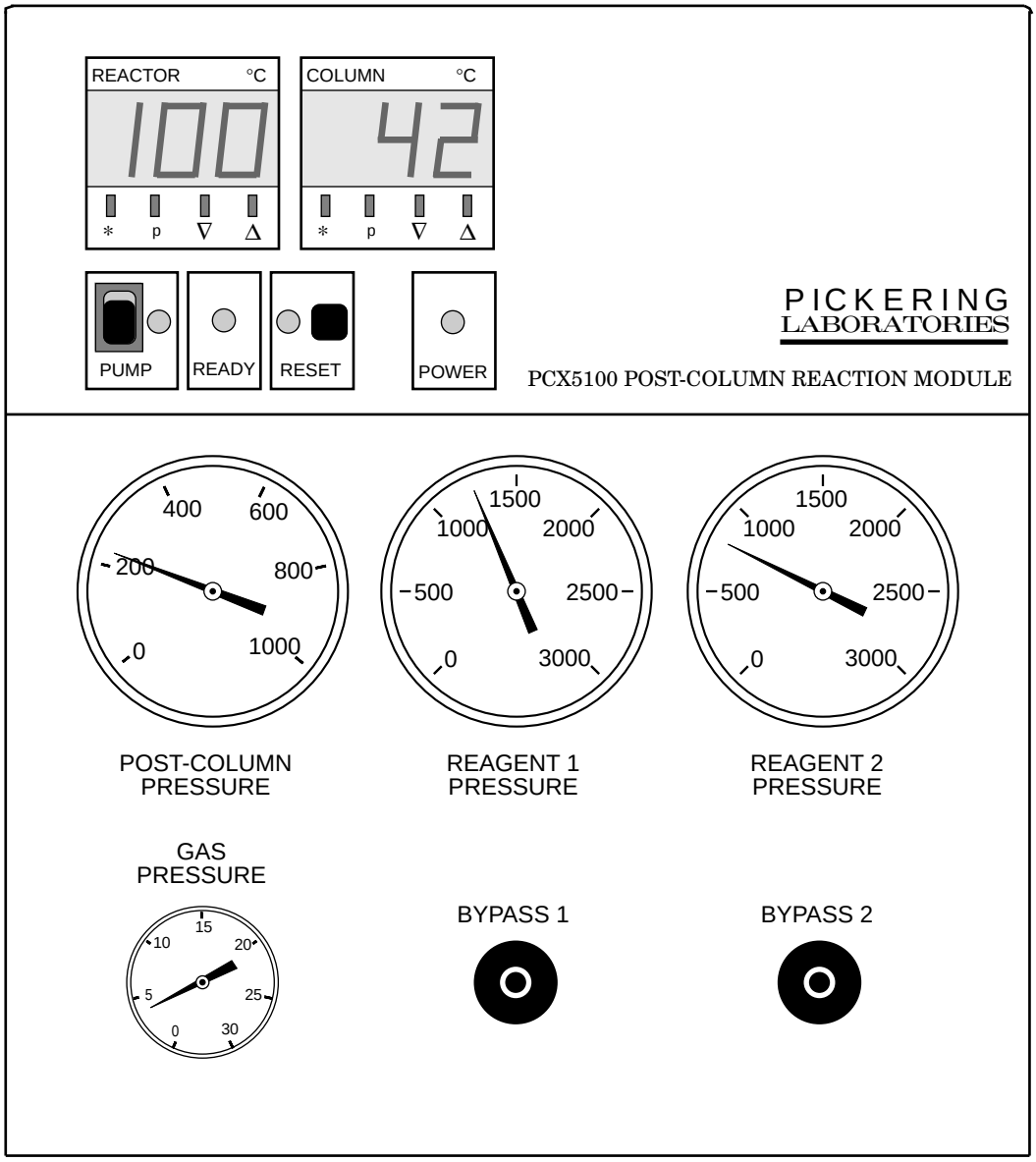


Figure 2-1

- Reagent 2 Pressure gauge operates the same as the "Reagent 1 Pressure" gauge.

- Gas Pressure gauge shows the pressure of inert gas supplied to the gas manifold. In normal operation, it shows 2–5 psi.

- Bypass 1 valve is used to purge and prime the first reagent pump. Attach the 20mL syringe to the Luer fitting in the center of the knob. Open the valve by turning it counterclockwise about one turn. Apply suction with the syringe to draw reagent through the pump. Use strong suction to remove bubbles from the "Reagent 1 Pressure" gauge, from the reagent pump, or from the reagent supply line. Close the valve by turning it clockwise; only gentle pressure is needed to close the valve. Keep the Luer fitting clean by rinsing it with water after use.

- Bypass 2 valve is used to purge and prime the second reagent pump, and it operates the same as "Bypass 1" valve.

- Reset button enables the controls for the heated reactor, column oven, reagent pump, and ready relay. The interlock system requires the pressure switch to sense over 500 psi before the PCX5100 can be enabled. This means that the LC pump must be running before pressing "Reset" can start the PCX5100. The "Remote Off" circuit on the rear panel must also be open (if connected to the LC). When reset, the corresponding indicator lamp lights.

- Pump switch controls power to the reagent pump. The ON position is indicated by a visible orange stripe. To power the pump, the switch must be in the ON position and the reset indicator lamp must be ON. The pump indicator lamp lights when the pump is ON.

- Temperature Controllers. The four-digit display normally shows the process temperature to the nearest degree. It also displays the temperature setpoint or the Option:Function list. There are four buttons below the digital display:

  * **p** ∇ Δ

  To view the setpoint, press the * button; the display will show the setpoint in flashing numbers as long as the * button is held down. To change the setpoint, press and hold the * button, and press the Δ button to increase or press the ∇ button to decrease. The process temperature display will return when you release the buttons.

Caution. Do not press the p button.

The **p** button is used by the factory to program the controller. ***Do not press the p button.*** If you should press the **p** button by "accident" (it is half-way recessed and requires extra efforts to activate it), press the **p** button a second time, and the process temperature will reappear.

There are some auxiliary displays. The green LED lamp located on the bottom right will light when heat is being applied. There are three LED lamps located just to the left of the numerical display. They indicate whether the process temperature is above, within, or below the setpoint range. The red lamp is lit when the temperature is not ready.

- The recommended maximum temperature for the column heater is 100˚C. A thermal safety switch shuts off the heater at ca. 110˚C.

- The recommended maximum temperature for the heated reactor is 130˚C. Above this temperature the reaction coil begins to lose strength. A thermal safety switch shuts off the heater at ca. 150˚C.

- Ready lamp lights when all these conditions are met: 1) the module is reset, 2) the pump switch is ON, 3) the reactor is within 2˚ of its set temperature, and 4) the column heater is within 2˚ of its set temperature. This lamp shows the state of the "ready" relay outputs on the rear panel.

Left Panel

- The liquid connections to the pressure switch are labelled "From LC Pump" and "To Injector." The pressure switch is part of the safety interlock system. The maximum pressure rating for this switch is 4,500 psi (310 bar). The switch requires 500 psi (34 bar) before the module can be reset.

Caution! Set the maximum pressure of the HPLC to no more than 4,500psi (310bar).

- The liquid connection "From Injector" is also the pre-column filter. The filter element is a 0.5µm frit (Cat. No. 3102-9047).

- A removable panel gives access to the electrical connections of the reagent pump and the thermostatted reactor, and the flow adjustment knobs of the post-column pump. It is removed by loosening the two captive thumb-screws along the bottom edge.

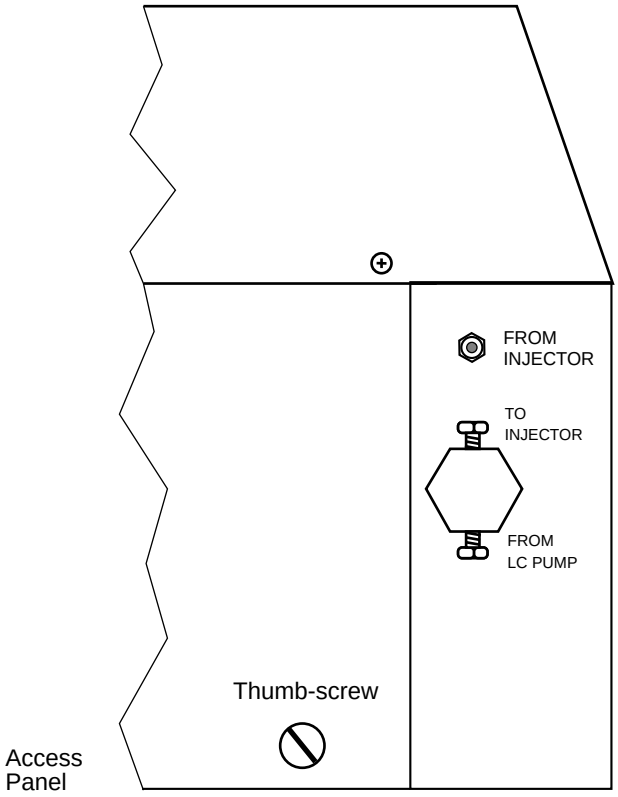


Figure 2-2

Right Panel

- "Gas In" fitting is where inert gas enters the system for pressurizing the reagent reservoirs. The internal gas regulator requires 45–75 psi (3–5 bar) to function properly.
- Gas is controlled by the toggle valve. Lever UP pressurizes the manifold. The "Gas Pressure" gauge on the front panel should read 2–5 psi in normal operation.
- Gas Out manifold is more than a simple distribution block. Each outlet has its own check valve to prevent back-flow of gas from the pressurized reagent bottles. The manifold also has a safety relief valve that opens at about 12 psi to prevent dangerous over-pressurizing of the reagent reservoirs. If the input pressure of gas is too low, the regulator sticks open and allow the gas to vent from the relief valve, rapidly depleting a gas cylinder.

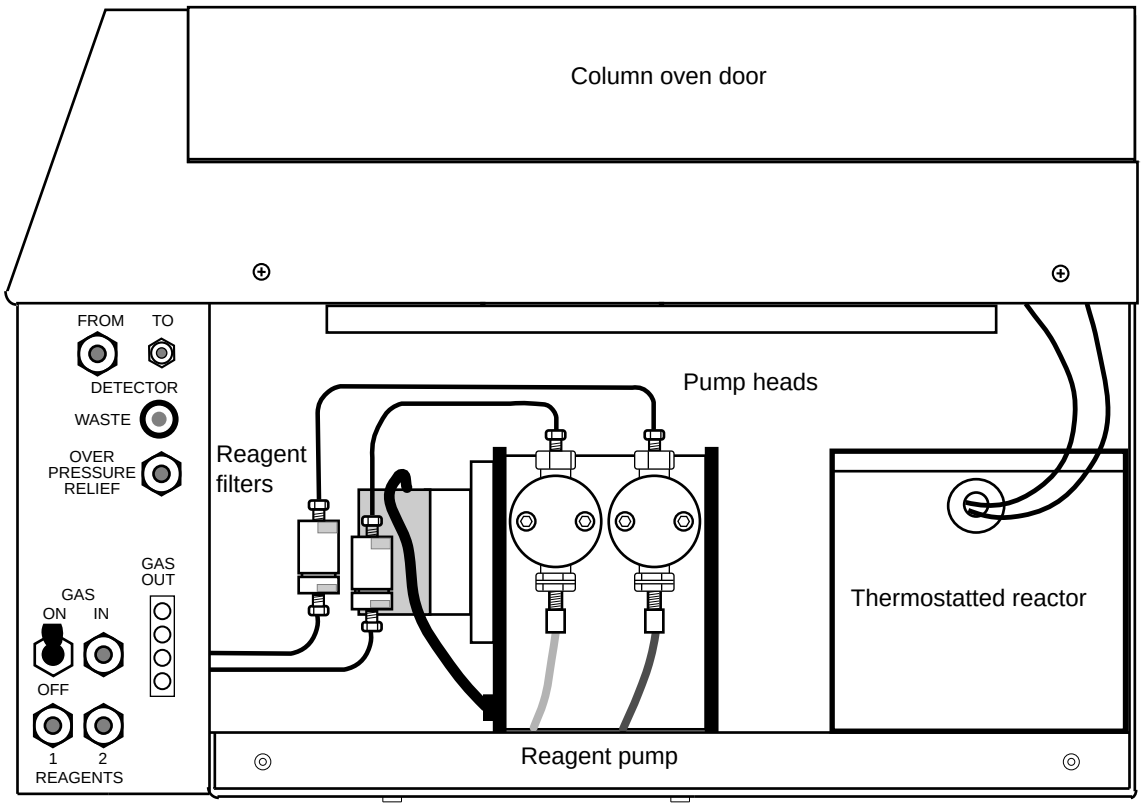
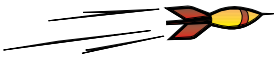


Figure 2-3

- Reagent 1 fitting supplies the first pump. Usually this reagent is either Hydrolysis Reagent or Oxidizing Reagent.
- Reagent 2 fitting supplies the second pump. Usually this reagent is OPA.
- To Detector bulkhead fitting connects to the fluorescence detector with 0.010 inch (0.25mm) ID tubing.
- From Detector fitting is where the outlet line of the detector connects. This is connected internally to the inlet end of the back-pressure regulator.
- Waste fitting is actually the outlet of the back-pressure regulator. The calibration screw is inside the fitting. Normally the pressure is factory set to 100 psi (7 bar). Connect a 0.020 inch (0.5mm) ID tubing to the waste container here.
- Over Pressure Relief is a safety relief valve that opens in case the post-column pressure reaches 525 psi (36 bar). This protects the soft fluorocarbon tubing of the reactors from rupture in the event of a blockage in the post-column system or other fault. Run a tubing from this fitting to a clean dry beaker. Any evidence of ***liquid*** in this tubing indicates ***a fault condition.***



- A removable panel gives access to the liquid ends of the pump and the reagent filters. The panel can be removed by loosening the two captive thumb-screws along the bottom edge.

Internal

- The duplex reagent pump with a piston-wash system is behind the right access panel. In normal operation, the pump requires no adjustment. It is calibrated at the factory to 0.30 mL/min for both channels. The micrometer knobs on back of the pump adjust the flow rates. One full turn of the knob changes approximately the flow rate by about 0.1 mL/min. Please see page **3-7** before turning on the post-column pump.
- The reagent filters are located just downstream of the outlet check-valves of the pump. The filters and elements are similar in appearance to the pre-column filter, but they are different. The reagent filter element is a 2µm frit (Cat. No. 3102-9132).

Rear Panel

- The power connector is a standard IEC 320 type connector. Use the appropriate power cord for your local wall outlet and electrical code. The 120V version comes with a standard North American cord set. The 240V version comes with a cord set used in much of continental Europe (France, Germany, Benelux, etc.), or your local reseller may have provided the correct local cord set. If your local power outlets are different, you will need to obtain the appropriate grounded cord set.

- The main power switch is located in the power connector assembly.

- The fuse holder is located in the power connector assembly. To change the fuse, first remove the power cord from the connector. Carefully pry out the fuse clip with a small screwdriver. Replace with the specified-type fuse.

  For 120V systems (PCX5100) use a fast-acting 3A, 250V, 5 x 20mm fuse, type GMA3 (Cat. No. 3543-0045).

  For 240V systems (PCX5102) use a fast-acting 1.6A, 250V, 5 x 20mm fuse, meeting IEC127 specifications (Cat. No. 3543-0044).

Warning. Ensure that the power cord is disconnected before replacing a fuse. Use only the specified-type fuse.

Attention. Assurez vous que le cable secteur n'est pas connecté avant de changer un fusible.

Warnung. Sicherungen dürfen nur bei nicht angeschlossenem Netzkabel ersetzt oder gewechselt werden.

Cuidado. Asegúrese que el cable de red está desconectado antes de instalar o cambiar un fusible.

Attenzione. Assicuratevi che il cavo di alimentazione sia scollegato prima di installare o sostituire un fusible.

Waarschuwing. Zorg dat de voedingskabel losgekoppeld is, voordat een zekering wordt geplaatst of vervangen.

Avvertimento. Fare atenzione che la corda del voltaggio sia staccata prima di cambiare valvole. Usa solo valvole di capacitá precisata dalla fattoria.

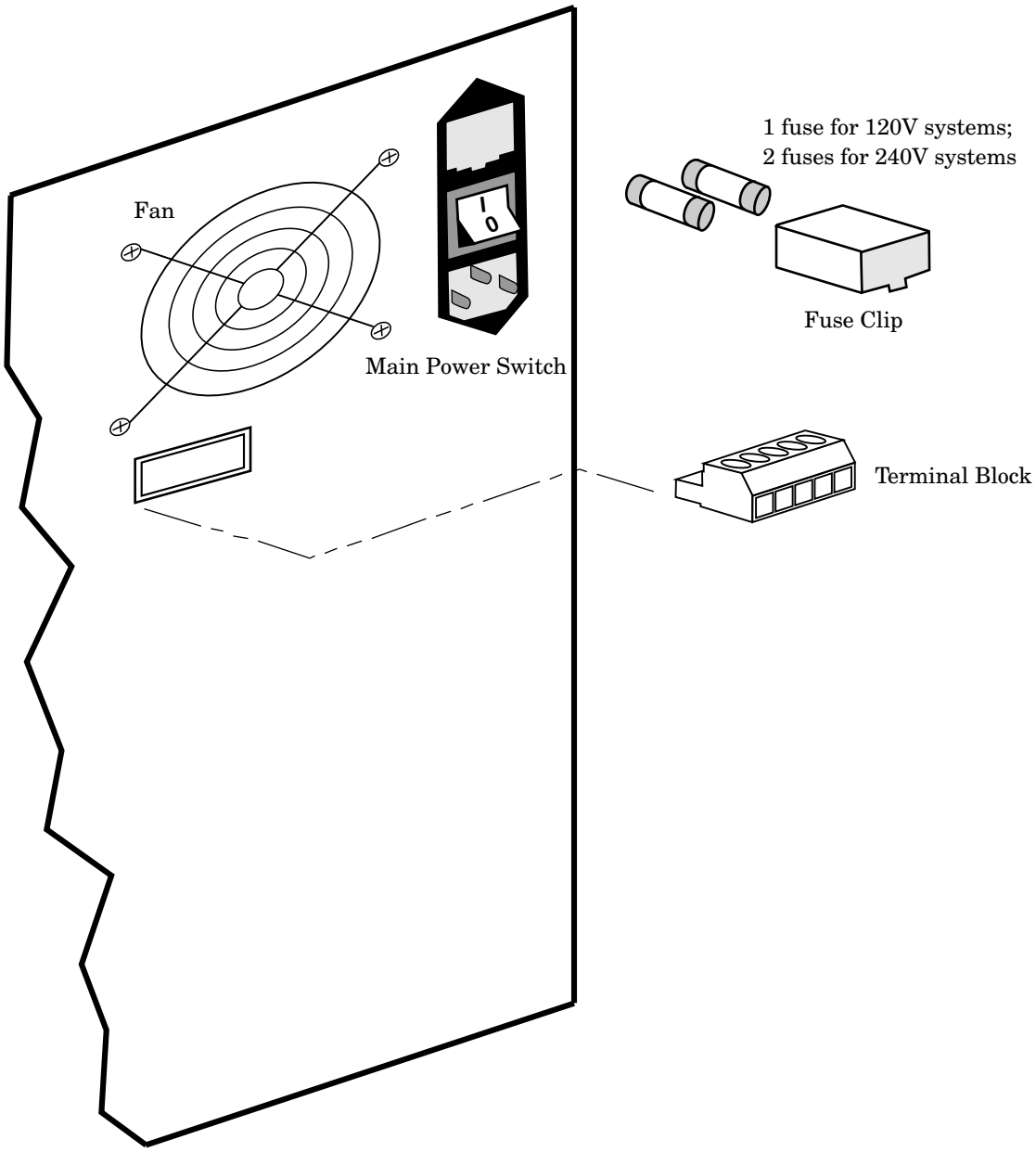

1 fuse for 120V systems;
2 fuses for 240V systems
Fan
Fuse Clip
Main Power Switch
Terminal Block

Figure 2-4

- The ready relay and remote off circuits connect via the removable terminal block. From left to right the terminals are:
  1. Ground
  2. "Remote off" input
  3. "Ready out" relay normally closed (RDY NC)
  4. "Ready out" relay common (RDY C)
  5. "Ready out" relay normally open (RDY NO)

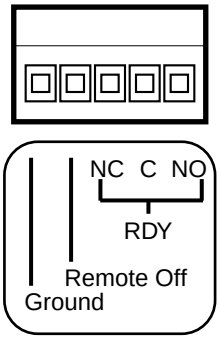


Figure 2-5

- The remote off input when momentarily grounded will turn off the reagent pump, column oven, and heated reactor. It can be actuated by a relay contact closure, TTL logic or other device capable of sinking 100µA to ground. The maximum input voltage is 10V. If held to ground, the remote off will prevent the "Reset" button from working.

- The ready out relay actuates when the module becomes ready. When the module is not ready, the normally closed circuit is connected to the common; when the module is ready, the normally open circuit is connected to the common. The relay contacts are rated at 0.5A and 100 VDC.

Column Oven

Column oven is located on the upper right side of the instrument. Simply lift the hinged lid to gain access to it. There is space for one analytical and guard column.

- The heater block is slotted to receive the analytical column.

- The last part of the lead-in capillary is embedded in the heating block to preheat the eluant for a more uniform temperature within the column. The lead-in capillary is 0.007 inch ID to minimize loss of efficiency.

Warning. The column heating block may become hotter than 70°C. For your safety, wear insulating gloves when the column oven is warm.

Attention. La résistance chauffante de la colonne peut dépasser une température de 70°C. Pour votre sécurité, prière de porter des gants isolants lorsque le four de la colonne est chaud.

Warnung! Der Heizblock des Säulenofens könnte heißer als 70°C werden. Für Ihre Sicherheit sollten Sie isolierende Handschuhe tragen, wenn der Säulenofen warm ist.

Atención. El bloque calefactor de columnas puede estar por encima de 70°C. Para su seguridad use guantes aislantes cuando el horno de columnas esté caliente.

Avvertimento. Il blocco della colonna potrá diventare molto caldo e superare ai 70°C. Per la sua protezione usa guanti con insulazione per questa applicazione.

Reagent Reservoirs

The PCX5100 includes two pressurized reagent reservoirs. The one with TFE tubing on the cap is used for either Hydrolysis Reagent or Oxidizing Reagent. The one with tan-colored Saran tubing on the cap is used for OPA Reagent.

Warning. For your safety, the bottles are coated with a tough plastic film and are rated to a maximum of 15 psig (1 bar). Do not use uncoated bottles.

Attention. Pour votre sécurité, les bouteilles sont recouvertes d'un film de plastique dur, et sont calibrées à un maximum de 15 psig (1 bar). Ne pas utiliser les bouteilles non recouvertes.

Warnung! Für Ihre Sicherheit wurden die Reagenzienflaschen mit einem festen Schutzüberzug aus Kunststoff versehen. Die Flaschen sind bis max. 1 bar (15 psig) zugelassen Flaschen mit beschädigtem Schutzüberzug dürfen nicht mehr benutzt werden Verwenden Sie keine Flaschen ohne Schutzüberzug!

Atención. Para su seguridad, las botellas están recubiertas con una resistente película plástica, y están constrastadas a 15 psig (1 bar). No utilice botellas sin recubrimiento.

Avvertimento. Per la sua protezione, le bottiglie sono construite forti con un percentuale du plastica, e sono usabili per un massimo di una Bar (15 psi). Non usare bottiglie normali.

- Gas inlet tubing on the reservoirs is marked with a black band for Saran tubing, and is dyed blue for TFE tubing. Just under the cap there is a pinhole drilled in the gas tubing to prevent liquid from creeping up the gas line in case of a slow leak in the gas system. Connect the gas tubing to the gas manifold using 1/4-28 nuts and reversed-ferrules.

- Reagent tubing is neither marked nor dyed and has a shut-off valve (Cat. No. 3104-0050). Connect the OPA reagent to the bulkhead fitting "Reagent 2" and connect the Hydrolysis or Oxidizing reagent to the bulkhead fitting "Reagent 1." Use 1/4-28 nuts and reversed-ferrules.

- The reservoir cap has a built-in vent valve. The large white knob is the valve; pull it up for CLOSED, and push it down for OPEN. If the gas is turned on, opening the vent valve will sparge the reagent. Closing the valve will pressurize the reservoir; this is the normal operating position. In the center of the knob there is a 1/4-28 fitting; you may optionally connect a tube here to carry vapors to an exhaust vent.

- When changing reagent, first turn off the gas with the toggle valve on the PCX5100. Then vent the reagent bottle by pushing down the valve. Now you can safely remove the cap. It is convenient to have extra bottles so that you can simply transfer the cap without setting it down and risking contamination.

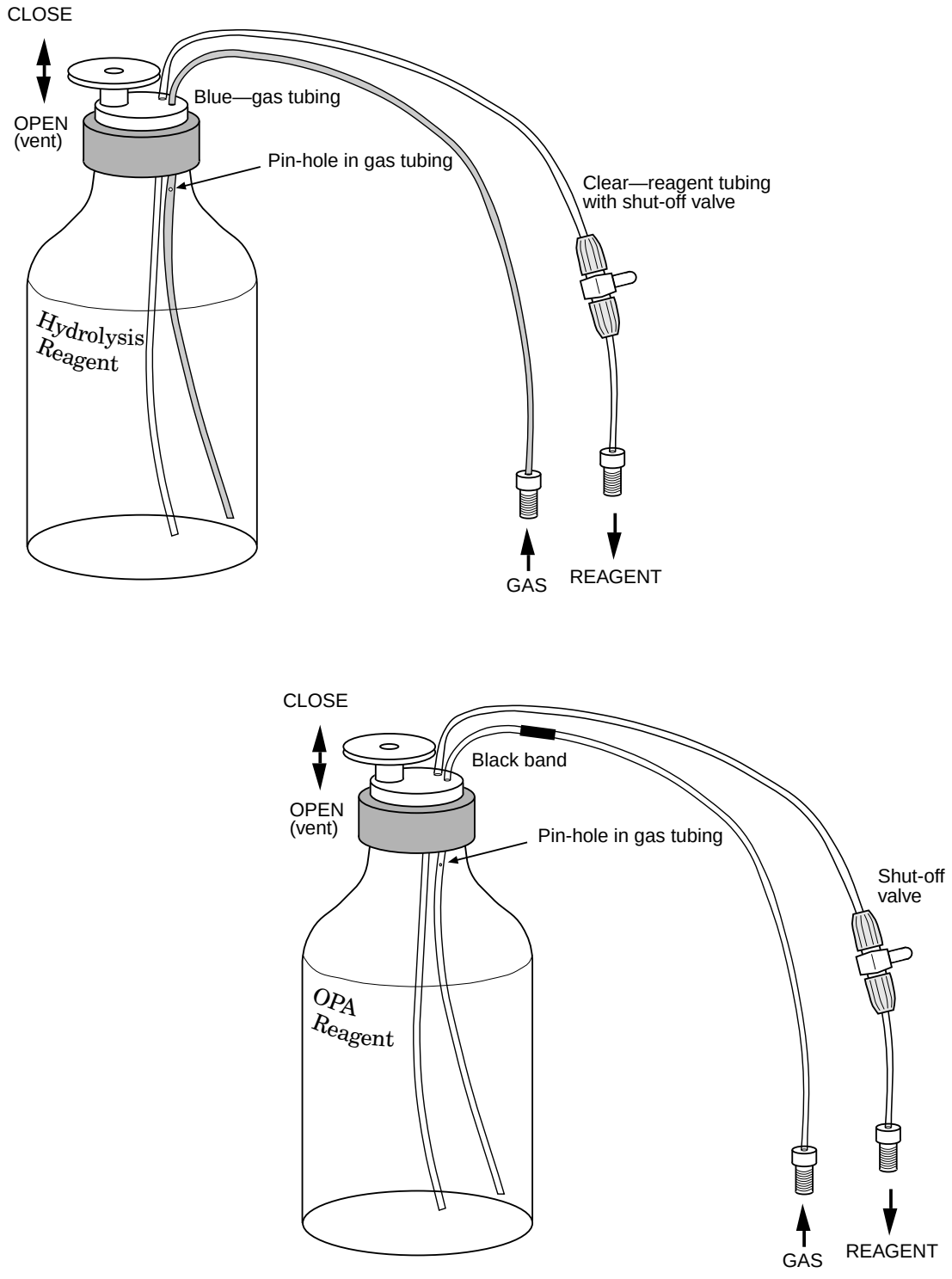

CLOSE
OPEN
(vent)
Blue—gas tubing
Pin-hole in gas tubing
Clear—reagent tubing
with shut-off valve
Hydrolysis
Reagent
GAS
REAGENT
CLOSE
OPEN
(vent)
Black band
Pin-hole in gas tubing
Shut-off
valve
OPA
Reagent
GAS
REAGENT

Figure 2-6

Specifications

Wetted Materials

PCX5100

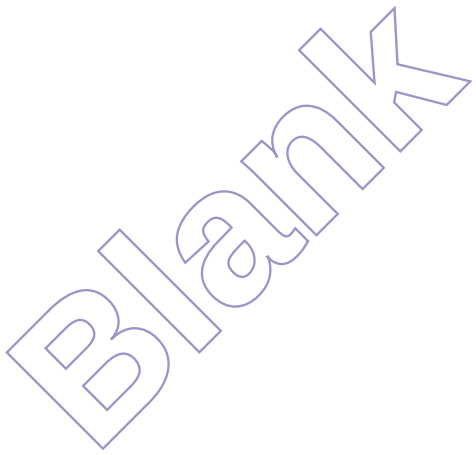
316 Stainless steel
Teflon FEP
Saran PVDC
Hastelloy-C
Kel-F CTFE
PEEK
Borosilicate glass
Sapphire
UHMW polyethylene

PCX5110

316 Stainless steel
Viton
Teflon FEP
Saran PVDC
Kel-F CTFE
PEEK
Tefzel ETFE
Borosilicate glass
Sapphire
UHMW polyethylene

Ratings

Dimensions:	32 cm x 27.5 cm x 45 cm (h x w x d)
Weight:	15 kg
Electrical Power:	100–120 V; 50/60 Hz; 1.5 A; grounded supply
	200–240 V; 50/60 Hz; 0.8 A; grounded supply
	Installation (overvoltage) category II, Pollution degree 2
Environmental:	Indoor use only
	Altitude up to 2,000 m
	Ambient temperature 5–40°C
	Relative humidity 80% @ 31°C, derated to 50% R.H. @ 40°C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Chapter 3
Installation

Read all installation instructions and MSDS before operating your post-column derivatization instrument and HPLC system. Check that you have all the items shown in the Packing List.

Site Requirements

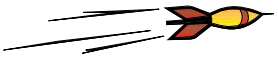
Standard Analysis System

- Pickering PCX5100 Post-Column Derivatization Instrument
- Pickering Carbamate and/or Glyphosate option package
- HPLC manual injector or autosampler
- Binary (or more) gradient HPLC pump
- HPLC fluorescence detector
- Integrator or data system

HPLC system requirements

The HPLC pumping system, the injector or autosampler, the fluorescence detector, and the integrator or data system must be supplied by the user.

- The HPLC pump must be capable of binary gradient elution.
- The injector should be able to inject a 10µL sample, preferably by full-loop injection.
- If drinking water is to be analyzed for carbamate insecticide residues, the injector should be able to inject at least 200µL, and preferably 400µL.



- If the system will be used for glyphosate analysis, be aware that the column regenerant is ***strongly alkaline.*** Any polymers or other materials in the HPLC pump, injector, needle seat, and detector must be compatible. For example, the standard rotor seal in Rheodyne injector valves is ***Vespel*** polyimide, which is not recommended at pH ≥ 9; a ***Tefzel*** or ***PEEK*** rotor seal must be installed.
- The PCX5100 contains an integral back-pressure regulator for the detector waste line. The pressure rating of the detector flowcell must be ≥ 110 psi (7.5 bar). If your detector flowcell is rated lower, consult Pickering Laboratories.
- Because the OPA reaction is extremely sensitive, the HPLC system must be thoroughly clean before using it with the PCX5100. Pay special attention to the cleanliness of eluant reservoirs and delivery tubings.

Space Requirements

Space requirements for the entire HPLC system are determined by the brand of HPLC pump and detector in use. Minimum benchtop space required for a standard system is approximately 5 feet (150cm) long by 2 feet (60cm) deep.

Electrical

In addition to the outlets required for the HPLC system, one grounded outlet will be needed.

Gas

Nitrogen, helium, or argon (in order of preference), is required to pressurize the reagent reservoirs. The PCX5100 requires gas pressure of 45–75 psi (3–5 bar) at the gas inlet. An ***adaptor*** from the gas regulator to 1/8 inch OD tubing is required. To minimize oxidation of the OPA reagent, use oxygen-impermeable tubing for the ***entire*** gas supply line (metal or Saran).

Miscellaneous Supplies

The user will need to provide adequate lengths of capillary tubing to connect HPLC pump and injector to pressure switch (0.010–0.020 inch ID), to detector inlet (0.010 inch ID), to detector outlet (0.010–0.020 inch ID), and to injector outlet (0.007–0.010 inch ID).

Chemicals & reagents required for carbamate analysis

Supplied by User

Important! These solvents and chemicals must be available in your laboratory before installing your Carbamate Post-Column Derivatization Instrument with the HPLC System.

- HPLC-grade methanol (from Fisher Scientific, J.T. Baker, or Merck). Additional filtration is not recommended.
- HPLC-grade water (also from Fisher Scientific, J.T. Baker, or Merck). Additional filtration is not recommended.
- Reagents for sample preparation.

Note: Water and methanol, even HPLC-grade from other vendors, may contain traces of amines or ammonia which will react with OPA/Thiofluor in the post-column system to cause interference. Water from laboratory purification systems (Milli-Q, Barnstead, etc.) also may not be acceptable and should be tested for suitability against Fisher HPLC-grade water. The age of the cartridge, the configuration (the activated charcoal cartridge should be placed after the ion-exchange resin cartridge), and the quality of the feed source determine acceptability. Water from qualified purification systems should be monitored on a regular basis, and proper maintenance procedures should be followed strictly.

Supplied with the carbamate post-column analysis system

Pickering Laboratories supplies the following reagents for system start-up. Additional reagents should be ordered to replenish the initial supply.

- Hydrolysis Reagent, 0.05M sodium hydroxide (Cat. No. CB130), 4 x 950mL
- OPA Diluent, 0.05M sodium borate buffer solution (Cat. No. CB910), 4 x 950mL
- *o*-Phthalaldehyde, 5g, chromatographic grade crystals (Cat. No. O120)
- Carbamate Test Mixture (Cat. No. 1700-0063) 2 x 1.5mL
- Thiofluor (Cat. No. 3700-2000), 2 x 10g, chromatographic grade crystals
- ChlorAC buffer (Cat. No. 1700-0063) for preservation of aqueous samples, 250mL

Chemicals & reagents required for glyphosate analysis

Supplied by User

- 5% Sodium hypochlorite for preparing oxidizing reagent or Clorox® (from local grocery stores)

Supplied with the glyphosate post-column analysis system

Pickering Laboratories supplies the following reagents for system start-up. Additional reagents should be ordered to replenish the initial supply.

- Glyphosate Eluant (Cat. No. K200), 4 x 950mL
- Glyphosate Column Regenerant (Cat. No. RG019), 1 x 950mL
- Hypochlorite Diluent (Cat. No. GA116), 4 x 950mL
- OPA Diluent, sodium borate buffer solution (Cat. No. GA104), 4 x 950mL
- *o*-Phthalaldehyde, 5g, (Cat. No. O120)
- Thiofluor, 2 x 10g, (Cat. No. 3700-2000)
- Glyphosate test mixture (Cat. No. 1700-0080), 1.5mL
- RESTORE (Cat. No. 1700-0140) for removal of metal ion contamination from guard & column

Unpacking

The PCX5100 instrument is shipped in two cartons. Report any carton damage to the carrier. Unpack both cartons and review the contents using the Packing List to ensure that your order is complete. If any items are missing, immediately contact Pickering Laboratories at (415) 694-6700 or by fax at (415) 968-0749.

Complete and mail the warranty registration included in this manual so that you will receive instrument and method updates from Pickering Laboratories.

Installation of the PCX5100

Layout of the HPLC with the PCX5100

The PCX5100 system flows left-to-right. The connection to the LC pump and injector are on the left. The connections to the detector are on the right. The connections for the gas lines and reagent lines are on the right. You will need ca. 4 inches (10cm) clearance on either side of the PCX5100 to make these connections. The column oven is in the upper right side of the instrument; the oven door swings up about 5 inches (12cm).

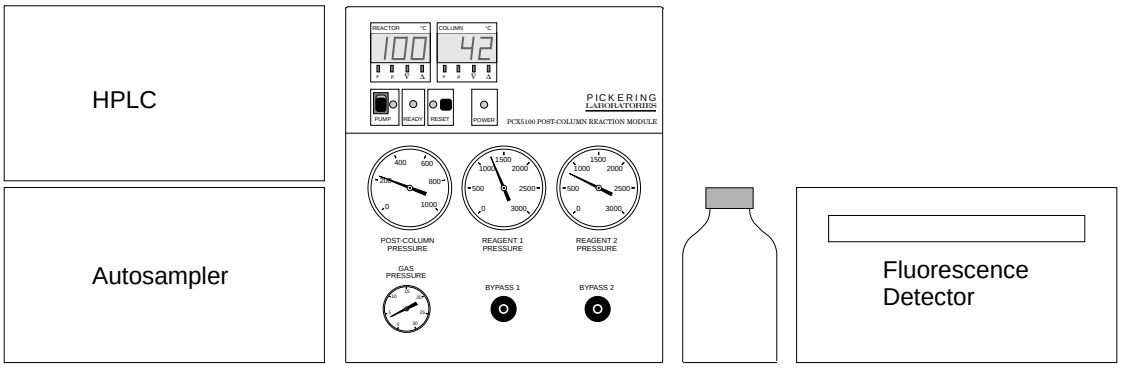


Figure 3-1

Note about fittings

The PCX5100 uses several styles of fittings. The external high-pressure fittings are all 10-32 x 1/16 inch Upchurch style. These fittings are compatible with Valco, Parker CPI, Swagelok, or various of the polymeric nuts and ferrules. The low-pressure gas and reagent fittings are 1/4-28 x 1/8 inch size. These can be used with either flared fittings or reversed-ferrule fittings. The fitting for the detector waste line is 1/4-28 x 1/16 inch and uses a reversed-ferrule type fitting. Pickering Laboratories supplies all the matching nuts and ferrules needed for normal assembly. Note that fittings and ferrules for the LC and detector are not supplied.

Upchurch, Parker, Valco style 1/4-28 reversed-ferrule

Figure 3-2. Note the direction of the ferrules: normal for Upchuch, Parker, & Valco; reversed for low-pressure 1/4-28.

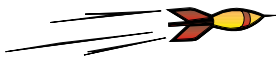
Inert Gas (N_2, He, or Ar) & Reagents Connections

Step 1 Using a piece of the tan-colored 1/8 inch Saran tubing, connect the "Gas In" port on the right side of the instrument to a supply of inert gas at 45–75 psi (3–5 bar). If you are using metal compression fittings, be careful not to over-tighten as the tubing can collapse or crack. Turn on the main gas supply. Switch the toggle valve up to the ON position to start gas flow. Let the gas system purge for about one minute. Switch the toggle valve down to OFF.

Step 2 Connect the gas lines of the reservoirs to the gas outlet manifold. On the OPA reservoir, the gas line will be marked with a small piece of black shrink tubing near the bottle cap. On the other reservoir, the gas line is dyed blue. Each of these tubings has a 1/4-28 nut and ferrule already in place. Plug any unused outlets of the manifold.

Note: Two types of gas tubing are used in this system: Saran and PTFE, a fluorocarbon material. The Saran tubing is impermeable to oxygen and is used for the OPA reagent reservoirs to prevent degradation of the OPA. Fluorocarbon tubing is 1000 times more oxygen permeable than Saran tubing. However Saran tubing is not compatible with NaOH.

Step 3 Connect the reagent lines of the reservoirs to the reagent inlets on the right side of the instrument. Connect the OPA reagent to port #2. Connect the other reagent to port #1. Each of these tubings has a 1/4-28 nut and ferrule already in place.



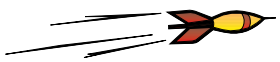
Important: The reagent lines and gas lines are not interchangeable.

Post-Column Interlock Connections

Step 1 Using 1/16 inch x 0.020 inch ID capillary tubing, connect the outlet of the HPLC pump directly to the pressure switch on the left side of the instrument. The connection is labelled "From Pump."

Step 2 Using 1/16 inch x 0.020 inch ID capillary tubing, connect the pressure switch to the inlet of the injector or autosampler. Use the fitting labelled "To Injector."

Note on usage: The post-column interlock monitors the eluent pump pressure and turns OFF electrical power to the post-column reactor, column oven, and reagent pump when the eluent pressure drops below 500 psig. (This pressure decrease may occur due to eluent pump malfunction, empty reservoirs, or a programmed shut-down after the last sample.) The interlock turns off the reagent pumps to prevent backflow of reagents into the column, which dissolves the silica and ruins the column. The interlock also defaults to OFF when a power loss occurs. The PCX5100 instrument does not automatically turn on as the eluent pressure rises above 500 psi. Press the RESET button to enable the instrument.



1 **Caution!** Operating the reagent pumps when the HPLC pump is not producing eluent flow can pump reagent into the analytical column causing irreversible damage.

2 Maintaining high temperature in the post-column reactor when there is no HPLC eluent flow can cause precipitation and complete blockage of the post-column reactor.

Connecting the injector

Connect the outlet of the injector (or autosampler) to the bulkhead fitting on the left side of the instrument labelled "From Injector." Use 1/16 inch x 0.007 inch ID or 1/16 inch x 0.010 inch ID capillary tubing. This fitting contains a replaceable 0.5µm filter element.

Reagent Reservoirs

Step 1 Assume that new reservoirs have not been cleaned. Wash the bottle with laboratory detergent and hot water. Rinse with methanol then with deionized water. Wipe down the dip tubes on the caps with methanol and a clean cellulose tissue. Avoid touching the tubings or the interior of the reservoir with your skin and do not leave caps and lines dangling without a reservoir because this will cause fluorescent contamination.

Note: Ensure that the reagent outlet and gas lines connected to the OPA reservoir are Saran (amber color). Saran tubing is necessary because of its low permeability to oxygen.

Step 2 Place the small tray in a convenient location and put the reservoirs in it.

Note: The reagent reservoirs are specially coated with a protective polymer to ensure operator safety if the reservoirs should become over-pressurized. Non-coated bottles must not be substituted in the PCX5100 system. Replacement or 2 L reagent reservoirs may be ordered directly from Pickering Laboratories.

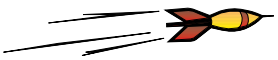
HPLC Detector Installation

Step 1 Install the HPLC fluorescence detector referring to the manufacturer's manual supplied with the instrument.

Step 2 Connect the inlet of the detector flowcell to the bulkhead union on the right side of the instrument labelled "To Detector." Use 1/16 inch x 0.010 inch ID capillary tubing.

Note to Hewlett-Packard 1046A end-users: Replace the 0.12mm ID inlet tubing (red) and heat-exchanger from the left side of the detector to the flowcell (behind the front panel of the detector) with a 0.25mm ID tubing (HP Cat. No. 79881-67302 or Pickering Cat. No. 3110-6045; blue tubing) to reduce the back-pressure.

Step 3 Connect a 1/16 inch x 0.020 inch ID tubing from the outlet of the flowcell to the fitting labelled "From Detector" using a 1/4-28 nut with a 1/16 inch reversed-ferrule.



Caution! The internal regulator provides 100 psi (7 bar) of back-pressure to the fluorometer and prevents outgassing at the flowcell. The pressure rating of the flowcell must be at least 110 psi (8 bar) so that the flowcell is not damaged. If your fluorescence detector has a flowcell pressure rating of less than 100 psi (7 bar), contact Pickering Laboratories. The back-pressure is adjustable with the hex-wrench that comes with your system. The minimum necessary backpressure is 75 psi (5 bar).

Step 4 Connect a 0.020 inch ID PTFE tubing to the "Waste" outlet on the right side of the instrument. Place the other end in an appropriately labelled waste container.

Step 5 Set the excitation and emission of the detector. The optimal excitation wavelength is 330nm and maximal emission wavelength is 465nm for most detectors. If your detector provides for an emission cutoff filter, use a 390nm filter.

Step 6 Connect the signal cable from the fluorometer to the input terminal of your data station. Ensure that the polarity is correct. (Refer to your HPLC instrument manual.)

Installing the analytical & guard columns

Install the analytical column and guard column in the column heater as follows:

Step 1 One set of PEEK tubing has been provided for installing each length of column. Select the column that you wish to install, and the corresponding tubing set.

Step 2 Remove the cover plate from the column block and set it aside.

Step 3 Connect the outlet of the guard column to the inlet of the analytical column.

Step 4 Loosely fit the inlet of the guard column to the eluent heat exchanger. Carefully lay the analytical column into its slot in the heating block. Tighten the connections.

Step 5 A loose end of tubing in the column oven leads to the first mixing tee. Attach the loose end to the outlet of the analytical column.

Step 6 Replace the cover plate.

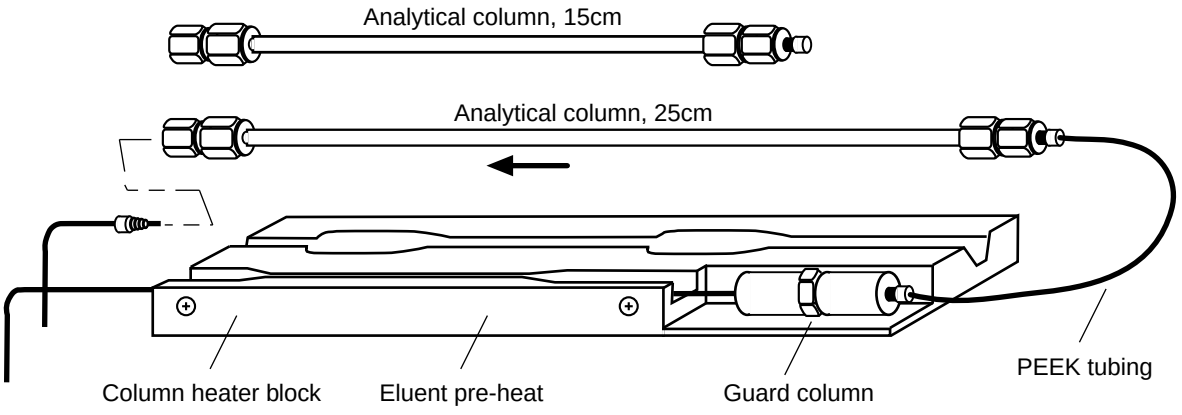


Figure 3-3

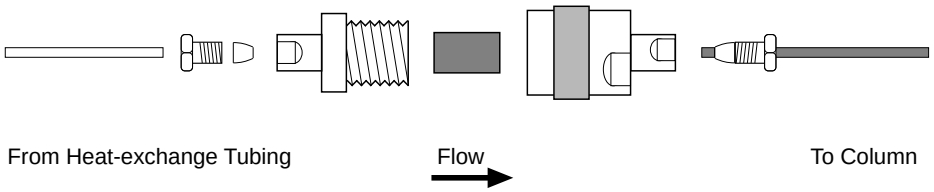


Figure 3-4. Direction of Carbamate guard column.

Reagent Pump

The reagent pumps have been calibrated to 0.30 mL/min at the factory and should not need further adjustment.

Piston-wash System

Important! Before starting the post-column pump, connect the piston-wash system as illustrated in Figure 3-5. Flush periodically with 80-20 water-methanol (4mL each time, at least twice a day). The piston-wash system is designed to flush the back-end of the primary seal which significantly extends seal life. However, if a pump with a piston-wash system is used without liquid in the piston-wash system, the secondary seal will wear out quickly (because it is dry). It can then scratch the piston and the scratched piston will in turn cause the primary seal to fail.

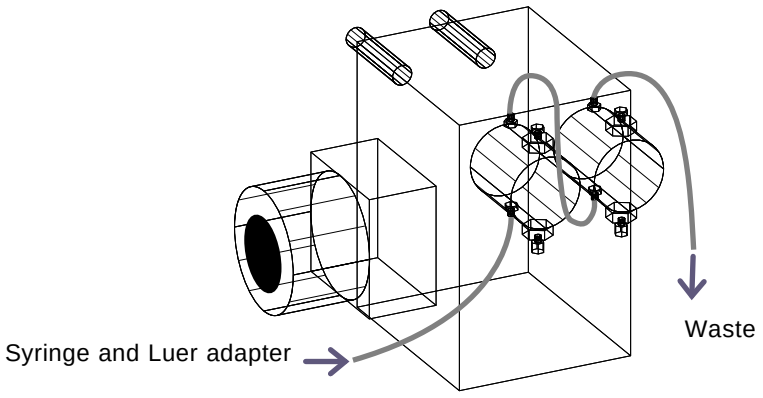


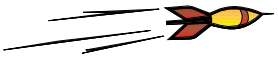
Figure 3-5. Cut the supplied c-flex tubing into 3 sections: one 8-inch and two 24-inch sections. Connect the 8-inch c-flec tubing between the outlet from pump #1 to the inlet of pump #2. The two 24-inch sections are for the connections between the Luer adapter and the inlet of pump #1 and from the outlet of pump #2 to waste.

System Testing & Verification

Read Chapter 4–System Operation to become familiar with the use of your instrument. At the beginning of Chapter 5 there are procedures for the initial system testing. The installation is not complete until the initial system tests have been performed satisfactorily.

The initial system tests consist of:

- Parameter log of pressures, temperatures, and flows under standard initial conditions (Appendix A)
- Chromatogram of test mix using Pickering standard conditions

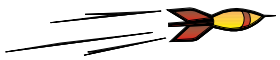


Important! If the system will not be used immediately after the installation, the system must be shut down properly and the reagents replaced with water / methanol. Flush the entire system with water / methanol as listed in Chapter 4–Shutdown Procedures.

Chapter 4
System Operation

Carbamate Analysis with the EC5100

Getting Started



Important! This chapter assumes that the PCX5100 has been installed according to the directions in Chapter 3. Do not operate the instrument until it has been properly installed, and you have read and understood the instructions in this section and the Material Safety Data Sheets (MSDS) for important safety information about the chemical reagents. You may use either the 15 cm or 25 cm column, but for the purposes of initial testing and training, install the 15 cm column. Later you may change to the other column.

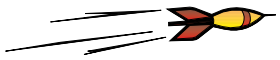
HPLC Mobile Phase (Supplied by User)

The Pickering Laboratories carbamate analysis requires two mobile phases: HPLC-grade water and HPLC-grade methanol. You may choose to use other conditions or eluants for your routine analysis, but during installation and checkout, we recommend you use these standard conditions.

- HPLC-grade methanol (Fisher Scientific, JT Baker, or Merck) must be used.
- HPLC-grade water (Fisher Scientific, JT Baker, or Merck) or high quality purified and deionized water may be used instead. For initial startup, use HPLC-grade bottled water to verify system performance. Water from some laboratory purification systems (Milli-Q, Barnstead, etc.) may not be acceptable for high-sensitivity carbamate analysis. The age of the cartridge, configuration, system maintenance, and quality of the feed source will determine acceptability. Ensure that an activated charcoal cartridge for the elimination of organics is present in the purification system. Place this cartridge after the ion-exchange resin cartridges.

Note! HPLC-grade mobile phases are filtered before bottling, so it is unnecessary to filter the mobile phases before use. Filtering with marginally clean glassware has been known to introduce large amounts of contaminating fluorescent compounds to the mobile phases. Degassing the mobile phases with an inert gas prior to operation of the PCX5100 system is recommended for optimum performance.

To prepare and degas the HPLC mobile phase, use this procedure:



Caution! Always wear gloves for this operation. Avoid touching the inside of reservoirs or handling the solvent filters with bare fingers since amino acid contamination present on hands causes high fluorescence background. Do not leave caps and lines dangling without a reservoir. To fill reservoir, transfer caps and lines into a spare bottle or an Erlenmeyer flask filled with deionized water.

Step 1. Fill eluant reservoir “B” with HPLC-grade methanol.

Step 2. Fill eluant reservoir “A” with HPLC-grade water.

Step 3. Place the filled eluant reservoirs on or near the HPLC pump.

Step 4. If your HPLC requires it, sparge (bubble) the eluants with helium.

Step 5. Prime the HPLC pump by withdrawing at least 30 mL of each solvent from the prime/purge port with the priming syringe that is supplied. An HPLC pump method can be configured to facilitate this step. Consult your HPLC manual.

Step 6. Close the prime/purge valve.

Step 7. Start the HPLC pump. Pump methanol through the column and system at 1.0 mL/min. Continue pumping until the entire post-column system is primed. The column back pressure should stabilize at approximately 700 psi (50 bar) for the 15 cm column or 1200 psi (80 bar) for the 25 cm column.

Post-column Reagent Preparation

The two derivatization reagents required for carbamate analysis are a hydrolysis reagent (NaOH) and *o*-phthalaldehyde reagent.

Note! During initial installation, the reagent bottles, lines, and pump should first be cleaned and primed with methanol to reduce possible fluorescence background.

To prepare and pressurize the post-column reagents, follow this procedure:

Step 1. Turn off the inert gas.

Step 2. Thoroughly wash the two reagent reservoirs and then rinse with methanol. Wipe down the dip tubes with methanol and a clean cellulose tissue.

Step 3. The hydrolysis reagent does not require preparation. Pour the hydrolysis reagent (Cat. No. CB130) directly into the reagent reservoir labeled Hydrolysis Reagent (Hydrolysis reagent reservoir cap has TFE lines). Put the cap on the reservoir. Close the vent valve.

Note! The preparation of the Hydrolysis Reagent by the user is not recommended because it is hard to obtain NaOH of adequate purity.

Step 4. Preparation of the OPA Reagent:

a. Pour the contents of one bottle (950 mL) of the OPA Diluent (Cat. No. CB910) into the reagent reservoir. (Save approximately 5 mL for step 4e.)

b. Put the cap on the bottle, open the vent valve, and turn on the gas supply. Thoroughly de-aerate the contents by sparging with inert gas. Continue bubbling for at least 10 minutes.

c. Dissolve 100 mg of OPA (Cat. No. O120) in approximately 10 mL of HPLC-grade methanol in a clean, dry container.

d. Turn off the gas supply and remove the cap from the bottle. Add the OPA solution to the deoxygenated Diluent in the reservoir.

e. Dissolve 2 g of Thiofluor (Cat. No. 3700-2000) in the reserved 5 mL of the OPA Diluent and add into the reservoir.

Note! If Thiofluor is not available, pipette 1 mL of 2-mercaptoethanol. Handling of 2-mercaptoethanol should be in the hood since it is volatile and has an unpleasant odor which will permeate the laboratory. 2-Mercaptoethanol should be replaced with Thiofluor (Pickering Laboratories brand of *N,N*-dimethyl-2-mercaptoethylamine hydrochloride), a non-volatile thiol salt.

f. Replace the cap and turn on the gas flow. Continue sparging for another minute. Close the vent valve. Gently swirl the reagent to complete the mixing.

Note! The preparation of the OPA Diluent by the user is not recommended because sodium borate (any grades) contains excessive amounts of heavy metal contaminants and insoluble matter. These impurities will eventually precipitate in the reactor and flowcell. The one year warranty does not cover damage caused by these contaminants.

Note! The Hydrolysis reagent remains stable indefinitely. The OPA reagent is sensitive to air oxidation and degrades over time. The PCX5100 modular system is designed to minimize this oxidation, resulting in a minimal loss of OPA reagent due to oxidation. When the OPA reagent reservoir is maintained under inert gas pressure, the OPA reagent maintains its activity for up to two weeks without significant loss of activity.

Priming the Reagent Pumps

Step 1. Ensure that the reagent and gas supply tubes for the reservoirs are connected to their proper fittings on the right side of the instrument.

Step 2. Connect a 20 mL disposable syringe to the Luer fitting in the center of one of the prime/purge valves.

Step 3. Open the prime/purge valve 1/2 to 1 full turn (CCW) and let the flow exit into the syringe.

Step 4. To purge air bubbles from the reservoir line, pump head, or reagent gauge, syringe suction may be applied. Draw liquid until no bubbles come through.

Step 5. Close the valve, remove the syringe, and wash the Luer fitting with a little water.

Step 6. Repeat the process for the other valve.

If priming the reagent pump is difficult, see Chapter 5 (Troubleshooting Guide p 5-16).

Turn on the PCX 5100

Step 1. The HPLC pump should be on and pumping methanol through the column at this time. If not, turn on the pump and wait until at least 500 psi (35 bar) of pressure develops.

Step 2. Turn on the main power at the rear of the PCX5100. Push the "Pump" switch onto the off-position (so that the orange stripe is not visible). If you are using the optional "Remote Off" circuit, make sure it is in the open or logic 1 state. Press the "Reset" button. The "Reset" lamp should become lit.

Step 3. Check that the column temperature setting is 42˚C and the reactor temperature setting is 100˚C. Press the * button on the corresponding temperature controller to view the setpoint and release it to show the actual (process) temperature (see page 2-3).

Step 4. After the temperature of the reactor shows over 50˚C, turn on the "Pump" switch. The two reagent gauges should begin pulsing with a maximum of about 1,000–1,500 psig.

Note! The pulsating pressure readings of the reagent pumps (approximately 500 psig swing) are normal. These pulsations are dampened by the liquids in the Bourdon tubes of the gauges and the flow restrictors (packed with diamond particles), located on the back of the gauge panel. The pulse dampening is very effective as indicated by post-column pressure gauge pulsations of less than 10 psig.

Note! Inspect all tubing connections in the post-column instrument to ensure there are no leaks.

Step 5. Wait for the temperatures to come up to setpoint. When all the temperatures are stable, the "Ready" lamp lights and the "RDY" relay switches.

Setting Up the HPLC Fluorescence Detector

Refer to your HPLC manual for setup details. Optimum conditions for most detectors are excitation at 330 nm and emission at 465 nm. If your detector has a selectable time-constant, use about 2 seconds.

Setting Up the Data Station or Integrator

Prepare the HPLC data station or integrator and set up a data handling method to accept data from the fluorescence detector. Initially, an area % method without naming peaks is adequate. This method should have a peak width of about 10 seconds and data end-time of about 27 minutes for the 15 cm column, or a data end time of about 45 minutes for the 25 cm column.

Setting Up the HPLC Pump Method

Pickering Laboratories recommends various gradient conditions depending on the column and type of sample. For the purposes of testing and set-up, use the 15 cm column with the 4th gradient on the next page. Note that the exact time of equilibration depends on the internal volume of your HPLC. When the column pressure is stable for at least one minute, the column has been re-equilibrated.

The First Chromatogram

Allow the column to equilibrate for about ten minutes under initial conditions. Inject 10 µL of Carbamate Test Mixture, and collect the first chromatogram.

Gradient Conditions for Carbamate Analysis

There are six possible gradients: two for the C_{18} 15 cm column (EC5100), two for the C_{18} 25 cm column (EC5100), and two for the C_8 25 cm column (EC5150). Two programs are for aqueous samples, three programs for methanolic samples, and one program for methanolic samples using a water-acetonitrile gradient. When using a C_8 25 cm column (EC5150), the water-acetonitrile gradient can be used as a confirmation method. The C_{18} columns operate at 42°C; the C_8 column at 37°C.

1846250 column (4.6 mm ID x 250 mm) with aqueous samples

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			100	0	1.0 mL/min
0	0	0	100	0	inject up to 400 µL water
1	0–1.7	1.7	100	0	concentrate sample on column
2	1.71	0.01	80	20	step change
3	1.71–45.7	44	25	75	linear gradient
4	45.71	0.01	0	100	step change
5	45.71–50	4.29	0	100	cleanout
6	50–	8–12	100	0	re-equilibration

1846150 column (4.6 mm ID x 150 mm) with aqueous samples

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			100	0	1.0 mL/min
0	0	0	100	0	inject up to 200 µL water
1	0–1	1	100	0	concentrate sample on column
2	1.01	0.01	82	18	step change
3	1.01–36	35	30	70	linear gradient
4	36.01	0.01	0	100	step change
5	36.01–38	2	0	100	cleanout
6	38–	5–10	100	0	re-equilibration

1846250 column (4.6 mm ID x 250 mm) with methanolic samples

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			80	20	1.0 mL/min
0	0	0	80	20	inject up to 10 µL methanol
1	0–1	1	80	20	isocratic
2	1–44	43	25	75	linear gradient
4	44.01	0.01	0	100	step change
5	44.01–49	5	0	100	cleanout
6	49–	5–8	80	20	re-equilibration

1846150 column (4.6 mm ID x 150 mm) with methanolic samples

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			82	18	1.0 mL/min
0	0	0	82	18	inject up to 10 µL methanol
1	0–0.5	0.5	82	18	isocratic
2	0.5–29	28.5	30	70	linear gradient
4	29.01	0.01	0	100	step change
5	29–31	2	0	100	Cleanout
6	31–	5–8	82	18	re-equilibration

0840250 column (4.0 mm ID x 250 mm) with methanolic samples

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			88	12	0.80 mL/min
0	0–2	2	88	12	inject up to 10 µL methanol
1	2–42	40	34	66	linear gradient
2	42–46	4	34	66	isocratic
4	46.1	0.1	0	100	step change
5	46.1–49	2.9	0	100	cleanout
6	49–	10–13	88	12	re-equilibration

0840250 column (4.0 mm ID x 250 mm) with methanolic samples using a water/MeCN gradient

Step	Times(min)	Interval	%Water	%MeCN	Comment
Equil.			90	10	0.80 mL/min
0	0–2	2	90	10	inject up to 10 µL methanol
1	2–46	44	49	51	linear gradient
2	46.1	0.1	30	70	step change
4	46.1–49	2.9	30	70	cleanout
5	49–	10–13	90	10	re-equilibration

Post-Column Conditions

For all the above programs the same post-column conditions apply

Reagent 1: 0.05 N NaOH (CB130)
Pump 1: 0.30 mL/min
Reactor 1: 500 µL at 100˚C

Reagent 2: OPA & Thiofluor in pH 9.1 borate buffer
Pump 2: 0.30 mL/min
Reactor 2: 100 µL at ambient temperature

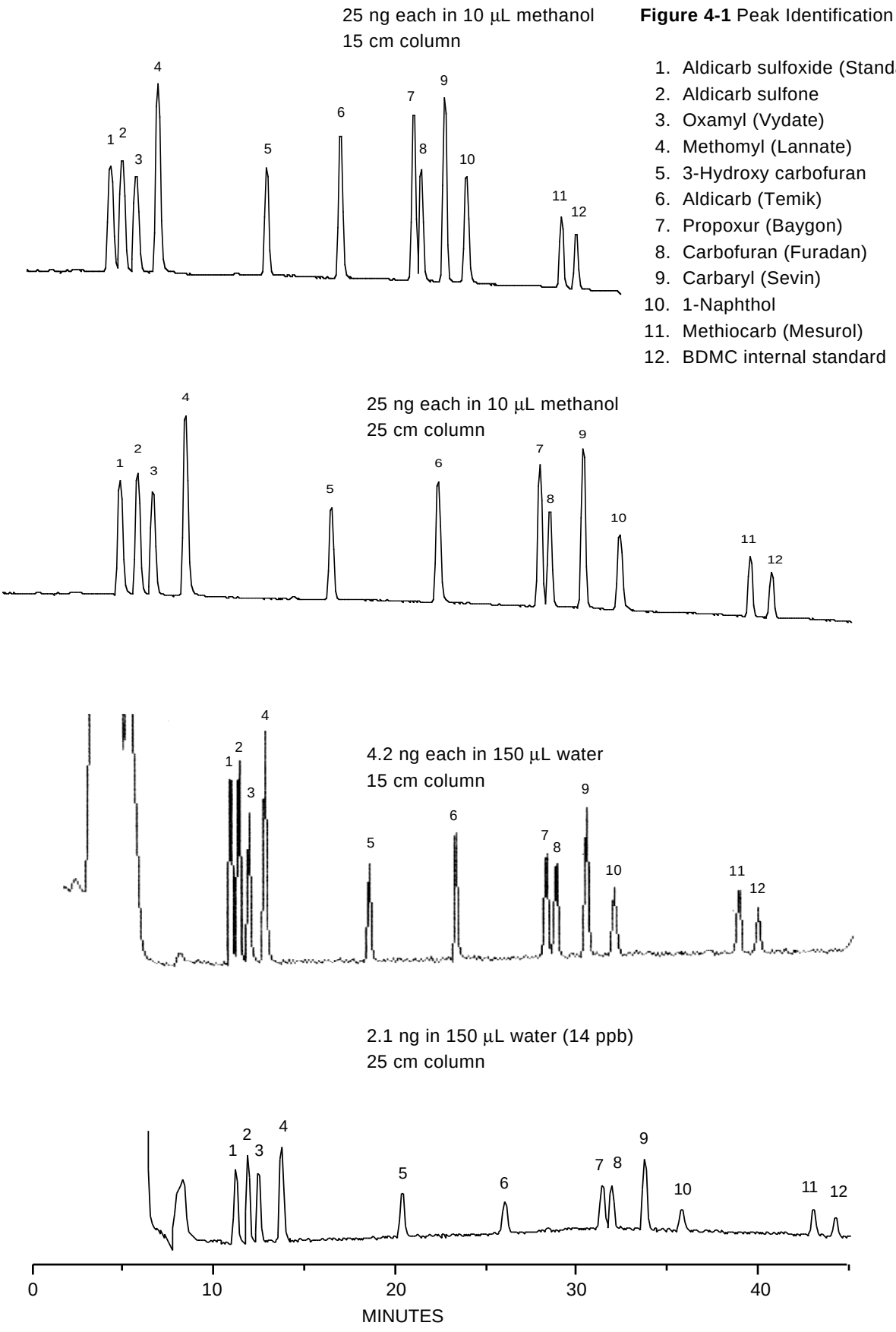


Figure 4-1 Peak Identification

1. Aldicarb sulfoxide (Standak)
2. Aldicarb sulfone
3. Oxamyl (Vydate)
4. Methomyl (Lannate)
5. 3-Hydroxy carbofuran
6. Aldicarb (Temik)
7. Propoxur (Baygon)
8. Carbofuran (Furadan)
9. Carbaryl (Sevin)
10. 1-Naphthol
11. Methiocarb (Mesurol)
12. BDMC internal standard

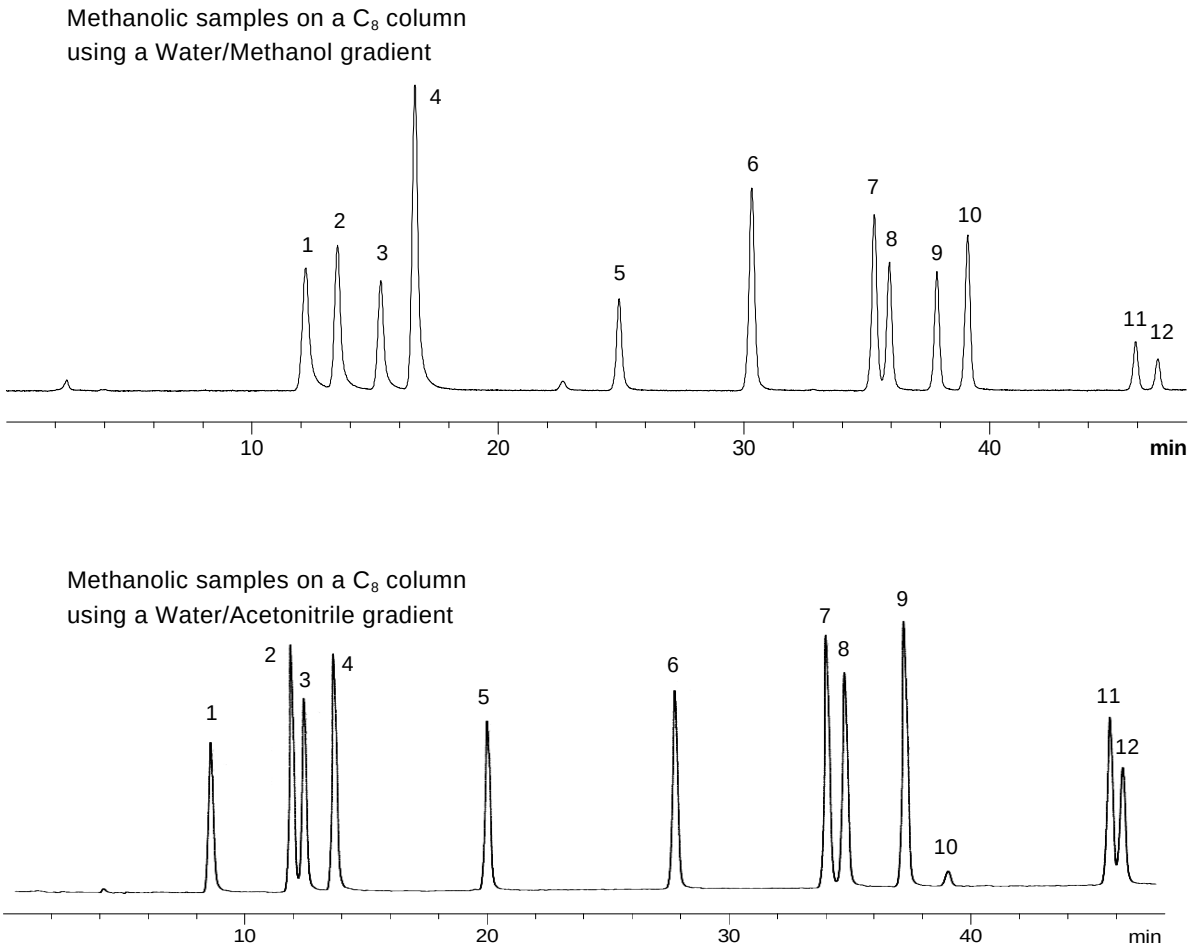


Figure 4-2 Peak Identification

1. Aldicarb sulfoxide (Standak)
2. Aldicarb sulfone
3. Oxamyl (Vydate)
4. Methomyl (Lannate)
5. 3-Hydroxy carbofuran
6. Aldicarb (Temik)
7. Propoxur (Baygon)
8. Carbofuran (Furadan)
9. Carbaryl (Sevin)
10. 1-Naphthol
11. Methiocarb (Mesurol)
12. BDMC internal standard

System Startup for Carbamate Analysis

Having accomplished the steps in Getting Started, you will find some of the following steps to be redundant. Use this procedure as a check list to review the logical sequence of tasks, and the instrument conditions to be observed prior to injecting the carbamate sample for analysis.

Step 1. Turn on the HPLC system, detector, injector, and data system. Start the HPLC pump with 100% methanol. If the 15 cm carbamate column is installed, the following readings should be observed on the PCX5100 instrument and LC system. The LC pump pressure will be higher if you are using the 25 cm column.

Instrument / Function	**Reading**
HPLC pump delivery	100% B (methanol)
HPLC flow	1.0 mL/min
column heater temperature	OFF
post-column reactor	OFF
HPLC pump pressure	850–1050 psi
post-column system pressure	180–190 psi
reagent 1 pressure	< 100 psi
reagent 2 pressure	< 100 psi

Step 2. If the above readings are displayed, set the HPLC system to deliver 82% water and 18% methanol. After approximately 10 minutes the PCX5100 instrument and HPLC system should display the following readings. (The 82% water: 18% methanol mixture has a higher viscosity than methanol alone and will result in higher pump and system pressures.) The following conditions are typical of a system without post-column reagent flow:

Instrument / Function	**Reading**
HPLC pump delivery	82% A (water) 18% B (methanol)
HPLC flow	1.0 mL/min
column heater temperature	OFF
post-column reactor	OFF
HPLC pump pressure	1800–2200 psi
post-column system pressure	300–325 psi
reagent 1 pressure	< 100 psi
reagent 2 pressure	< 100 psi

Step 3. Turn ON the main power switch. The lights on the temperature controllers should come on but the heaters remain off. Turn OFF the "Pump" switch. Press the "Reset" button on the front. This sends power to the heated reactor and column oven. Allow the reactor to reach 50˚C before turning on the reagent flow (Step 4).

- Ensure that the reagent pump is turned OFF.
- Press the "Reset" button.
- Wait for the temperatures to stabilize.

The following HPLC system readings should now be displayed. (Pressure of the pump and PCX 5100 instrument will have dropped by about 100 psi due to the decreased resistance of the post-column reactor heated to 100˚C.)

Instrument / Function	Reading
HPLC pump delivery	82% A (water) 18% B (methanol)
HPLC flow	1.0 mL/min
column heater temperature	42˚C
post-column reactor	100˚C
HPLC pump pressure	1200–1500 psi
post-column system pressure	180–200 psi
reagent 1 pressure	< 100 psi
reagent 2 pressure	< 100 psi

Step 4. Switch the reagent pump on. Prime the pump and purge the reagent lines. Observe a small increase in the fluorescence signal.

- Press the "Pump" switch ON so that the orange stripe becomes visible.
- Attach the 20 mL plastic syringe to the Luer fitting on "Bypass 1." Open the valve 1 turn. Draw about 10 mL of reagent into the syringe. Close the valve. Discard the liquid in the syringe.
- Repeat the operation for "Bypass 2."

Function	Reading
eluant composition	82% A (water), 18% B (methanol)
eluant flow rate	1.0 mL/min
column heater temperature	42˚C
reactor temperature	100˚C
HPLC pump pressure	1300–1600 psi
post-column system pressure	210–250 psi
reagent 1 pressure	max. 600–1100 pulsating
reagent 2 pressure	max. 600–1100 pulsating

As the OPA reagent appears in the detector, you should see an increase in the baseline signal, typically 50–100%. When all pressures are stable and the fluorescence detector baseline is flat, the system is ready for injection of a carbamate sample.

Shutdown Procedures

Upon completion of the analyses, use one of the following three procedures to shut down the PCX 5100 system properly. These procedures can prevent potential column damage, reaction coil blockage, high background fluorescence, reagent precipitation, or other problems.

Short Term (Up to 3 days)

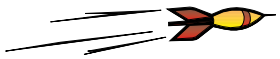
- Turn off the PCX 5100 either manually or via the "Remote Off" function.
- Set the HPLC pump at 1 mL/min of methanol to flush the system until the detector baseline drops.
- Set the HPLC pump to ≤ 0.1 mL/min methanol.
- Turn off the detector lamp.
- You may also program a slowdown method to accomplish all the above steps.

Step	Time (min)	%MeOH	Flow (mL/min)
0	0	100	0.02
1	5	100	0.02
2	5.1	100	1
3	15	100	1
4	15.1	100	0.02

Note! The automatic valves prevent reagents from back-flowing onto the column. The inert gas should be left on to preserve the OPA reagent.

Medium Term (Up to 6 days)

- Turn off the PCX 5100 either manually or via the "Remote Off" function.
- Set the HPLC pump at 1 mL/min of methanol to flush the system until the reactor temperature drops below 60˚C. This takes about an hour.
- Disconnect the outlet of the detector at the "From Detector" fitting, relieving pressure on the post-column system.
- Relieve the pressure in the reagent gauges by briefly opening the bypass valves.
- Let the system drain for 1–2 minutes.
- Turn off the fluorescence detector and HPLC pump.



Caution! The medium term shutdown should be performed prior to any work on the HPLC or PCX 5100. Failure to do so could defeat the safety systems.

Long Term (7 days or more)

- Set the HPLC to pump methanol at 1 mL/min.
- Turn off the reagent pump. Set the reactor temperature to < 60˚C.
- Turn off the gas at the toggle valve and vent the reservoirs.
- Replace both reagents with water and draw 10 mL through each prime/purge valve.
- Replace the water with water / methanol (1 / 1).
- Turn the reagent pump on and flush the system until the temperature of the reactor has fallen below 60˚C.
- Turn off the PCX 5100.
- Relieve the pressure in the reagent gauges by briefly opening the bypass valves.
- Let the system drain for 1–2 minutes.
- Turn off the inert gas source.
- Turn off the HPLC system.
- Remove the column and guard column and plug them. (When removing the column, disconnect the outlet fitting first.) Replace them with a tubing and unions so there are no open lines.

Glyphosate Analysis with the EG5100

Getting Started

Important! This section assumes that the PCX 5100 has been installed according to the directions in Chapter 3. Do not operate the instrument until it has been installed properly, and you have read and understood the instructions in this section.

HPLC Mobile Phase

The Pickering Laboratories glyphosate analysis requires two mobile phases: K200 eluant and RG019 column regenerant. Do not use water or eluant containing any organic modifiers such as methanol, acetonitrile, etc., for the glyphosate column. Do not exceed a flow rate of 0.4 mL/min.

To prepare and degas the HPLC mobile phase, use this procedure:

Note! Pickering mobile phases are filtered before bottling, so it is unnecessary to filter the mobile phases before use. Filtering with marginally clean glassware has been known to introduce large amounts of contaminating fluorescent compounds to the mobile phases. Always wear gloves for this operation. Avoid touching the inside of reservoirs or handling the solvent filter with bare fingers since amino acid contamination present on hands causes high fluorescence background. Do not leave caps and lines dangling without a reservoir. To fill reservoir, transfer caps and lines into a spare bottle or an Erlenmeyer flask filled with deionized water.

Step 1. Remove any stainless steel inlet frit or sinker from the HPLC reservoirs. Titanium, polymeric, or ceramic frits are acceptable but it is better to run without any inlet frits.

Step 2. Fill eluant reservoir "B" with RG019 column regenerant (0.005 M KOH).

Step 3. Fill eluant reservoir "A" with K200 glyphosate eluant (0.005 M K^+, pH 2.00 phosphate buffer).

Step 4. Place the filled eluant reservoirs on or near the HPLC pump.

Step 5. If your HPLC requires it, sparge the eluants with helium. Do not use stainless steel frits in the sparging line. Do not use continuous sparging with buffers, because the composition will change with time.

Step 6. Prime the HPLC pump by withdrawing at least 30 mL of each solvent from the prime/purge port with the priming syringe that is supplied. An HPLC pump method can be configured to facilitate this step. Consult your HPLC manual.

Step 7. Close the HPLC prime/purge valve and flush the HPLC for at least 30 min without the glyphosate column attached (>1 mL/min; 50% A / 50% B).

Step 8. Stop the HPLC pump. Connect guard and column according to Figure 3-1 (page 22).

Step 9. Start the HPLC pump. Pump K200 through the column and system at 0.40 mL/min. Do not exceed a flow rate of 0.4 mL/min for the glyphosate column and guard. Continue pumping until the entire post-column system is primed. The column back pressure should stabilize at approximately 1500 psi (100 bar).

Post-column Reagent Preparation

The two derivatization reagents required for glyphosate analysis are a hypochlorite reagent (NaOCl) and *o*-phthalaldehyde.

Note! During initial installation, the reagent bottles, lines, and pump should first be cleaned and primed with methanol to reduce possible fluorescence background. Do not leave caps and lines dangling without a reservoir. To fill reservoir, transfer caps and lines into a spare bottle or an Erlenmeyer flask filled with deionized water.

To prepare and pressurize the post-column reagents, follow this procedure:

Step 1. Turn off the inert gas.

Step 2. Thoroughly wash the two reagent reservoirs and then rinse with methanol. Wipe down the dip tubes with methanol and a clean cellulose tissue.

Step 3. Preparation of the Oxidizing Reagent.

a. Pour one bottle (950 mL) the Hypochlorite Diluent (GA116) directly into the reagent reservoir labeled Oxidizing Reagent (Reservoir cap has TFE reagent and gas lines).

b. Add 100 µL of 5% sodium hypochlorite solution (Clorox) to the diluent. The exact amount will depend on the actual hypochlorite concentration of the stock solution. When you get your first chromatograms, you will be able to adjust the amount to optimize the relative peak areas of glyphosate versus AMPA. Figure 4-2 shows a typical response curve.

c. Cap the reservoir, close the vent valve, and swirl the solution to mix it thoroughly.

Note! The hypochlorite concentration slowly decreases with time. This will manifest itself as a change in the relative peak areas of glyphosate and AMPA. It will remain usable for several days, but we recommend you calibrate daily.

Caution! Do ***not*** use calcium hypochlorite in the oxidizing reagent. This will cause plugging of the post-column reactor. ***The one year warranty does not cover damage caused by calcium hypochlorite-based reagents.*** The EPA Draft Method 547 is wrong on this point; $Ca_3(PO_4)_2$ is insoluble in water.

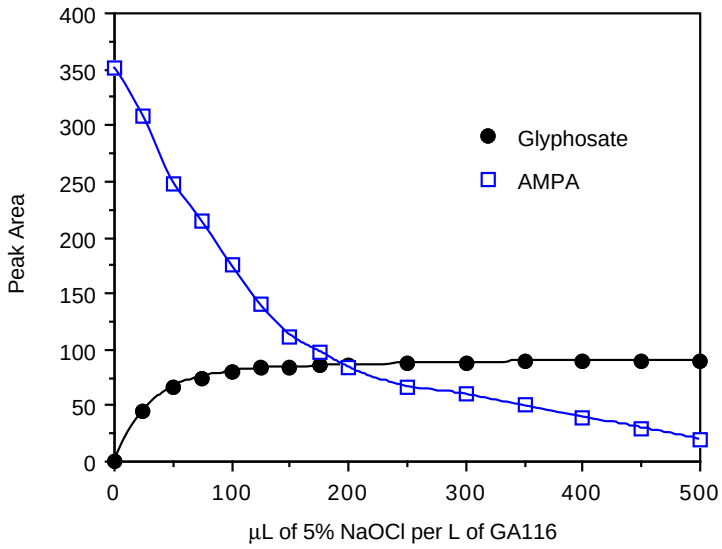


Figure 4-2

Step 4. Preparation of the OPA Reagent:

a. Pour the contents of one bottle (950 mL) of the OPA Diluent (Cat. No. GA104) into the reagent reservoir. (Save approximately 5 mL for step e.)

b. Put the cap on the bottle, open the vent valve, and turn on the gas supply. Thoroughly de-aerate the contents by sparging with inert gas. Continue bubbling for at least 10 minutes.

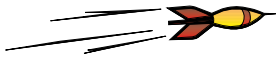
c. Dissolve 100 mg of OPA (Cat. No. O120) in approximately 10 mL of HPLC-grade methanol in a clean, dry container.

d. Turn off the gas supply and remove the cap from the bottle. Add the OPA solution to the deoxygenated Diluent in the reservoir.

e. Dissolve 2 g of Thiofluor (Cat. No. 3700-2000) in the reserved 5 mL of the OPA Diluent and add into the reservoir.

Note! If Thiofluor is not available, pipette 1 mL of 2-mercaptoethanol. If 2-mercaptoethanol is used, this step should be performed in the hood since it is volatile and has an unpleasant odor which will permeate the laboratory. 2-Mercaptoethanol should be replaced with Thiofluor (Pickering Laboratories brand of *N,N*-dimethyl-2-mercaptoethylamine hydrochloride), a nonvolatile thiol salt.

f. Replace the cap and turn on the gas flow. Continue sparging for another minute. Close the vent valve. Gently swirl the reagent to complete the mixing.



Caution! The preparation of the OPA Diluent by the user is not recommended because sodium borate (any grades) contains excessive amounts of heavy metal contaminants and insoluble matter. These impurities will eventually precipitate in the reactor and flowcell. ***The one-year warranty does not cover damage caused by these contaminants.***

Note! The OPA reagent is sensitive to air oxidation and degrades over time. The PCX 5100 modular system is designed to minimize this oxidation, resulting in a minimal loss of OPA reagent due to oxidation. When the OPA reagent reservoir is maintained under inert gas pressure, the OPA reagent maintains its activity for up to two weeks without significant loss of activity.

Priming the Reagent Pumps

Step 1. Connect a 20 mL plastic syringe to the Luer fitting in the center of one of the prime/purge valves.

Step 2. Open the prime/purge valve 1/2 to 1 full turn (CCW) and let the flow exit into the syringe.

Step 3. To purge air bubbles from the reservoir line, pump head, or reagent gauge, syringe suction may be applied. Draw liquid until no bubbles come through.

Step 4. Close the valve, remove the syringe, and wash the Luer fitting with a little water.

Step 5. Repeat the process for the other valve.

If priming the reagent pump is difficult, see Chapter 5 (Troubleshooting Guide p 5-16).

Turn on the PCX 5100

Step 1. The HPLC pump should be on and pumping K200 at this time. If not, turn on the pump and wait until at least 500 psi (35 bar) of pressure develops.

Step 2. Turn on the main power at the rear of the PCX 5100. The temperature controllers should turn on. Push the "Pump" switch to OFF (so that the orange stripe is not visible). If you are using the optional "Remote Off" circuit, make sure it is in the open or logic 1 state. Press the "Reset" button. The "Reset" lamp illuminates.

Step 3. Check that the column temperature setting is 55˚C and the reactor temperature setting is 36˚C. Press the * button on the corresponding temperature controller to view the setpoint and release it to show the actual (process) temperature (see page 2-3).

Step 4. Turn on the "Pump" switch. The two reagent gauges should begin pulsing with a maximum of about 600–1000 psi.

Note! The pulsating pressure readings of the reagent pumps (approximately 500 psig swing) are normal. These pulsations are dampened by the liquids in the Bourdon tubes of the gauges and the flow restrictors (packed with diamond particles), located on the back of the gauge panel. The pulse dampening is very effective as indicated by post-column pressure gauge pulsations of less than 10 psig.

Note! Inspect all tubing connections in the post-column reaction instrument to ensure there are no leaks.

Step 5. Wait for the temperatures to come up to setpoint. When all the temperatures are stable, the "Ready" lamp lights and the "RDY" relay switches.

Setting up the Fluorescence Detector

Refer to your detector manual for setup details. Optimum conditions for most detectors are excitation at 330 nm and emission at 465 nm. If your detector has a selectable time-constant, use about 2 seconds.

Setting up the Data Station or Integrator

Prepare the HPLC data station or integrator and set up a data handling method to accept data from the fluorescence detector. Initially, an area % method without naming peaks is adequate. This method should have a peak width of about 20 seconds and data end-time of about 20 minutes

Setting up the HPLC Pump Method

These are the recommended conditions for glyphosate analysis using the 1954150 column and 1953020 guard column. The column temperature is 55˚C.

Step	Times (min)	Interval	%K200	%RG019	Comment
Equil.			100	0	0.40 mL/min (Maximum)
0	0	0	100	0	inject ≤ 50 µL
1	0–15	15	100	0	isocratic
2	15–17	2	0	100	step change
3	17–	8–12	100	0	re-equilibration

The exact time of equilibration depends on the internal volume of your HPLC. When the baseline and column pressure are stable for two minutes, the column has been re-equilibrated.

Post-column Conditions

Reagent 1: 100 µL of 5% NaOCl (Clorox) in GA116 Diluent
Pump 1: 0.30 mL/min
Reactor 1: 500 µL at 36˚C

Reagent 2: *o*-Phthalaldehyde and Thiofluor in GA104 borate buffer
Pump 2: 0.30 mL/min
Reactor 2: 100 µL at ambient temperature

The First Chromatogram

Allow the column to equilibrate for about 20 minutes under initial conditions. Inject 10µL of Glyphosate Text Mixture, and collect the first chromatogram.

Shutdown Procedures

Upon completion of the analyses, use one of the following three procedures to shut down the EG5100 system properly. These procedures can prevent potential column damage, reaction coil blockage, high background fluorescence, reagent precipitation, or other problems.

Short Term (Up to 3 days)

- Turn off the PCX 5100 either manually or via the "Remote Off" function.
- Continue the HPLC pump at 0.40 mL/min of K200 to flush the system until the detector baseline drops.
- Set the HPLC pump to ≤ 0.1 mL/min K200 so that the column pressure is below 500 psi (35 bar).
- Turn off the detector lamp.
- You may also program a slowdown method to accomplish all the above steps.

Step	Time (min)	%K200	Flow (mL/min)
0	0	100	0.02
1	5	100	0.02
2	5.5	100	0.25
3	15	100	0.25
4	15.1	100	0.02

Note! The automatic valves prevent reagents from back-flowing onto the column. The inert gas should be left on to preserve the OPA reagent.

Medium Term (Up to 6 days)

- Turn off the PCX 5100 either manually or via the "Remote Off" function.
- Set the HPLC pump to 0.40 mL/min of RG019 for 5 min. Excessive flushing will require an equally excessive re-equilibration when you start up again.
- Disconnect the outlet of the detector at the "From Detector" fitting relieving pressure on the post-column system.
- Relieve the pressure in the reagent gauges by briefly opening the bypass valves.
- Let the system drain for 1–2 min.
- Turn off the fluorescence detector and HPLC pump.

Caution! The medium term shutdown should be performed prior to any work on the HPLC or PCX 5100. Failure to do so could defeat the safety systems.

Long Term (7 days or more)

- Turn off the reagent and HPLC pumps.
- Turn off the gas at the toggle valve and vent the reservoirs.
- Replace both reagents with water and draw 10 mL through each prime/purge valve.
- Replace the water with water / methanol (1 / 1).
- Set the HPLC to pump 100% RG019 at 0.4 mL/min
- Turn on the reagent pumps and flush for 5 minutes
- Turn off the PCX5100 and the HPLC pump.
- Relieve the pressure in the reagent gauges by briefly opening the bypass valves.
- Let the system drain for 1–2 min.
- Turn off the inert gas source.
- Remove the column and guard column and plug them. (When removing the column, disconnect the outlet fitting first.) Replace them with a tubing and unions so there are no open lines.
- Replace the eluants with waterand draw 10 mL through the HPLC prime/purge valve.
- Replace the water with water / methanol (1 / 1). Turn on the HPLC pump for 15 minutes.
- Turn off the HPLC system.

Combination Carbamate/ Glyphosate Systems

The PCX 5100 can be used for either carbamate or glyphosate analysis. To change from one to the other, you will need to change the reagents, column, eluants, and temperatures. Refer to the instructions above for the details.

Changing from carbamate to glyphosate

- Please read page 3-1 for the HPLC System Requirements for ***glyphosate analysis!*** The HPLC components must be compatible with ***high pH regenerant*.**
- Because the reactor is so slow to cool, this is best performed first thing in the morning after the system has been cooling off overnight.
- Perform the medium-term shutdown at the end of the day before the conversion.
- You may change the reactor temperature to 36°C and column temperature to 55°C at this time or wait until the next day.
- Remove the carbamate column and guard column and plug them. When removing the column, disconnect the outlet fitting first.
- Remove any stainless steel inlet frits or sinkers from the HPLC reservoirs.
- Change the HPLC eluants from water and methanol to K200 and RG019.
- Flush the HPLC pump, injector, and the inlet lines of the PCX 5100 with K200 and RG019 for at least 30 min at > 1 mL/min without the glyphosate column and guard attached. Do not allow methanol into the glyphosate column.
- Change the reagents from CB130 and CB910 to GA116 and GA104. The buffering capacity of CB910 is inadequate to neutralize K200, so you must use GA104.
- Turn off the HPLC pump
- Install the glyphosate column and guard.
- Change HPLC program and start the HPLC pump to a ***maximum*** of 0.4 mL/min of K200.
- Turn on the PCX 5100 and press the "Reset" button. Immediately, before the reactor can heat up, lower the temperature setting from 100˚C to 36˚C.
- Set the column temperature to 55˚C.
- Prime the reagent pumps by drawing 10–20 mL through the bypass valves. Start the reagent pumps.
- Allow the system to equilibrate and flush itself for at least one hour before using it to collect data.

Changing from glyphosate to carbamate

- Perform the medium-term shutdown.
- Remove the glyphosate column and guard column and plug them.When removing the column, disconnect the outlet fitting first.
- Change the HPLC eluants from K200 and RG019 to water and methanol.
- Flush the HPLC pump, injector, and the inlet lines of the PCX 5100 with methanol without the carbamate column and guard attached for at least 30 min. Do not allow either of the glyphosate eluants onto the carbamate column.
- Turn off the HPLC pump
- Install the carbamate column and guard.
- Change HPLC program and start the HPLC pump.
- Turn on the PCX 5100 and press the "Reset" button. Immediately, before the column oven can heat up, lower the temperature setting from 55˚C to 42˚C. Set the reactor temperature to 100˚C.
- Change the reagents from GA116 and GA104 to CB130 and CB910.
- Prime the reagent pumps by drawing 10–20 mL through the bypass valves. Start the reagent pumps.
- Allow the system to equilibrate and flush itself for at least one hour before using it to collect data.

Chapter 5
Routine Maintenance & Troubleshooting

Your Pickering PCX5100 will require some routine maintenance to stay in top condition. Ordinarily, little maintenance is needed beyond good operating procedures.

Initial System Testing

The initial system testing is part of the installation process. Part of this testing is to establish standard conditions so that you can return to them for diagnostic purposes in the event of later problems.

Test Chromatogram

Set up the HPLC and the PCX5100 as recommended in Chapter 4. For carbamate analysis use the gradient for methanolic samples with either 15 or 25 cm column. The sample should be 10µL of Carbamate Test Mixture 1700-0063. For glyphosate analysis the sample is 10µL of Glyphosate Test Mixture 1700-0080. These conditions are close approximations to those used by Pickering for column and instrument testing.

Collect two chromatograms to be sure that the system is stable and repeatable. Compare your chromatograms to the test chromatogram supplied with the Pickering column. Your chromatograms should not be significantly different. If there is a problem, see the later portion of this section for troubleshooting. Keep copies of your test chromatograms and the Pickering test chromatogram on file.

Parameter Log

Make copies of the blank forms in Appendix A and complete the parameter log on the photocopy. Your system should have come with a similar log from factory testing. Use the same conditions as for the test chromatogram above. Report the pressures for the system equilibrated under initial conditions. The pressures reported for Reagent 1 and Reagent 2 should be the maximum swings of the pointers. Although the parameters will not be identical to the factory, they should be similar. Keep a daily log of the four pressures for diagnostic use. See page 5-11: Interpretation of Pressures.

There is also a sheet for you to record the HPLC system parameters. Include all the settings for the pump, injector, detector, and integrator. Keep copies of this document as it will be very helpful for troubleshooting.

Test Chromatogram & Parameter Log for User-Defined Conditions

Typically your conditions for routine analysis will be different than the conditions used for testing. You may be using a different sample, sample volume, standard solution, gradient, or even column. Set up the system for injection of your calibration solution, and collect two chromatograms. The only standard for comparison is your expectations.

Fill out the parameter log for your initial conditions if they are different than the Pickering standard conditions. Record all the LC settings for your method.

Keep copies of these chromatograms and logs for future use. We suggest posting this information near your instrument.

Post-Column Band-Spreading Test

This test measures the amount of increased band-spreading due to the post-column system. It exploits the fact that 1-naphthol is fluorescent without derivatization. The general scheme is to analyze the test mixture with the post-column reagents turned off. First, test with the column connected directly to the detector (bypassing the PCX5100); second, test with the column effluent flowing through the post-column reactors.

This test is best performed on a system that has been shut down and at least partially cooled down. A normal carbamate analyzer works for this test. If you have a glyphosate analyzer, you will need to change the eluants and column.

Step 1. Conditions Isocratic program, 40% water, 60% methanol
Flow rate: 1.0 mL/min
Sample: 10µL of Carbamate Test Mix, 1700-0063
Detector: excitation 330nm, emission 465nm

The integrator or data system should be set up similarly to normal conditions, but the fluorescence of the 1-naphthol will only be 20% as much as the same amount under post-column conditions; the alkaline PC conditions enhance the native fluorescence.

Step 2. You need to decide on a criterion of peak width. Generally the width at half-maximum is the most consistent measure. There are several ways to measure it. If your computer data station calculates peak width, use that. The ratio of area over height is a good approximation, and some data stations report this number. If you are using an integrator that only reports areas, you can measure the height in millimeters, and calculate a ratio; this number has arbitrary units, but it is more precise than a direct measurement of the width. However you decide, record the method and all relevant parameters (e.g. detector sensitivity, attenuation, chart speed, chart scale).

Step 3. Install the column in the PCX5100. This can be your normal carbamate column or any C18 column in good condition.You may have a column reserved especially for quality control or diagnostic purposes and use it here.

Step 4. Disconnect the detector inlet from the PCX5100. Disconnect the column outlet from the PCX 5100. Connect the detector to the outlet of the column.

Step 5. Turn ON the HPLC system. Set the "Pump" switch OFF on the PCX5100. Turn ON the main power on the PCX5100 and press the "Reset" button. Immediately, lower the temperature setting on the reactor to below 55˚C before the reactor can warm up. Wait for the column to come to equilibrium.

Step 6. Collect three chromatograms of the test mixture. You should see a single peak around 4–6 minutes. The retention times and width parameters should agree within 2%. If not, keep trying until you have three consistent runs in sequence. The precision of the height and area is not important for this test. Calculate the average width (Wd).

Step 7. Turn OFF the LC pump. Restore the normal post-column connections. Turn the LC pump back ON and press the "Reset" button. Set the reactor to the normal operating temperature—100˚C. Wait for the entire system to equilibrate, about 15 min.

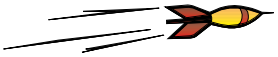
Step 8. Collect three consistent chromatograms with the effluant passing through the post-column reactors. Calculate the average width (Wpc).

Step 9. The ratio of Wd/Wpc should be 0.80 to 0.85 for a post-column system in good condition. If the ratio falls below 0.75, the PCX5100 needs either cleaning or repair. If there is band-spreading elsewhere in the system (column, injector, detector) the ratio will be artificially high, so be certain that the rest of the system is operating properly. You can also check for column degradation by comparing the historical value of Wd to the current one for that column. Be sure that the conditions are consistent when making this comparison.

Step 10. Keep records of the test results and conditions for future comparison.

Precautions & Problem-Prevention

General



- Use Pickering Laboratories reagents and eluants. The quality of the chemicals is excellent and the cost is low relative to the worth of your analytical results. ***The one year warranty does not cover damage caused by poor-quality reagents and eluants not purchased from Pickering Laboratories.***
- Use the proper start-up and shutdown procedures consistently (see Section 4).
- Frequently observe the pressures and check for leaks. You should be able to identify a problem before it becomes serious. Keep a daily log of the four pressures.

Mobile Phase

- Avoid touching the interior of the mobile phase reservoirs and the dip tubes with your fingers. Amino acids in fingerprints will cause contamination. Gloves are suggested.
- Do not leave caps and lines dangling without a reservoir. To fill reservoir, transfer caps and lines into a spare bottle or an Erlenmeyer flask filled with deionized water.
- Use HPLC-grade methanol and water (Fisher Scientific, JT Baker, or Merck) for carbamate analysis to avoid problems with baseline drift, spurious peaks, and noise.
- Use bottled HPLC-grade water if possible (Fisher Scientific, JT Baker, or Merck), especially during the initial system start-up. If water from a water purification system is used, ensure the system has an activated charcoal unit to eliminate organics, and that the charcoal cartridge is placed after the ion-exchange cartridges. (Many ion-exchange resins leach out OPA-positive contaminates that cause unacceptable fluorescence background.)
- The water in the solvent reservoir should be changed every 3 to 4 days to prevent possible bacterial growth.
- Avoid purging the system with 100% acetonitrile as precipitation of borate salt in the reactor might occur. Do not exceed 70% acetonitrile if it will be used as the mobile phase. (Methanol is recommended as the organic mobile phase for the Pickering Laboratories column and it is less expensive. Reagent precipitation problems rarely occur using methanol as the flushing solvent.)
- When switching a system between glyphosate and carbamate modes, be sure to flush the HPLC and injector with compatible mobile phase before connecting the column. Eluants for one analysis will damage the column for the other.

Column Maintenance and Precautions

- Always protect the analytical column by use of the pre-column filter and guard column.
- Check for leaks daily at column fittings. In particular, glyphosate eluants are corrosive.
- If the column back-pressure is high (> 2000psi), isolate the source of the high pressure—guard, analytical column, or the 0.5µm in-line filter. Replace items causing the increased back-pressure (Back-pressure from filter and guard should be < 200psi).

Carbamate Columns

- During shutdown, flush the column with pure methanol. Do not store the column in water.
- The analytical column can be back-flushed with methanol at 1 mL/min to clear partial blockage. (Do not disassemble or attempt to replace column inlet frit as this will void the column warranty.) Disconnect the outlet of the column during the back flush operation.
- Organic contaminants can be washed off the column by first washing with methanol then with dichloromethane. Wash again with methanol before use.
- The column is temperature-controlled to reduce baseline shift (caused by viscosity changes during gradient formation), to reduce back-pressure, and to improve retention time reproducibility.
- Use the Pickering Laboratories carbamate analysis column, which is specifically designed and tested for the separation of carbamates in the EPA Methods.

Glyphosate Column

- During shutdown, flush the column with RG019 for 5 minutes but no more than 10 minutes. Do not store the column in the eluant.
- Contamination usually occurs on the guard column. Wash it separately from the analytical column. This will save much time in the washing and re-equilibration.
- Contaminants of special concern: iron and other polyvalent cations, organic dyes, surfactants, detergents, and lipids. They may cause irreversible damage.
- Organic solvents will cause the resin in the column to swell. This leads to high back-pressure and broadened peaks. The column sometimes can be regenerated.
- Use Pickering eluants with the Pickering column, as they are designed to work together.

The PCX5100 has two safety systems to prevent accidental backflow of reagents onto the column. The pressure interlock requires that the HPLC pump deliver at least 500psi before the reagent pump can be engaged. The second is a pair of automatic valves that prevent gas pressure from pumping reagents back through the column during extended shutdowns. However, there are ways that the safety systems can be bypassed accidentally. For example, residual pressure in the gauges immediately after shutdown will take some time to leak down to zero. Follow these procedures to avoid such accidents:

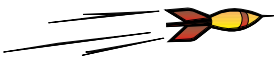
- Never disconnect any fittings between the HPLC pump and the column until the post-column system has been shut down and ***depressurized.***
- Any leaking-fittings between the HPLC pump and the column can permit backflow in the event of an unattended shutdown.
- When removing the column, remove the ***outlet*** fitting first.
- Always follow the proper shutdown procedures. See Section 4.

Sample and Standard Precautions

- The test mixtures for carbamates and glyphosate are for qualitative use only. They are not recommended for calibration purposes.
- Filter all sample through a 0.45µm membrane filter. Some samples may require even more stringent filtration, especially if colloids are present.
- Aqueous samples must always be properly buffered. Consult EPA Methods 531.1 or 547 for details.
- For carbamate analysis with methanolic samples, inject ≤ 10µL. Large amount of organic solvents can cause peak distortion. For small aqueous sample volumes (< 20µL) either of the two Pickering columns can be used. For volumes up to 500µL, only the 25cm column should be used, and a gradient delay time should be programmed into the analysis (0% organic) to trap the sample onto the head of the column.

Reagent Precautions

- Always wear gloves during the preparation of reagents. The Hydrolysis Reagent and Thiofluor cause skin irritation. Also fingerprints contaminate reagents.
- The hydrolysis reagent is stable and can be replaced as it is used. The OPA reagent is sensitive to air oxidation, degrades over time, and should be prepared fresh for optimum sensitivity. OPA reagent is stable for at least two weeks when properly prepared and pressurized with inert gas.
- Thiofluor is extremely hygroscopic. Always keep in a tightly closed container.
- The preparation of the OPA Diluent by the user is not recommended because sodium borate (any grades) contains excessive amounts of heavy metal contaminants and insoluble matter. These impurities will eventually precipitate in the reactor and flowcell. ***The one year warranty does not cover damage caused by these contaminants.*** If you must prepare your own borate buffer for the OPA reagent, ***do not use sodium tetraborate*** as suggested by the EPA methods. Instead, use molar equivalents of boric acid and sodium hydroxide, because they are available in higher purity (ACS-grade or better) and have very little insoluble matter.



Reactor Precautions

- Do not operate the heated reactor above the boiling point of the eluant unless the back-pressure regulator is connected to the waste line of the detector. Boiling inside the reactor causes precipitates to form.
- Do not operate the reactor above 130˚C. This can weaken and deform the PTFE tubing.
- Do not operate with a post-column pressure above 600 psi.

Electrical Precautions

- Always use the correct fuse.
- Do not alter the programming of the two temperature controllers. If you accidentally change the programming, contact Pickering Laboratories for advice.

Routine Maintenance

Reagent Pump

The PCX5100 uses a custom-made Eldex reagent pump with piston wash. The piston seals require periodical replacement. The length of service to be expected from the seal depends on a wide variety of factors, including if proper shutdown procedures were followed, how often the system was turned on and off, and if the piston wash is wetted. It is critically important that the seal be replaced immediately upon failure, or better yet, before failure, because the reagent can leak into the mechanical housing of the pump and cause corrosion. When a leak occurs, you may notice fluid on the side of the pump. However, a leak may not always be visible, particularly at slow flow rates. A litmus paper can be placed up the drain slot (both sides of the pump); the litmus paper should be removed periodically and checked for color changes to see if leakage has occurred.

You will need to purchase a seal kit (3106-1310; including two seals, two back-up washers, a 5/32" hex wrench, and a seal installation tool). Extra seals and a preventive maintenance kit may also be ordered from Pickering Laboratories (see Appendix B). Additional tools needed for piston seal change are 1/4" and 1/2" open-end wrench. Open-end wrenches can be purchased at local hardware stores.

Step 1. Flush the Eldex pump with water, then shut down the PCX5100, and let the reactor cool. Turn off the gas valve and vent the reservoirs. Place them at a level below the pump to prevent siphoning of the reagents.

Step 2. Remove right access panel by loosening two captive screws along bottom edge.

Step 3. Disconnect the reagent inlet line from the bottom of the pump head (it is best to repair one pump head at a time). Wipe up any spilled liquid. Disconnect the outlet tubing from the top of the pump head (***stabilize*** the outlet check-valve with a 1/2" wrench when removing the 1/16" fitting to prevent the check-valve from moving).
Optional but highly recommended: remove the pump from the chassis by 1) loosening the two captive screws and 2) unplugging the electrical connector.

Step 4. Remove the pump head from the pump as follows (Figure 5-1): with a 5/32" hex wrench, remove the two bolts while ***holding*** the pump head against the pump housing (the pump head is under spring tension). Gently pull the pump head ***straight*** out from the pump housing, in line with the axis of the piston. ***Do not tilt*** the pump head sideways; the piston may break.

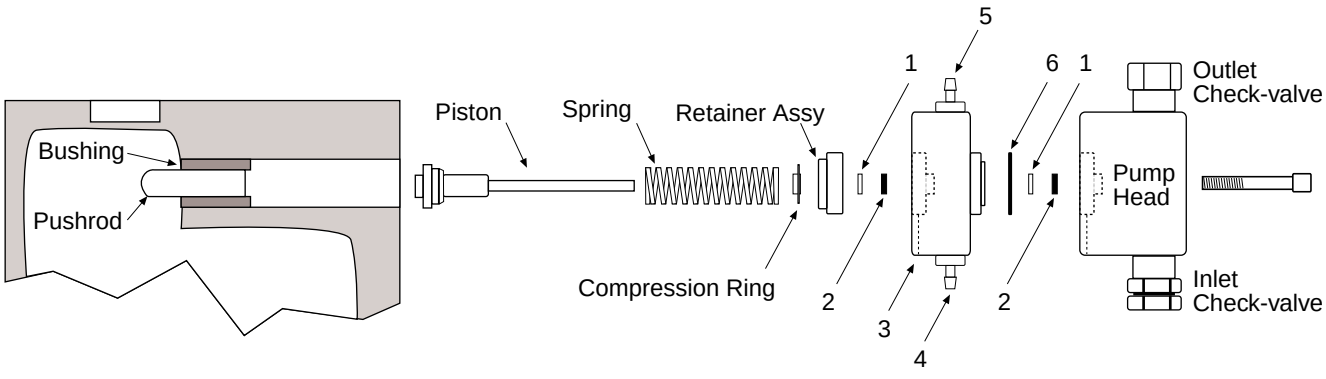


Figure 5-1. Pump assembly with wash system. Pump assembly with wash system. 1) Back-up Washer; 2) Piston Seal; 3) Wash Cylinder; 4) Inlet Port of the Wash System; 5) Outlet Port of the Wash System; 6) O-ring

Step 5. Inspect the piston. If the piston has scratches on the sapphire, or significant corrosion on the stainless steel piston holder, it should be replaced. Clean any deposits on the sapphire with soapy water, deionized water, and then methanol. If the sapphire cannot be cleaned, the piston should be replaced. Set the cleaned piston in a safe place.

Step 6. Remove the retainer assembly from the pump head. Insert the hooked end of the installation tool into the pump head through the back-up washer and piston seal. Discard the seal but the back-up washer is reusable. Do not scratch the walls of the seal cavity with the tool.

Step 7. Inspect the retainer. Clean the retainer if necessary. If there is evidence of wear, or the piston does not fit snugly in the retainer, or the retainer grips the piston too tightly, the retainer should be replaced.

Step 8. This step is only ***necessary*** if there are signs of ***corrosion*** on the stainless steel piston holder in step 5. Remove the pushrod from the pump housing with round-end tweezers. Inspect for corrosion damage; clean or replace the pushrod if necessary. Coat the pushrod with a light film of SAE 30 oil and re-install with the tweezers.

Step 9. Inspect the pump head, paying special attention to the cavity for the piston seal. Any scratches or irregularities will require replacement of the pump head. The whole pump head may be cleaned with soapy water, and then with deionized water in a sonicator bath.

Step 10 Insert the installation tool (blunt end) into the retainer assembly and then into the new backup washer and new piston seal. When inserting the new piston seal, the piston seal should lay flat on a hard surface with the spring side down. Insert the tool into the pump head as shown in Figure 5-2. Now the spring side should face the pump head. Keep the tool perpendicular with the face of the pump head. Press gently and evenly on the edge of the retainer assembly with both thumbs. Withdraw the installation tool.

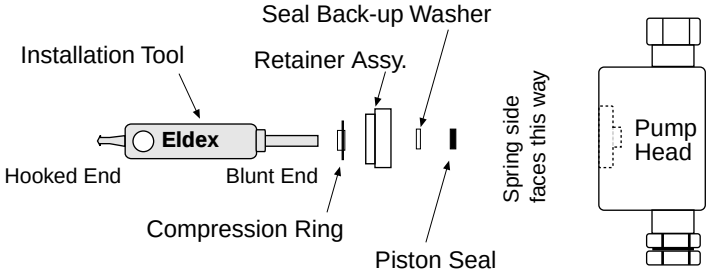


Figure 5-2. Piston seal replacement with installation tool

Optional. The piston seal (Figure 5-3) in the wash system also requires periodic replacement. However, it needs not be changed as often as the primary seal. Follow steps 6 and 10 if you desire to replace the seal in the wash system.

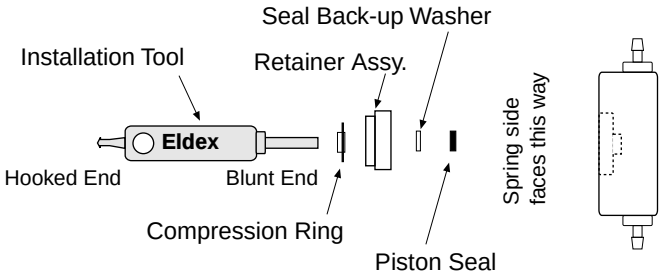


Figure 5-3. Piston seal replacement in the wash system with installation tool

Step 11 Reassemble the pump head and wash system as shown in Figure 5-1. Hold the pump assembly firmly in place and einstall it on the pump, in line with the axis of the piston; ***do not tilt*** the pump head sideways.

Step 12 Repeat steps 4–11 for reagent 2 side. If the pump was removed from the chassis in step 3, replace the pump. Reconnect the tubings to the pump head (***stabilize*** the outlet check-valve with a 1/2" wrench when tightening the fitting to prevent the check-valve from moving). Relocate the reservoir and turn on the gas. Start the PCX5100 and prime the pump.

Check-valves

Always work with check-valves in a ***clean area*** to prevent dust and dirt from entering the pump. The check-valves are the hexagonal-shaped components on the pump head (Figure 5-1). Inlet check-valves can be distinguished from outlet check-valves by the groove on the hexagonal part of the inlet check-valve. Tools needed for check-valve repairs: 1/4" & 1/2" open-end wrench, 5/32" hex wrench. Open-end wrenches are not included.

Cleaning Check-valves

Cleaning check-valves is easy and very effective and should be your first consideration.

Step 1 Flush the pump with water, then shut down the post-column system, and let the reactor cool. Turn off the gas and vent the reservoir. Place it at a level below the pump to prevent siphoning of the reagent.

Step 2 Remove the right access panel by loosening two captive screws along bottom edge.

Step 3 Disconnect the reagent inlet line from the bottom of the pump head. Wipe up any spilled liquid. Disconnect the outlet tubing from the top of the pump head (stabilize the outlet check-valve with a 1/2" wrench when removing the fitting to prevent the check-valve from moving).

Step 4 Remove the pump head from the pump as follows (Figure 5-1): with a 5/32" hex wrench, remove the two bolts while ***holding*** the pump head against the pump housing (the pump head is under spring tension). Gently pull the pump head straight out from the pump housing, in line with the axis of the piston. ***Do not tilt*** the pump head sideways; the piston may break.

Step 5 Place the pump head in a beaker of soapy water (do not remove the check-valves). Suspend the beaker in a sonicator bath and turn it on maximum power, for 30 minutes

Step 6 Replace the soapy water with deionized water and sonicate for 10 minutes.

Step 7 Reconnect the tubings to the pump head (stabilize the outlet check-valve with a 1/2" wrench when tightening the fitting to prevent the check-valve from moving). Replace the access panel. Relocate the reservoir and turn on the gas. Start the post-column system and prime the pump.

Removing Existing Check-valves

Step 1 Follow steps 1–4 from the Cleaning Check-valves section.

Step 2 To prevent the internal components of the valve from falling out upon removal, keep the pump head in its normal position when removing the inlet check-valve; turn the pump head ***upside down*** when removing the ***outlet*** check-valve. Remove the check-valves with a 1/2" wrench (counter-clockwise). After removal, keep the valve oriented so the translucent washers faces upwards.

Installing New Check-valves

Step 1 Unscrew the shipping nut and remove the metal shipping washer.

Step 2 Insert the new inlet check-valve into the pump head and tighten by hand until just finger-tight. Tighten with a 1/2" wrench 1/8 to 1/4 turn more. ***Do not overtighten!*** The sapphire seats may crack.

Step 3 Turn the pump head ***upside down;*** insert the new outlet check-valve into the pump head and tighten by hand until just finger-tight. Tighten with a 1/2" wrench 1/8 to 1/4 turn more. ***Do not overtighten!***

Step 4 Follow step 7 from the Cleaning Check-valves section.

Rebuilding Check-valves

Step 1. Remove the check-valve cartridge from its housing (Figure 5-4).

Step 2. Insert the smaller dowel pin provided (1/8" od x 1-1/4" long) into the hexagonal end of the valve and press out the internal components of the check-valve assembly using a steady pressure. Do not hammer parts through with the dowel pin or hammer on the dowel pin. Do not allow the valve parts to fall out onto a hard surface.

Step 3. Reassemble the check-valve by placing the valve insert in the valve housing using the larger dowel pin (3/16" od). Make sure that the valve insert is oriented correctly.

Step 4. Press a new Kel-F® seal into the valve housing.

Step 5. Slide the check-valve cartridge into the valve housing making certain the cartridge is oriented correctly.

Step 6. Press a second Kel-F seal into the valve housing. The Kel-F seal will extend approximately 0.02–0.03" from the valve housing.

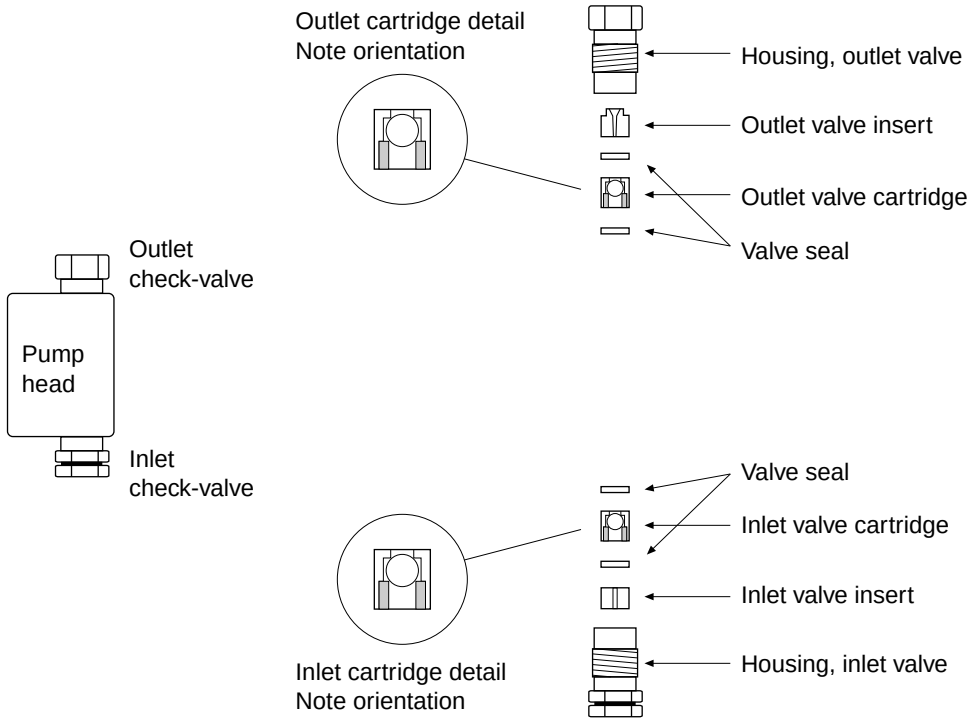
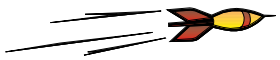


Figure 5-4. Check-valve assemblies

Pre-column Filter

The filter is located inside the fitting on the left side of the PCX5100 labelled "From Injector."



Step 1. Be sure that the PCX 5100 is shut down and ***depressurized*** before changing the filter.

Step 2. Disconnect the capillary tubing from the fitting. Use a 7/16" wrench to remove the end of the fitting. Remove the filter element.

Step 3. The correct filter element is 3102-9047, a 0.5µm porosity, 0.063" diameter frit. It is superficially similar to the reagent filter, so take care not to confuse them.

Step 4. ***Wet*** the new filter element with water, and replace the fitting. Be careful that the filter element does not fall out of the fitting while installing it. Tighten the fitting firmly.

Step 5. Reconnect the capillary tubing.

Reagent Filters

These filters are located directly above the pump heads inside the PCX5100 cabinet.

Step 1. Be sure that the PCX5100 is shut down and ***depressurized*** before changing a filter.

Step 2. Turn off the gas valve and open the vent valves on the reservoirs. Place the reservoirs at a level below the pump to prevent siphoning of the reagent.

Step 3. Disconnect the tubings from the top and bottom of the filter. Use two 7/16 inch wrenches to disassemble the filter. Remove the filter element.

Step 4. The correct filter element is 3102-9132, a 2µm, 0.188 inch diameter frit. It is superficially similar to the pre-column filter, so take care not to confuse them.

Step 5. Replace the filter element, and replace the fitting. Be careful that the element does not fall out of the fitting while installing it. Tighten the fitting firmly.

Step 6. Reconnect the tubings to the filter. The arrow on the side of the filter shows the direction of flow.

Ambient Reactor

The C_{18} phase can be stripped with repeated injections of low pH samples (water samples stabilized with chloroacetic acid or ChlorAC) and can accumulate in the ambient reactor. Part required: ambient reactor, Cat. No. 1100-2927.

Step 1. Shutdown the post-column system and let the reactor cool for at least 30 min.

Step 2. Remove both access panels.

Step 3. Disconnect two fingertight fittings (Figure 6-9)

I. Right side; back of "To Detector" fitting

II. Left side; lower left Tee

Step 4. Install new ambient reactor.

Note: Do not overtighten the fittings.

Step 5. Reinstall side access panels.

Fuse

The line fuse is on the back panel in the power inlet module between the cord connector and the power switch.

Step 1. Remove the cord from the power inlet.

Step 2. Use a small flat screwdriver to pry up the fuse holder then pull it out.

Step 3. Only use the correct type of fuse: for the 120V instrument, ***one each,*** GMA3 type 3A, 250V, 5 x 20mm, fast acting; for the 240V instrument, ***two each,*** IEC127 type, 1.6A, 250V, 5 x 20mm, fast acting.

Step 4. Reinstall the fuse holder and the power cord.

Troubleshooting Guide

Advice

Rules of Dolan and Snyder [see references]

- Rule of One: Make one change at a time.
- Rule of Two: Confirm the problem before fixing it.
- Substitution Rule: Swap in a good part for a questionable one.
- Put it Back: If swapping does not fix it, put the original back in.
- Write it Down: Changes or modifications, incidents.
- Crystal Ball: Preventive maintenance saves more time in the long run.
- Buffer Rule: Remove buffers from LC when not in use.

General Procedure for Troubleshooting

- Examine the system front to back. Fix all leakages.
- Verify that all settings, eluants, reagents, valves, etc. are according to specifications.
- Have there been any changes in the system?
- Compare against reference conditions: standard sample, column, parameter log as appropriate.
- Gather information: observations, manuals, books, technical assistance.
- Test your conclusions about the nature of the problem.
- Start working.

Interpretation of Pressures

The ***most useful*** diagnostic tool is a pressure log. Note that it is important to record all four pressures under initial conditions. Each permutation indicates a specific problem.

Condition	Column	Post-Column	Reagent 1	Reagent 2
Normal	1200	250	1500	1500
Pre-column filter blocked	↑	—	—	—
Heated reactor obstructed	↑	↑	↑	—
Ambient reactor obstructed	↑	↑	↑	↑
Reagent 1 not pumping	—	↓	↓	—
Reagent 2 not pumping	—	↓	—	↓
Restrictor 1 blocked	—	—	↑	—
Restrictor 2 blocked	—	—	—	↑

Most Common Problems with Post-column

High post-column pressure—caused by
- obstruction of flow path by deposits
- over-tightened fittings pinching a Teflon tube closed
- obstruction of detector flowcell
- heat exchanger in detector is too restrictive
- defective back-pressure regulator

High background signal—caused by
- Contaminated eluant
- bacterial growth
- fingerprints
- water purifier needs service
- Contaminated reagent(s)
- defective chemicals

Reagent backflows into column—caused by
- Not following proper shutdown procedure
- Not shutting down and depressurizing post-column before working on the HPLC
- Leaking fittings between column and HPLC pump
- Defective reagent control valves

Air in reagent pump or flow conditioners—check for
- Reagent pressure is low
- Some peaks disappear or change relative intensity
- Noisy baseline with 2 second period
- Reagent pressure is low
- Pump takes too long to come up to pressure

Poor peak shape—caused by
- Column worn out
- Guard column dirty
- Bad column
- Deposits in post-column flow path
- Partial obstruction of flowcell
- Too strong a solvent or too large a sample injected
- Bad tubing connection: wrong style nut, too large tubing, wrong type union
- Reagent flow rate(s) too high
- Strange injector problems

Deposits in reactor—caused by
- Dissolved silica reprecipitating (carbamate column)
  - NaOH backflow into column
  - Corrosive samples
  - Backflushing a dirty column into the system
- Contaminated reagents
- Hard water samples
- Degradation of Teflon tubing
- Greasy samples
- Using calcium hypochlorite as the oxidant in glyphosate determination
- Preparing your own reagents with poor quality chemicals.

High column pressure—caused by
- Filter is clogged—replace the frit
- Guard column is clogged—replace it
- Worn HPLC pump seal or worn injector rotor seal
- Unfiltered samples
- Particulate matter in eluant reservoirs
- Post-column pressure is high
- Column is damaged—replace it
- Organic solvent in glyphosate column—wash column

Noisy baseline—check for
- Is there a pattern or rhythm in the noise?
- Match the frequency of the noise to one of the pumps. The Pickering pump has a 2 second period. Most HPLC pumps have a period of 5–30 seconds. The problem is related to the pump with the matching frequency.
- If the noise is random, check your detector.
- If the background signal is also elevated, check for chemical contamination, or an error in formulation.
- OPA reagent is too old or oxidized.

Reagent pump stops or delivers wrong flow rate
- Check pump setting
- Check reagent pressurization
- Lubricate pump (LDC Minipump only)
- Check pump seal for leakage
- Do not open the restrictor. It is supposed to be full of gray-green powder.

Peaks disappear or diminish, Carbamates

1 All disappear except 1-naphthol and carbaryl
- OPA reagent expired
- Error in preparing OPA reagent (no thiol, no OPA, wrong pH)
- Reagent 2 pump air-locked

2 All disappear except 1-naphthol
- Out of Hydrolysis Reagent
- Reagent 1 pump air-locked

3 Some peaks small or missing, others normal size
- Reactor at wrong temperature
- Mis-adjusted reagent pumps
- Error in preparing a reagent

4 All peaks diminish, caused by a dirty flowcell, autosampler, or deteriorated samples
- Test with a second fluorescent detector. If a second fluorescent detector is not available, use an UV-Vis detector set at 330nm absorbance.
- Change the rotor seal of the autosampler or use a manual injector.
- Prepare fresh standards from neat reference material. Solution standards , even stored in ampoules, are not reliable (especially when dissolved in acetonitrile!)

Peaks disappear or diminish, Glyphosate

1 Iron contamination of column from samples, long storage of the column, stainless steel inlet frits in the eluant reservoirs, or corrosion in system

- Flush guard and column with the Glyphosate Restore solution.
- Remove stainless steel frits from the eluant reservoirs.
- Clean or replace any corroded parts.

2 Glyphosate peak too small or gone, but AMPA present

- Oxidizing reagent too weak, too old, NaOCl stock solution too old
- Reactor at wrong temperature

3 Oxidizing reagent too strong (AMPA vanishes)

4 Reagent pump mis-adjusted

5 Using CB910 instead of GA104 for OPA Diluent

What to do if…

Reactors or mixing tees have deposits

- Mineral deposits from hard-water samples or reagents can usually be dissolved by pumping 20% nitric acid through the reactor. The Pickering pumps and most (but not all) HPLC pumps will tolerate this. Columns and autosamplers probably will not tolerate this.
  a. Start HPLC pump at < 0.5 mL/min (H_2O for carbamates column; K200 for glyphosate column).
  b. Replace both post-column reagents with deionized water. Run post-column pumps for 5–10 min.
  c. Stop post-column pumps. Replace deionized water with 20% nitric acid and run post-column pumps for 10–15 min.
  d. Reverse the order of washing with water and then replace with the post-column reagents.

Note: The washing solution can be stored in Erlenmeyer flasks or spare bottles. Pressurizing the washing solution is not necessary.

- Grease deposits can be dissolved by turning off the reagents and pumping methanol through the system (Carbamate column only). Stronger solvents such as acetone, methylene chloride, or tetrahydrofuran (THF) may be needed.
- Silica deposits are too hard to remove. Replace the reactor(s). Carefully clean or replace other components in the flow path. You must remove all the silica before the system will work again. This will probably entail major repair.

NaOH backflows onto a carbamate column

- ***Do not restart the system.*** Dissolved silica or C_{18} phase will reprecipitate in the post-column reactors, or flowcell. Most often, these additional complications require replacement of both reactor coils.
- Replace the column.
- Call Pickering Laboratories for help.

Organic solvent in a glyphosate column

- This procedure usually works but may not work every time.
    a. Shut down the PCX 5100 and remove the analytical and guard columns.
    b. Flush out all organic solvents from the LC and injector.
    c. Backflush both columns with K200 glyphosate eluant. Use a very slow flow rate so that the back pressure does not exceed 2000 psi.
    d. Keep flushing until the pressure drops. Keep raising the flow rate until the pressure is normal at 0.40 mL/min and 55˚C.
    e. Reinstall the analytical (in reversed-direction) and guard column and test them.

Glyphosate peak is a doublet.

- Add 2–4 µL Glyphosate RESTORE (Cat. No. 1700-0140) to the sample before injections.

Glyphosate and AMPA peaks are late and broad due to contamination

- Usually only the guard column is contaminated. We suggest you buy a spare guard column to minimize down-time.
- Wash guard in reversed-direction with Glyphosate RESTORE.
    a. Remove the analytical column after ensuring no residual post-column pressure.
    b. Reverse the guard column and pump RESTORE through the guard at 0.4 mL/min for a minimum of 15 min, directing the effluent to waste.
    c. Pump K200 eluant through the guard long enough to displace RESTORE.
    d. Reconnect the column and guard in the normal directions and restart the HPLC and post-column systems.

When Priming the Reagent Pump is Difficult
Sometimes the reagent pump may be very difficult to prime. This can happen after a pump has been shipped, serviced, stored for a long time, or after putting a new bottle of reagent on. Almost always this is due to a trapped bubble inside the pump. Ordinary priming will not always remove the bubble, especially if it is caught inside the piston seal or inside one of the gauges. There are two ways to overcome this.

The first and simplest way is to first prime the pump with a low-surface tension liquid, then change to the normal reagent. Degassed methanol will work well. Simply use the priming syringe to draw about 5 mL of methanol through the pump, then draw 10 mL of reagent through the pump. If there is air in a gauge, the pump may take several minutes to come up to final pressure.

The second and more thorough method is vacuum priming. You will need a liquid shut-off valve (Pickering 3104-0050 or Omnifit 1101 or equivalent) to perform this. Newer PCX5100 (April 94) has built-in liquid shut-off valves (Start with Step 2).

a. Disconnect the reagent reservoir, and install the liquid shut-off valve in the reagent supply tubing.

b. This can be performed with the pump on or off, it makes little difference.

c. Connect the 20 mL priming syringe to the bypass. Open the Bypass valve. Draw liquid until no bubbles come through.

d. Close the liquid shut-off valve. Empty the syringe, and reconnect it to the bypass.

e. Pull a vacuum with the syringe. Hold the vacuum until no more bubbles come out. This causes the trapped bubbles to expand.

f. While still holding a vacuum, open the liquid shut-off valve. This sweeps the expanded bubbles out.

g. Wait until about 5 mL of liquid has collected, then close the bypass valve.

   Optional: before closing the bypass valve, use the syringe to apply pressure until the pressure gauge moves. Close the valve while holding pressure on the system.

h. If the pump is not on, turn it on.

i. The pressure should come up within a few seconds. If it does not begin pulsing within 30 sec., repeat steps 4–8. If you can not prime the pump after 2 or 3 applications of vacuum, then there is some other problem. Check for leaking piston seals, dirty check valves, loose fittings, or other defects.

Cleaning the Post-column System

Always wear safety glasses or goggles, laboratory coat, gloves, and other appropriate safety-clothing. Please read and understand the instructions in the MSDS shipped with the chemicals. If the MSDS are missing, please contact Pickering Laboratories and we can fax you a copy instantly.

Fittings

Clean any leaks from fittings thoroughly with water and dry with paper towels, especially if the solution is a buffer or hydroxide. Standing salt and hydroxide solution is corrosive.

External

Soak up spills with rags, paper towels, or sponge. Clean spill-area with a wet towel and dry thoroughly. Do not spray water directly into the instrument.

Vacuum clean the post-column system once a year, especially the foam filter in the front underside. Alternatively, the foam filter can be removed, cleaned with soap and water, air-dried, and replaced.

Internal

The side access panel can be removed by removing two captive screws along the bottom edge. There is an advantage and also a disadvantage to leaving the side panels off. With the side panels off, you can spot any leakages quickly but it is more dusty because the air flow no longer travels through the foam filter. If the side panels were left off, vacuum clean the inside as well as outside.

Soak up spills and leaks with rags, paper towels, or sponge. Clean spill-area with a wet towel or water from a squeeze bottle and dry thoroughly. Do not spray water directly into the electrical part of the post-column pump.

Chapter 6
Major Repairs

Major repairs are usually made on an exchange basis. Contact Pickering Laboratories to get a Returned Goods Authorization number before returning any components. Write this RGA number prominently on the package and packing slip.

Generally, it is best to return the whole post-column system (except the reagent reservoirs) for repair or recertification. This chapter covers disassembly of the post-column system. Pickering Laboratories recommends disassembly of the post-column system ***only as a last resort for repair.*** Generally, chemical problems can be solved with chemistry. Please consult Pickering Laboratories before disassembly.

Warning. Disassembly of the post-column system should be carried out by qualified service personnel only. Ensure that the power cord is disconnected before disassembly of the post-column system. Operation of the instrument with the top cover off will expose hazardous live voltages.

Le démontage du système poste-colonne ne doit être effectué que par du personnel qualifié. S'assurer que le câble électrique est débranché avant de démonter le système. Danger d'électrocution en cas d'utilisation du système sans couvercle.

Warnung! Reparaturen, die nur mit Eingriffen in das System durchgeführt werden können, sollten ausschließlich durch ausgebildete Servicetechniker durchgeführt werden. Stellen Sie sicher, daß vor dem Offnen des Gehäuses das Gerät vom Netz getrennt wird. Die Inbetriebnahme des Gerätes mit offenem Gehäuse kann lebensgefährlich sein.

Atención. El desmontaje del sistema post-columna sólo puede ser efectuado por personal cualificado. Asegúrese que el cable de alimentación esté desconectado antes de desmontar el sistema post-columna. La operación del instrumento sin la cubierta superior expondrá a voltajes activos peligrosos.

Avvertimento. Disunire il sistema della colonna dovrebbe essere fatto solamente da personale technico. Deve essere sicuro che questa macchina sia spenta e il voltaggio staccato prima di disunire la colonna e altri pezzi. Usare questa macchina senza essere completamente chiusa é pericoloso perché il voltaggio é in posizione che facilmente viene in contatto con il technico.

Removal of the column oven will require removal of the outer case.

Warning. The column heating block may become hotter than 70°C. For your safety, wear insulating gloves when the column oven is warm.

Attention. La résistance chauffante de la colonne peut dépasser une température de 70°C. Pour votre sécurité, prière de porter des gants isolants lorsque le four de la colonne est chaud.

Warnung! Der Heizblock des Säulenofens könnte heißer als 70°C werden. Für Ihre Sicherheit sollten Sie isolierende Handschuhe tragen, wenn der Säulenofen warm ist.

Atención. El bloque calefactor de columnas puede estar por encima de 70°C. Para su seguridad use guantes aislantes cuando el horno de columnas esté caliente.

Avvertimento. Questo blocco che scalda la colonna puo diventare piu caldo di 70°C. Per la sua protezione usa guanti insulanti quanto il blocco e la colonna sono caldi.

Step 1. Shut down the PCX5100 completely and let the reactor cool. Disconnect the power cord. Remove both access panels.
Step 2. Remove the five screws holding the top cover. Lift the cover off.
Step 3. Disconnect the liquid connections to the part.
Step 4. Disconnect the electrical cable.
Step 5. Remove the screws securing the part to the chassis. Remove the part.
Step 6. Install the new part. Reassemble the PCX5100 in reversed-order of disassembly.

Diassembly of Front Panel

Note: Pickering Laboratories recommends disassembly of the post-column system only as a last resort for repair. Generally, chemical problems can be solved with chemistry. Please consult Pickering Laboratories before disassembly. Tools needed: 1/4" open-end wrench, 5/16" nut-driver, and a Phillips screw driver. Tools are not included.

Step 1. Shutdown the post-column system completely and let the reactor cool for at least 30 min. Disconnect the power cord.
Step 2. Remove both access panels.
Step 3. Remove the five screws holding the top cover (two on each side and one on back). Lift the cover off.
Step 4. Disconnect both inlets and outlets to the post-column pumps.
Step 5. Disconnect five electrical connections at the printed-circuit board (Fig. 6-1): from two temperature controllers, pump on/off switch, ready LED lights, and the pressure sensing switch. Note the orientation of the connectors to facilitate reassembly.
Step 6. Detach the two fittings on the pressure regulator (Figure 6-2).
Step 7. Remove two bolts with an 1/4" open-end wrench, one on each side at the bottom (Figures 6-3 and 6-4) of post-column system.
Step 8. Remove two nuts (Figures 6-3 and 6-4) on inside panel with a nut-driver.
Note: Do not remove the two support bars (Figure 6-3).
Step 9. The front panel can now be separated from the rest of the post-column system.
Note: The inlet and outlet tubings for the HPLC column can be bent slightly to facilitate this final step.

Reassembly of Front Panel

Step 1. Fit the front panel into the post-column system; make sure the two threaded pins are aligned.
Note: Avoid crimping the electrical cable from the pressure sensing switch.
Step 2. Return the inlet and outlet tubings for the HPLC column through the opening at the front of the column oven.
Step 3. Partially tighten the nuts with the nut-driver.
Step 4. Tighten the two bolts at the bottom of post-column system, then fully tighten the nuts from step 3.
Step 5. Attach the fittings to the pressure regulator (Figure 6-2).
Step 6. Reconnect the five electrical cables from the pressure sensing switch, two temperature controllers, reagent pump on/off switch, and LED lights.
Note: The electrical cables from the left and right temperature controllers should be connected to their respective sides in the printed-circuit board (Figure 6-1).
Step 7. Connect inlets and outlets to the post-column pumps.
Step 8. Install side access panels and top cover.
Note: Installing access panels first will assure a better fit of the top cover.

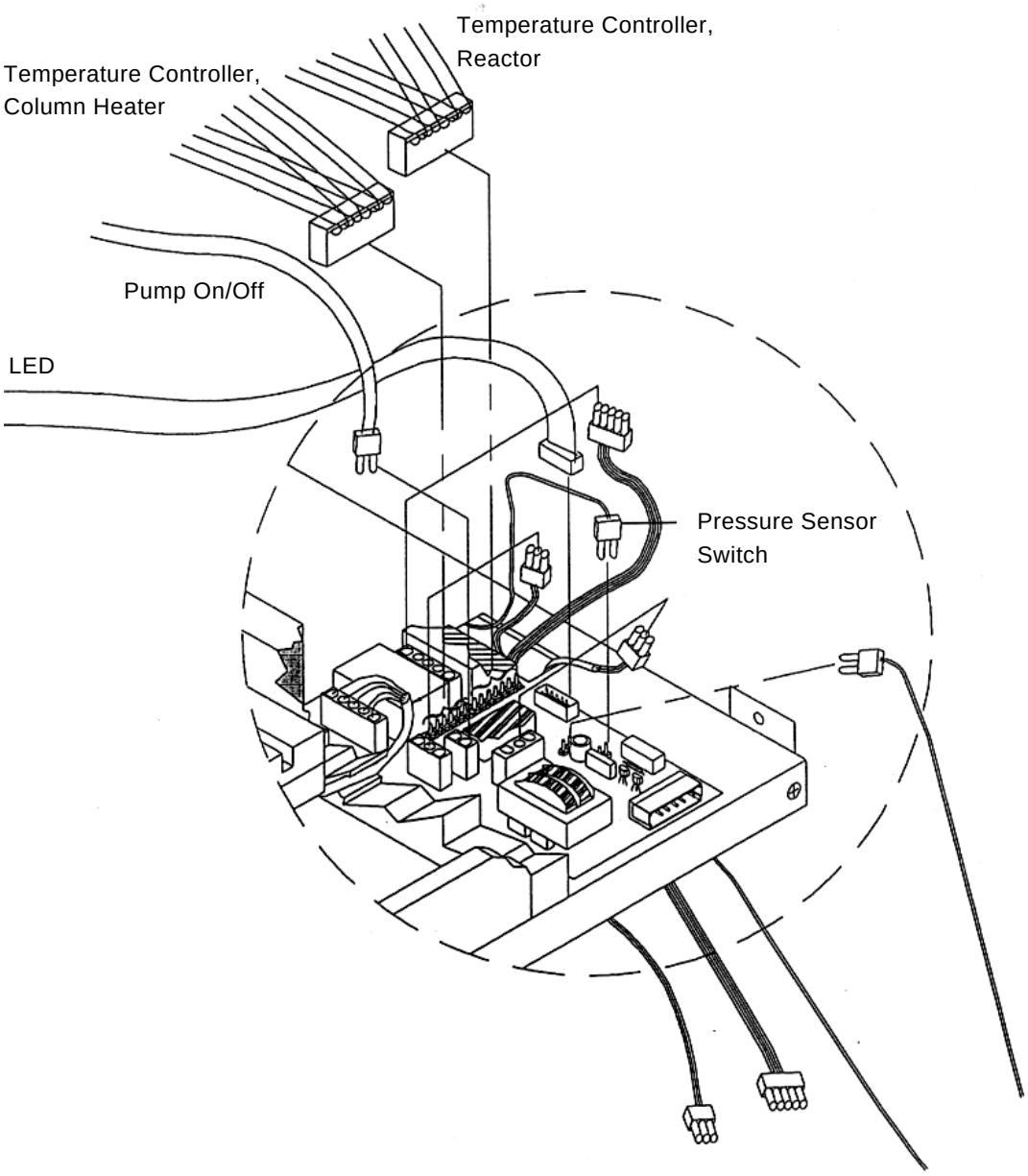


Figure 6-1. Electrical connections at the printed circuit board

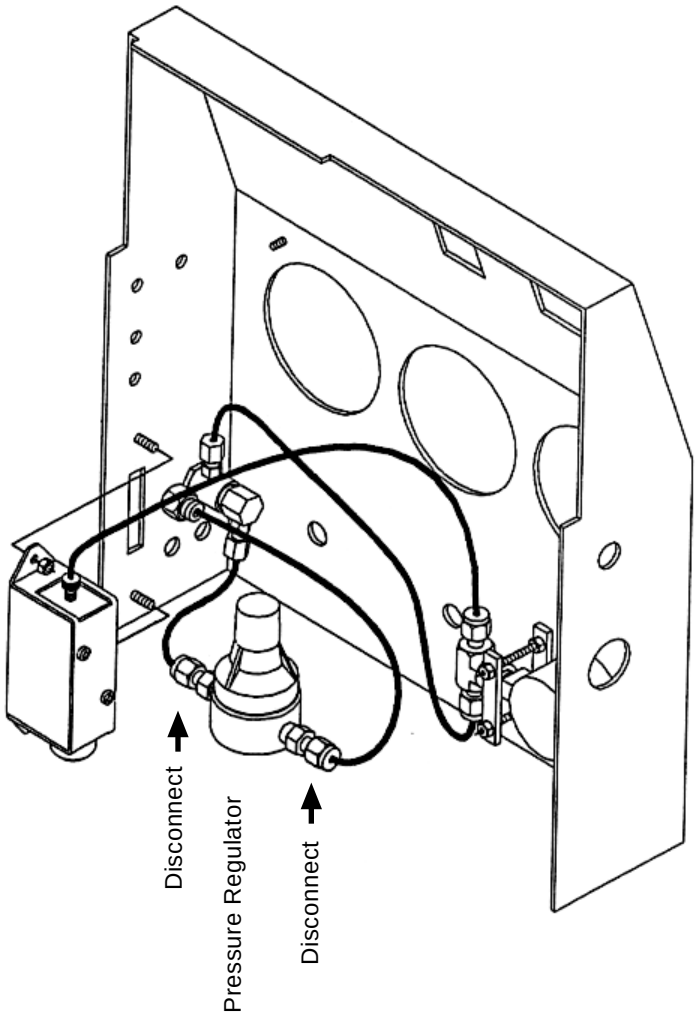


Figure 6-2. Gas Pressure System

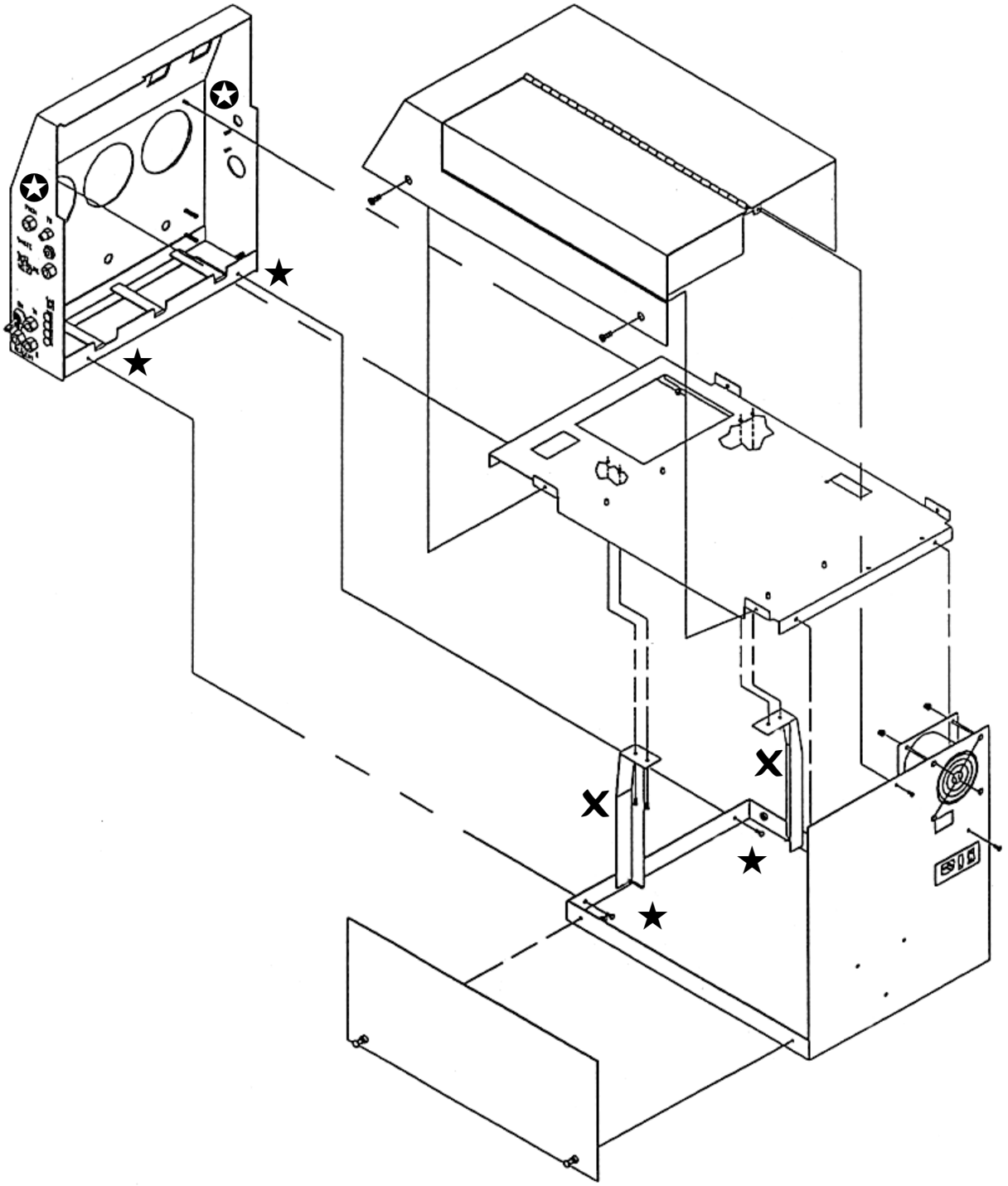


Figure 6-3. Bolts in step 7 for diassembly of front panel are marked with ★. Nuts in step 8 are marked with ✪. Do not remove the support bars marked with ✖.

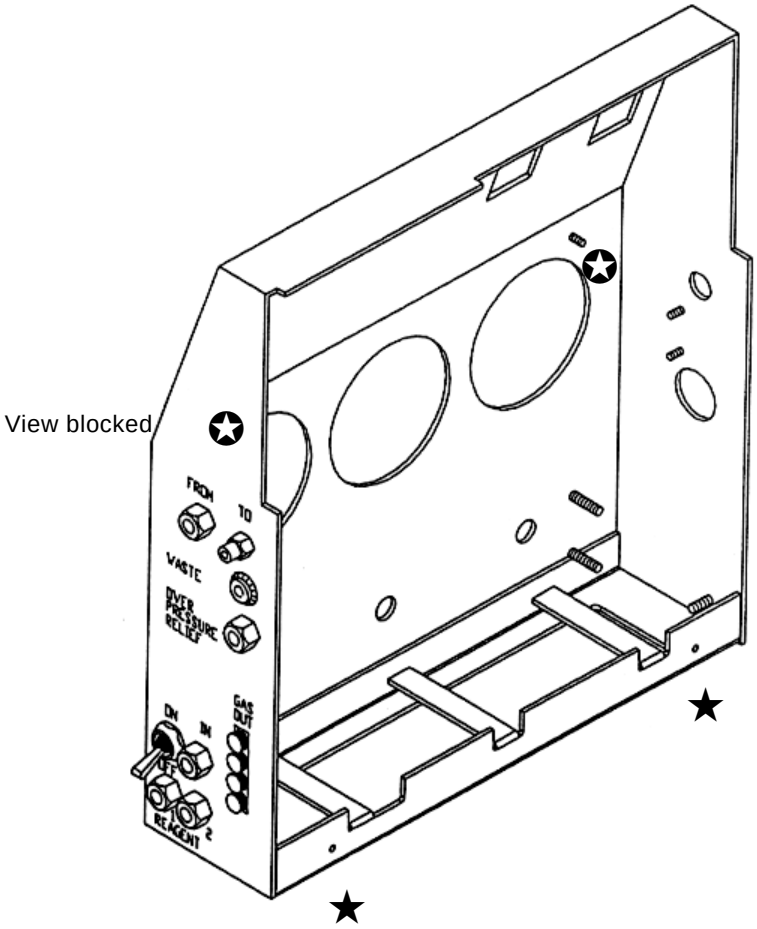


Figure 6-4. Close-up of front panel. Bolts in step 7 for diassembly of front panel are marked with ★. Nuts in step 8 are marked with ✪.

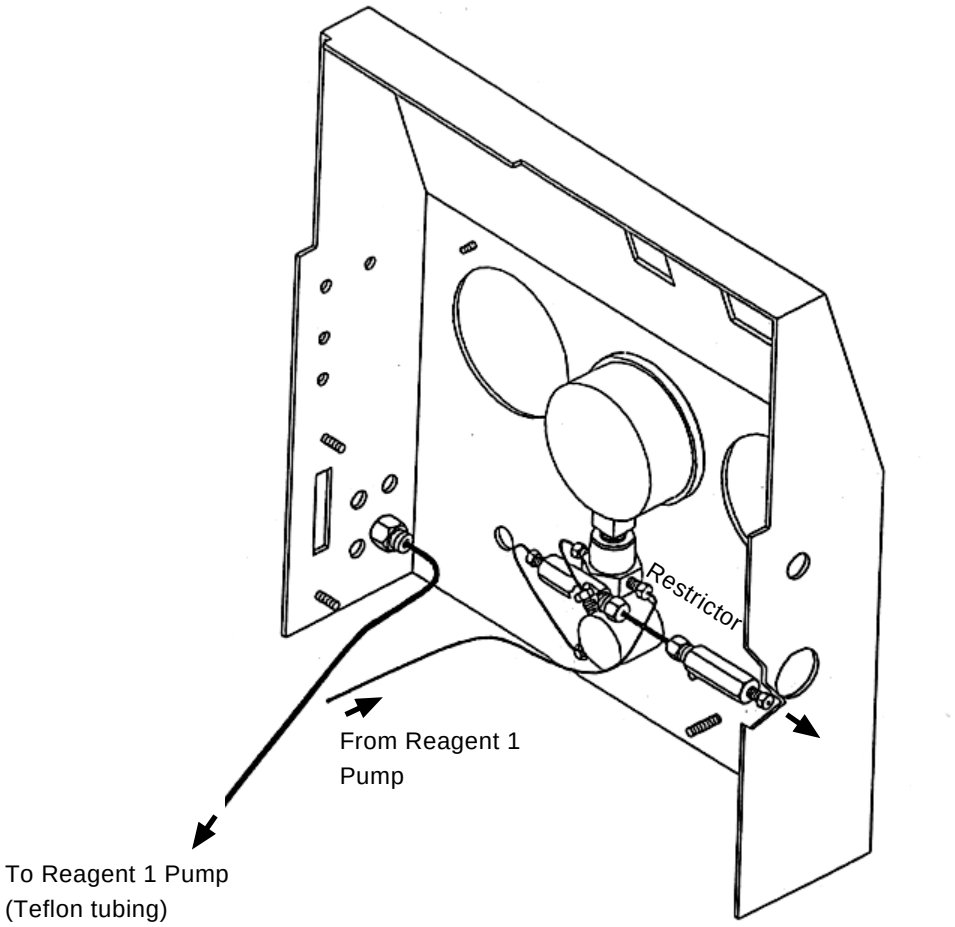
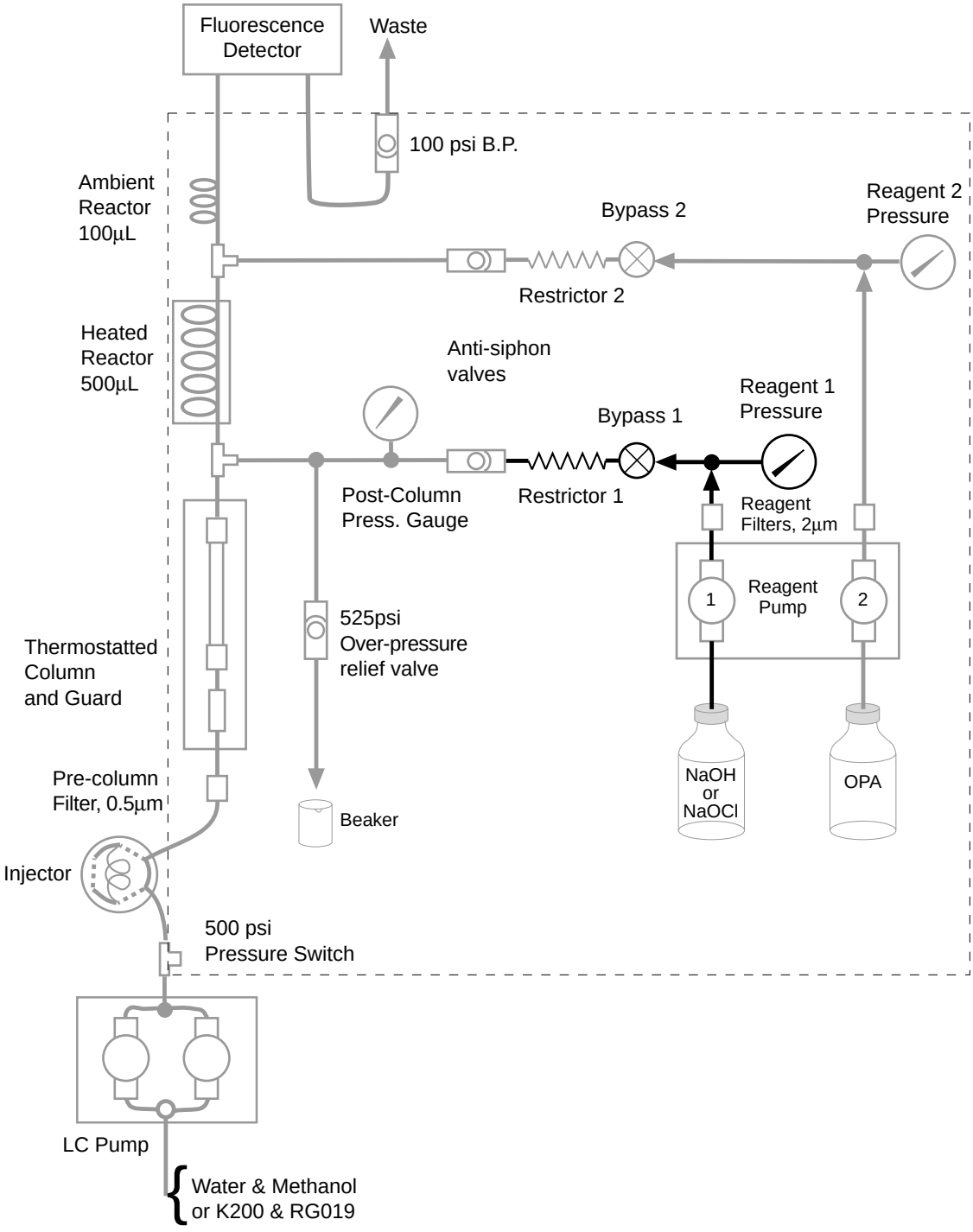


Figure 6-5. Post-column reagent 1 flow path up to Restrictor 1; includes pressure gauge 1, and prime/purge valve 1


Fluorescence Detector
Waste
100 psi B.P.
Ambient Reactor 100µL
Bypass 2
Reagent 2 Pressure
Restrictor 2
Heated Reactor 500µL
Anti-siphon valves
Reagent 1 Pressure
Bypass 1
Post-Column Press. Gauge
Restrictor 1
Reagent Filters, 2µm
1
Reagent Pump
2
Thermostatted Column and Guard
525psi Over-pressure relief valve
NaOH or NaOCl
OPA
Pre-column Filter, 0.5µm
Beaker
Injector
500 psi Pressure Switch
LC Pump
Water & Methanol or K200 & RG019

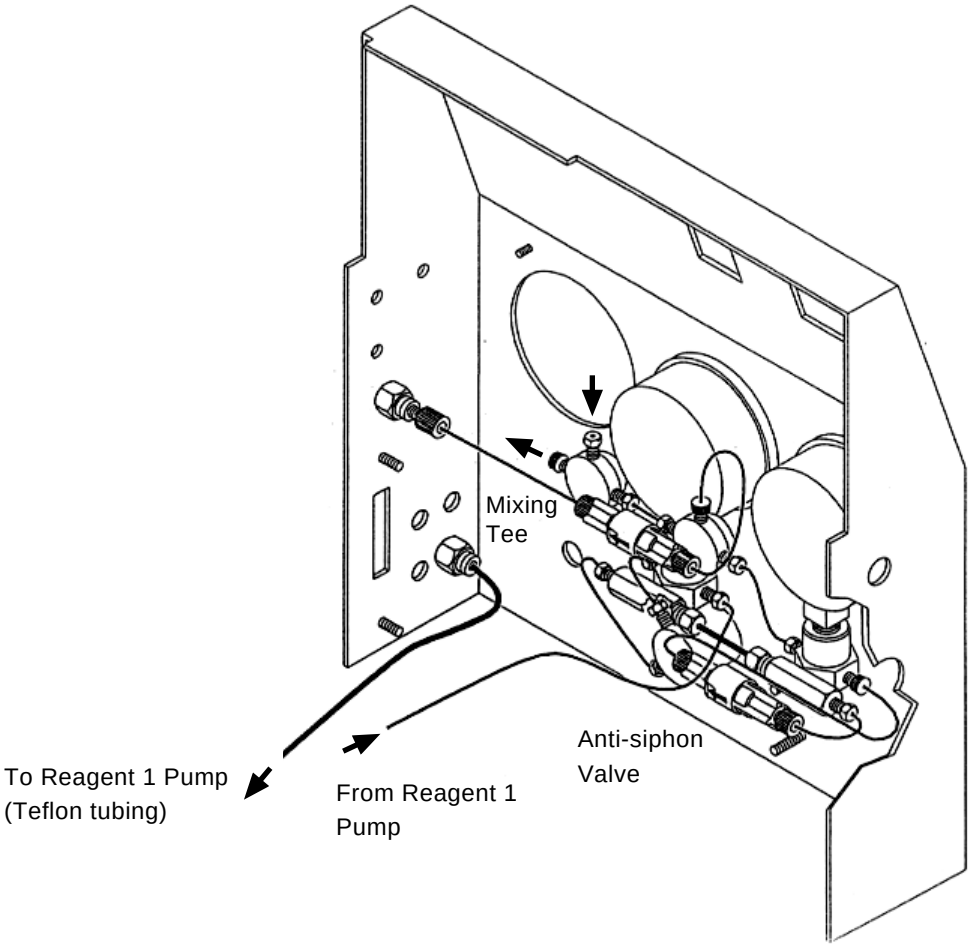
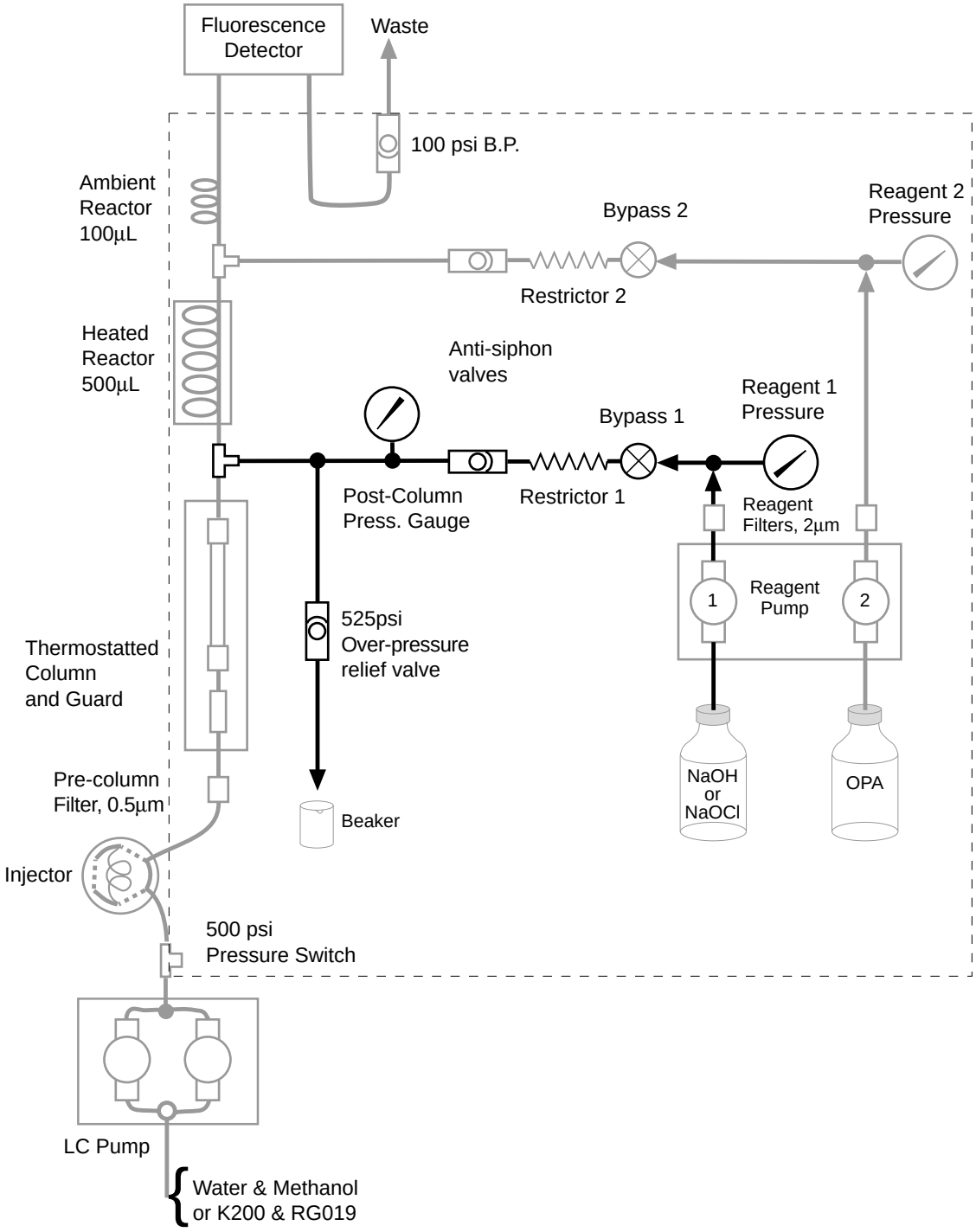


Figure 6-6. Post-column reagent 1 flow path up to the first mixing Tee; includes post-column pressure gauge, anti-siphon valve, and the 525 psi over-pressure relief valve


Fluorescence Detector
Waste
100 psi B.P.
Ambient Reactor 100µL
Bypass 2
Reagent 2 Pressure
Restrictor 2
Heated Reactor 500µL
Anti-siphon valves
Reagent 1 Pressure
Bypass 1
Post-Column Press. Gauge
Restrictor 1
Reagent Filters, 2µm
1
Reagent Pump
2
525psi Over-pressure relief valve
Thermostatted Column and Guard
NaOH or NaOCl
OPA
Pre-column Filter, 0.5µm
Beaker
Injector
500 psi Pressure Switch
LC Pump
Water & Methanol or K200 & RG019

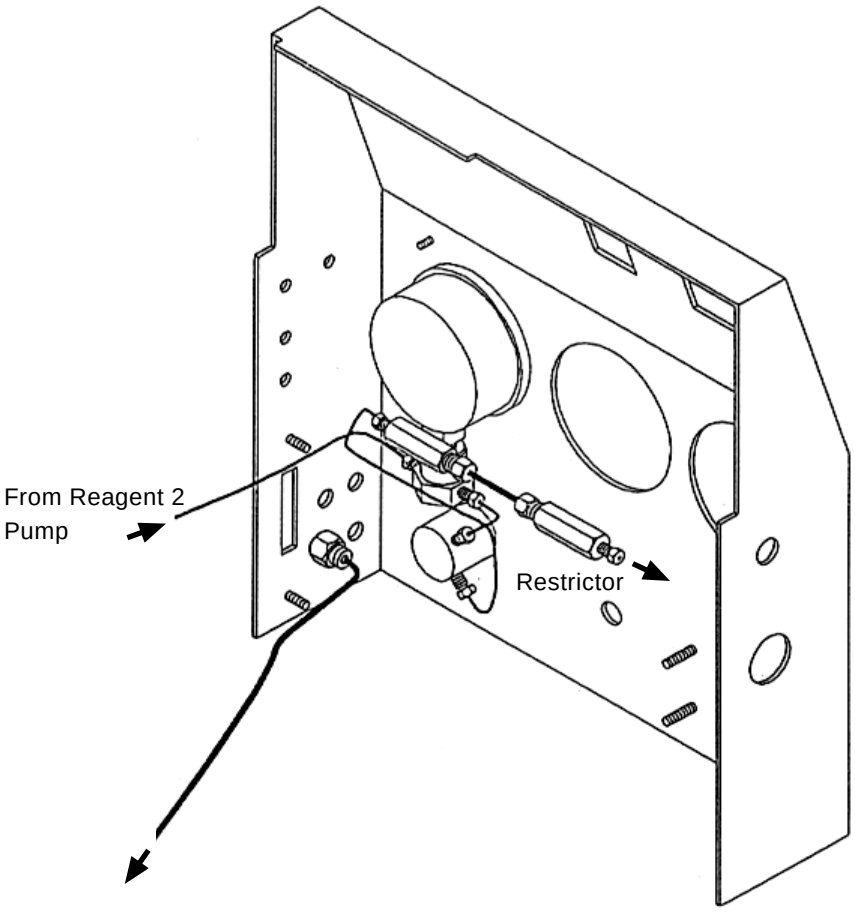
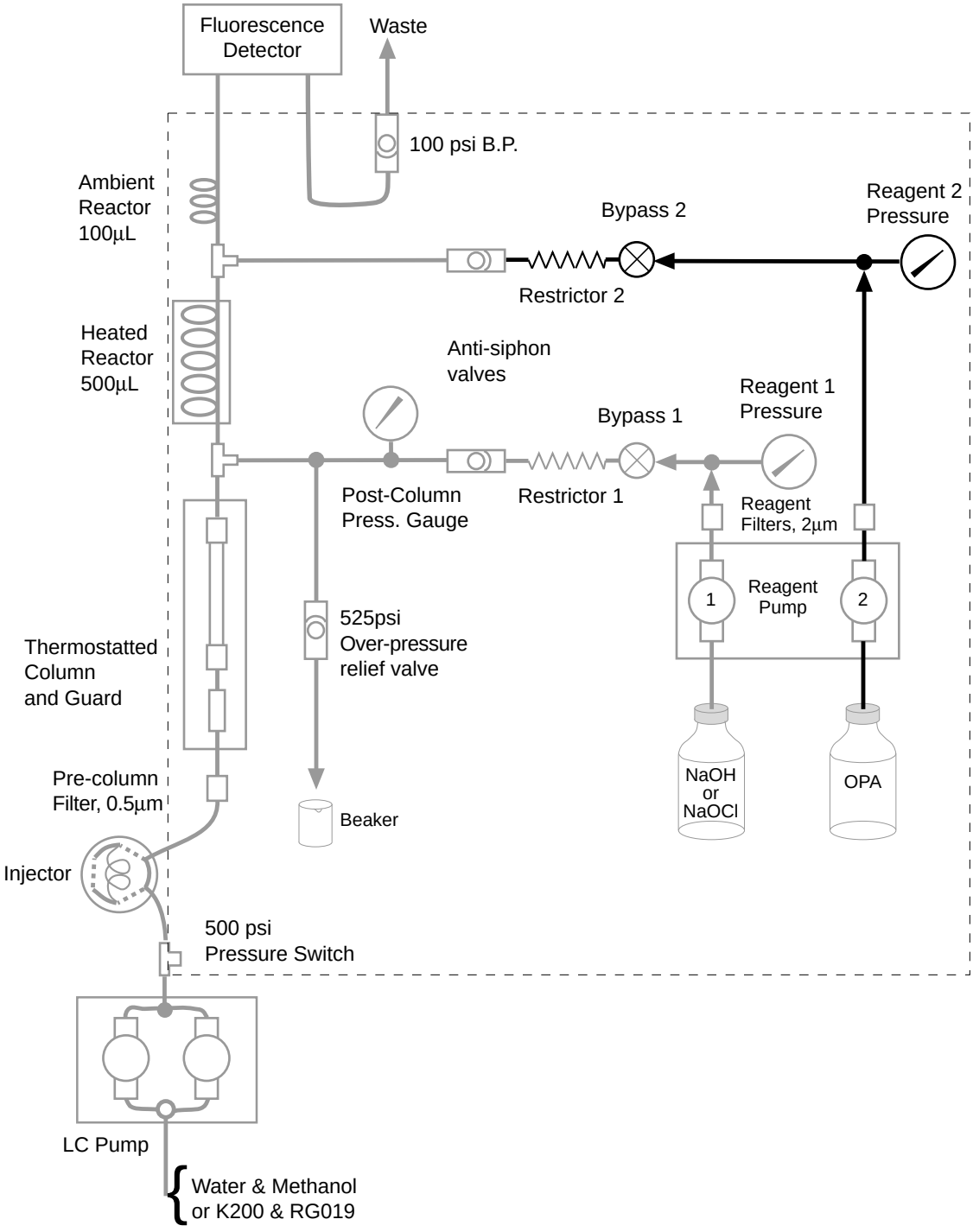


Figure 6-7. Post-column reagent 2 flow path up to Restrictor 2; includes pressure gauge 2, and prime / purge valve 2


Fluorescence Detector
Waste
100 psi B.P.
Ambient Reactor 100µL
Bypass 2
Reagent 2 Pressure
Restrictor 2
Heated Reactor 500µL
Anti-siphon valves
Reagent 1 Pressure
Bypass 1
Post-Column Press. Gauge
Restrictor 1
Reagent Filters, 2µm
1
Reagent Pump
2
525psi Over-pressure relief valve
Thermostatted Column and Guard
NaOH or NaOCl
OPA
Pre-column Filter, 0.5µm
Beaker
Injector
500 psi Pressure Switch
LC Pump
Water & Methanol or K200 & RG019

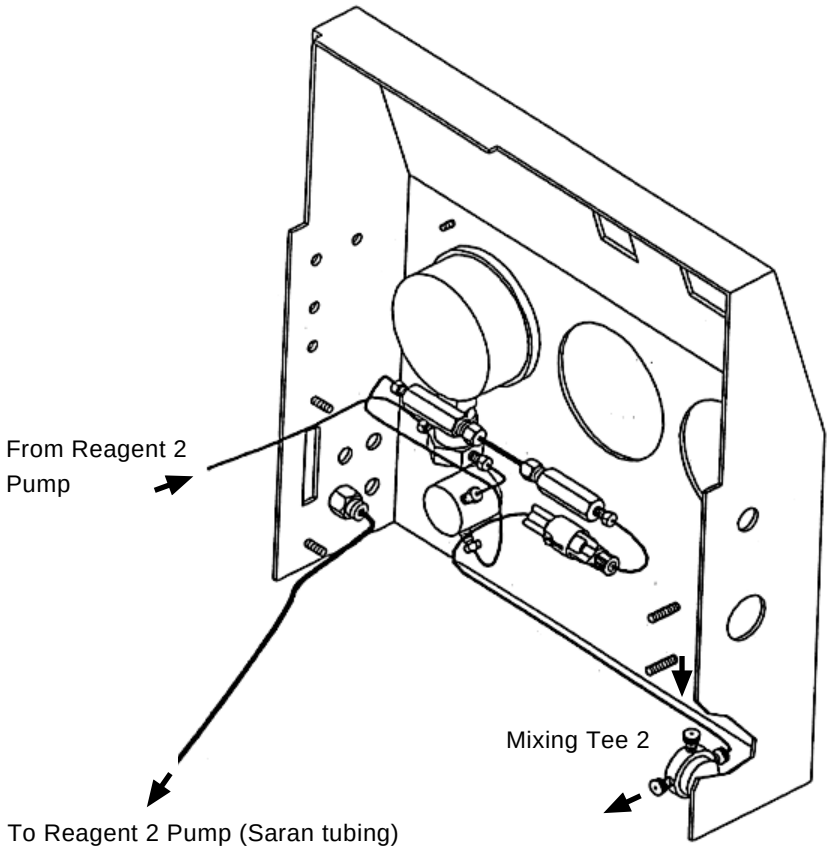
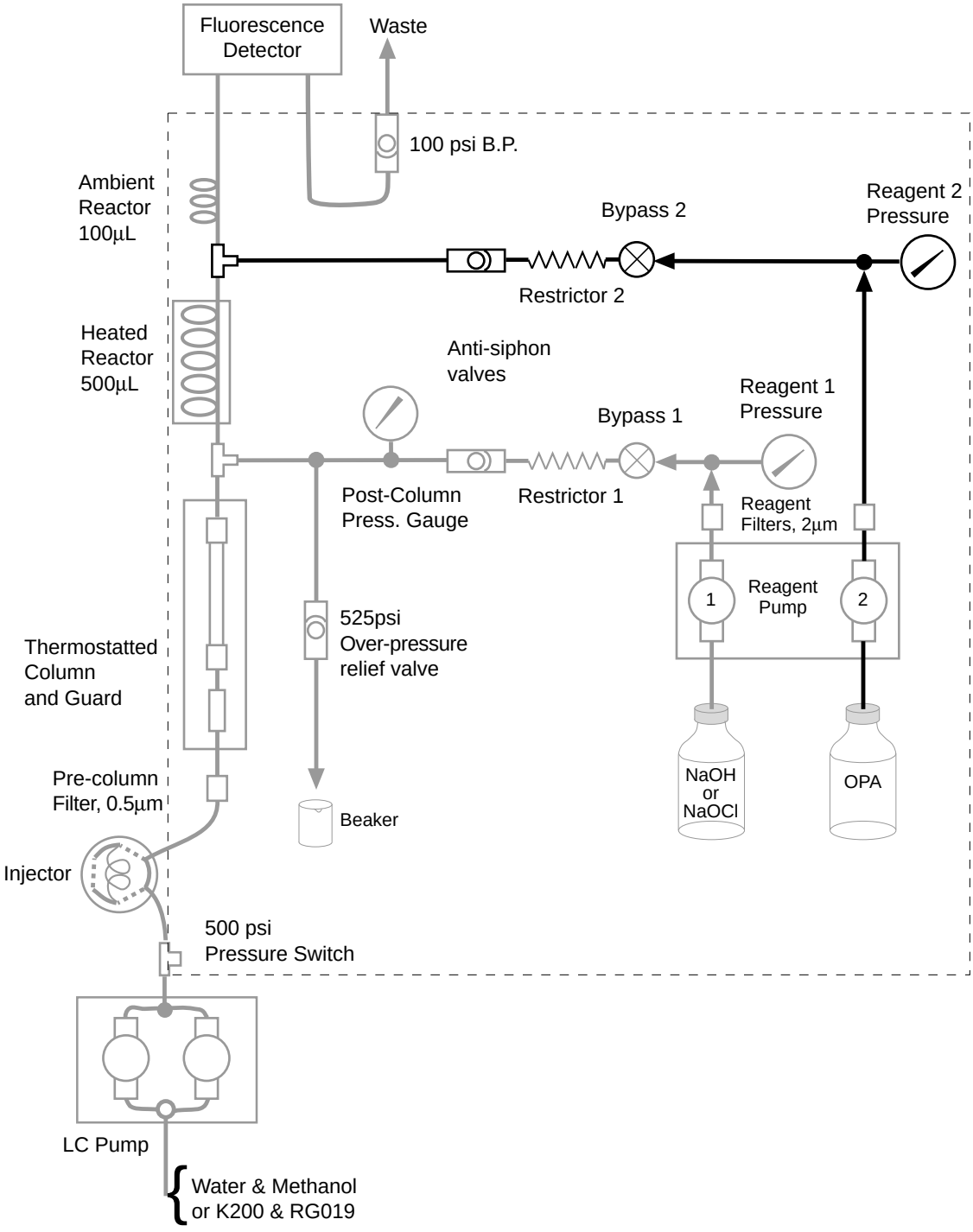


Figure 6-8. Post-column reagent 2 flow path up to the second mixing Tee; includes anti-siphon valve


Fluorescence Detector
Waste
100 psi B.P.
Ambient Reactor 100µL
Bypass 2
Reagent 2 Pressure
Restrictor 2
Heated Reactor 500µL
Anti-siphon valves
Reagent 1 Pressure
Bypass 1
Post-Column Press. Gauge
Restrictor 1
Reagent Filters, 2µm
1
Reagent Pump
2
525psi Over-pressure relief valve
Thermostatted Column and Guard
NaOH or NaOCl
OPA
Pre-column Filter, 0.5µm
Beaker
Injector
500 psi Pressure Switch
LC Pump
Water & Methanol or K200 & RG019

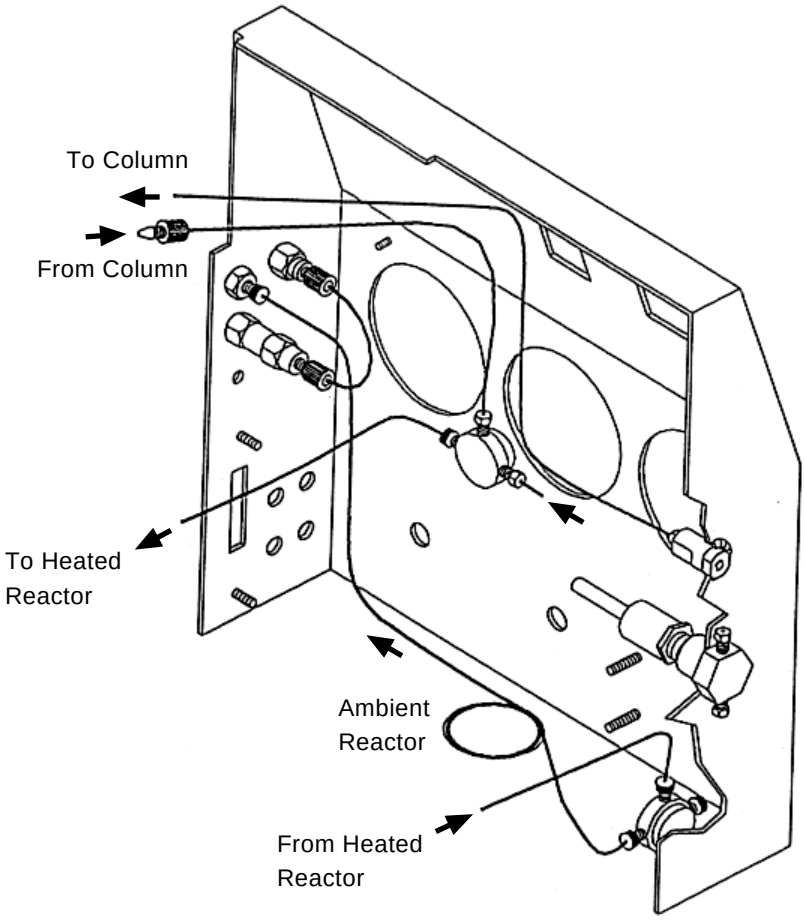
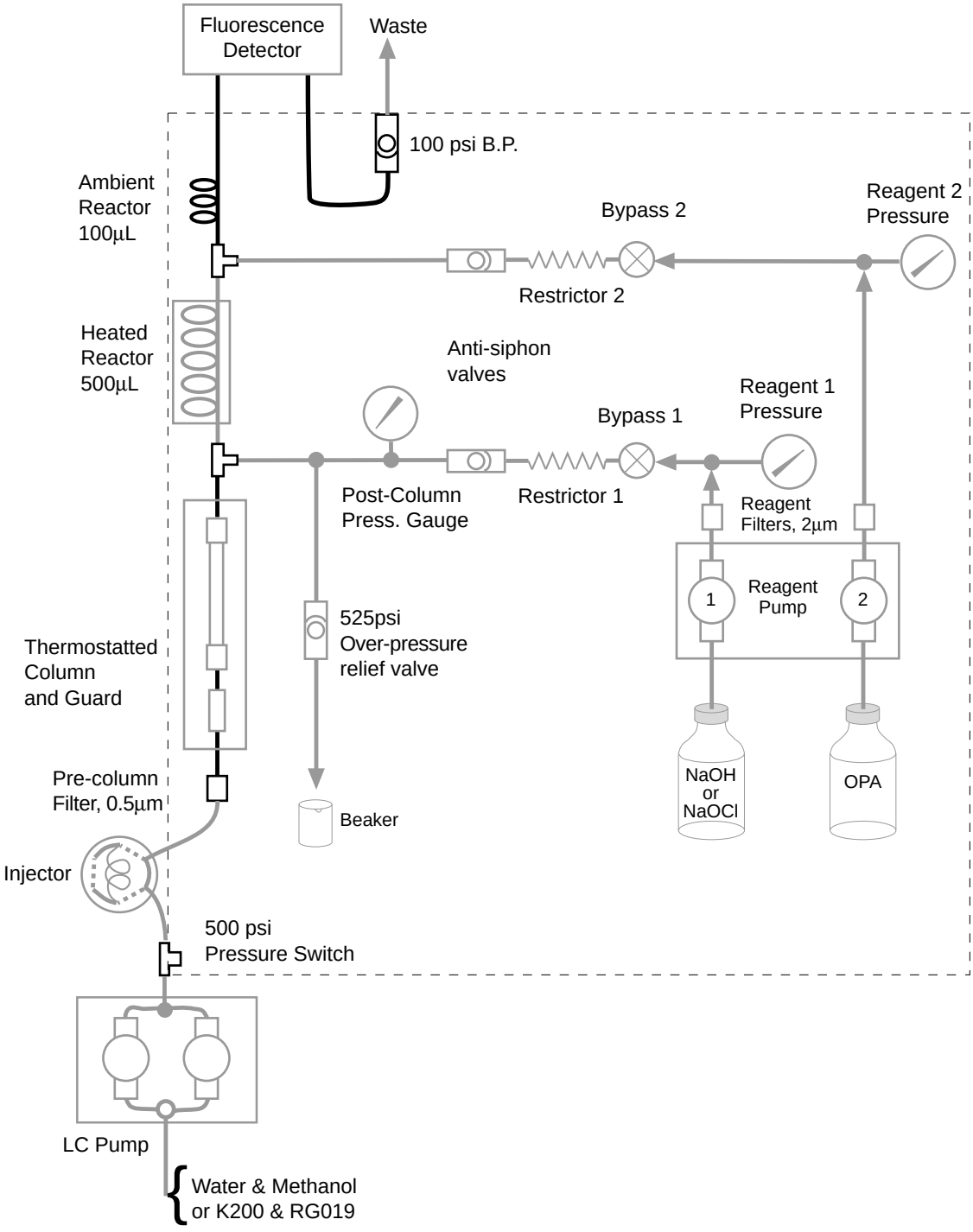
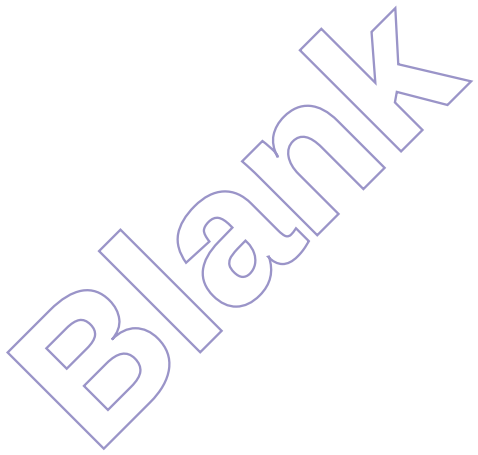


Figure 6-9. HPLC column and post-column flow path; includes pressure sensing switch, inlet filter, first mixing Tee, inlet and outlet tubings for the heated reactor, second mixing Tee, ambient reactor, outlet from post-column to detector, and built-in 100 psi back-pressure regulator


Fluorescence Detector
Waste
100 psi B.P.
Ambient Reactor 100µL
Bypass 2
Reagent 2 Pressure
Restrictor 2
Heated Reactor 500µL
Anti-siphon valves
Reagent 1 Pressure
Bypass 1
Post-Column Press. Gauge
Restrictor 1
Reagent Filters, 2µm
1
Reagent Pump
2
525psi Over-pressure relief valve
Thermostatted Column and Guard
NaOH or NaOCl
OPA
Pre-column Filter, 0.5µm
Beaker
Injector
500 psi Pressure Switch
LC Pump
Water & Methanol or K200 & RG019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Appendix A

PCX 5100 Parameter Log

Reagent Pump Calibration

Pump 1

Micrometer	Flow Rate	Medium

Pump 2

Micrometer	Flow Rate	Medium

Normal Pressures and Flows at Initial Conditions

	Flow Rate	Pressure
Analytical Pump		
Reagent Pump 1		
Reagent Pump 2		
Post-Column		

	Temperature
Analytical Column	
Heated Reactor	
Room	

Tested by: ..... Date: .....

Analytical Conditions

LC Program: .....

Initial Composition: ..... Initial Flow Rate: .....

Gradient Program:

Column: .....

Type: ..... ID: ..... Temperature: .....

Injector/Autosampler: .....

Sample: ..... Volume: ..... Solvent: .....

Manual/Auto: ..... Other Parameters: .....

Detector: .....

Excitation: ..... Band: ..... Emission: ..... Band: .....

Range: ..... PMT: ..... Lamp: ..... Resp. time: .....

Integrator or Data Station: .....

Range or Scale: ..... Units: ..... Attenuation: .....

Chart Speed: ..... Peak Threshold: ..... Width: .....

Run Time: .....

Operator: ..... Date: ..... Reference: .....

Post-Column Band-Spreading Test

Test Conditions

LC

Eluent: 60 / 40; methanol / water
Flow rate: 1.0 mL/min
Sample: Pickering carbamate test mixture, Cat. No. 1700-0063, 10 µL
Column: Type ..... ID .....
Temperature: 42°C
Detector: Excitation ..... Emission ..... Lamp ..... PMT gain ..... Range/Attn .....
Integrator: Range / Attn ..... Units ..... Multiplier ..... Chart speed .....
Method for calculating peak width:

Part 1: Column connected directly to the detector
Post-column pumps off, reactor temperature ≤ 55°C

Run #	Retention time	Peak width
Average		$=W_d$

Part 2: Post-column reactors connected to column and detector
Post-column pumps off, normal reactor temperature .....

Run #	Retention time	Peak width
Average		$=W_{pc}$

Ratio: W_d / W_{pc} = ...

Operator: ..... Date: .....

Post-Column Delay

The post-column delay volume is about 600 µL. The delay time can be calculated as:

$$t = [0.5/(f_e + f_{R1})] + [0.1/(f_e + f_{R1} + f_{R2})]$$

where f_e is eluent flow rate, f_{R1} is first reagent flow rate, and f_{R2} is second reagent flow rate; 0.5 is the volume of the first reactor and 0.1 is the volume of the second.

Appendix B

Recommended Consumables and Spare Parts

For routine maintenance and minimal interruptions to your operation, always keep the necessary consumables and spare parts available.

Carbamate Reagents

Part Number	Description
O120	*o*-Phthalaldehyde, Chromatographic Grade crystals, 5 g
3700-2000	Thiofluor, Chromatographic Grade crystals, 10 g
CB910	OPA Diluent for Carbamate Pesticide Analysis, 4 x 950 mL
CB130	Hydrolysis Reagent for Carbamate Pesticide Analysis, 4 x 950 mL
1700-0063	Carbamate Test Mixture, qualitative sample, 12 components, 1.5 mL, 2.5 µg/mL
1700-0132	ChlorAC™ Buffer for preservation of aqueous carbamate samples, 250 mL

Glyphosate Reagents

Part Number	Description
O120	*o*-Phthalaldehyde, Chromatographic Grade crystals, 5 g
3700-2000	Thiofluor, Chromatographic Grade crystals, 10 g
GA104	OPA Diluent for glyphosate analysis, 4 x 950 mL
GA116	Hypochlorite Diluent for glyphosate analysis, 4 x 950 mL
K200	Eluent for glyphosate analysis, 4 x 950 mL
RG019	Column Regenerant for glyphosate analysis, 4 x 950 mL
1700-0080	Test mixture, 2.5 µg/mL each glyphosate and AMPA, 1.5 mL
1700-0140	RESTORE for removal of metal ion contamination from glyphosate column and guard, 250 mL

Columns & Guards

Part Number	Description
0840250	C_8 Carbamate column, 4.0 mm ID x 250 mm
1846150	C_{18} Carbamate column, 4.6 mm ID x 150 mm
1846250	C_{18} Carbamate column, 4.6 mm ID x 250 mm
18ECG002	Replacement Carbamate Guard Cartridges - (Qty. 2)
1954150	Glyphosate column, 4.0 mm ID x 150 mm
1953020	Glyphosate guard column, 3.0 mm ID x 20 mm

Spare Parts

Part Number	Description
3102-9047	Replacement frit, 0.5µm (for pre-column filter)
3102-9132	Replacement frit, 2µm (for reagent filters)
1100-2927	OPA Reactor, 0.011" ID TFE tubing
1100-0281	0.5mL Coil Assembly only, no heater
1100-2660	Heated Reactor, 0.5mL, 120V for PCX5100
1100-2661	Heated Reactor, 0.5mL, 240V for PCX5102
1100-0200	Restrictor, for OPA, NaOCl, & NaOH reagent, with nuts & ferrules
3106-1330	Seal (1) for reagent pump
3106-1310	Seal Kit for reagent pump, includes 2 seals and seal installation tool
3106-1314	Inlet Check Valve for reagent pump
3106-1316	Outlet Check Valve for reagent pump
3106-1320	Piston, sapphire, for regular reagent pump
3106-1332	Piston, sapphire, for reagent pump with **piston-washing system**
3106-1322	Piston Guide / Retainer
3106-1324	Liquid End Assembly
3107-0137	Reagent Bottle, coated, 1 liter borosilicate, no cap
3107-0138	Cap for Reagent Bottle 3107-0137, without tubing
2103-0463	Tubing, Saran, 1/8" OD x 0.063" ID, per 3ft (90cm)
3104-0081	Seal Kit for bypass valve
3101-0060	Nut, Fingertight for 1/16" plastic tubing
3102-1202	Nut, male, Upchurch type, 10-32, 1/16"
3102-2102	Ferrule, Upchurch type, 1/16"
3102-1402	Nut, male, Valco type, 10-32, 1/16"
3102-2402	Ferrule, Valco type, 1/16"
3103-1030	Tubing, stainless steel, 1/16" OD x 0.010" ID x 30cm
2101-0212	Tubing, TFE, 1/16" OD x 0.011" ID, per 3ft (90cm)
2101-0225	Tubing, TFE, 1/16" OD x 0.025" ID, per 3ft (90cm) (waste line)
3101-0007	Nut, 1/4-28 x 1/16"
3101-0008	Ferrule, for 1/4-28 x 1/16"
3101-0005	Nut, 1/4-28 x 1/8"
3101-0006	Ferrule, for 1/4-28 x 1/8"
3102-1518	Nut, Lite-Touch, for 10-32, 1/16"
3102-2507	Ferrule, Lite-Touch, for 10-32, 1/16"
3543-0045	Fuse for PCX5100, 120V
3543-0044	Fuse for PCX5102, 240V

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Carbamates

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- M. W. Dong, F.L. Vandemark, W.M. Reuter, and M.V. Pickering, "Carbamate pesticides analysis by liquid chromatography," *Amer. Environ. Lab.,* **2**(3) (1991) 14–27.

- K.M. Hill, R.H. Hollowell, and L. D. Dal Cortivo, "Determination of N-methylcarbamate pesticides in well water by liquid chromatography with post-column fluorescence derivatization," *Anal. Chem.,* **56** (1984) 2465–2475.

- H. Frister, H. Meisel, and E. Schlimme, "OPA Method Modified by Use of N,N-Dimethyl-2-mercaptoethylammonium Chloride," *Fresenius Z. Anal. Chem.,* **330** (1988) 631–633.

Glyphosate

- J.E. Cowell, "Analytical Residue Method for N-Phosphono-methylglycine and Aminomethylphosponic acid in Environmental Water," *Monsanto Method Number 86-63-1,* 1987

- Environmental Protection Agency Draft Method 597: "Analysis of Glyphosate in Drinking Water by Direct Aqueous Injection LC with Post-Column Derivatization."

Instrumentation

- M.V. Pickering, "Assembling an HPLC post-column system: practical considerations," *LC•GC,* **6,** 11 (1988) 994–997.†

- M.V. Pickering, "Modifying HPLC equipment to tolerate corrosive solutions," *LC•GC,* **6,** 9 (1988) 800–809.†

- J.W. Dolan and L.R. Snyder, "Troubleshooting LC Systems," Humana Press, Clifton, NJ (1989).

† Reprints available from Pickering Laboratories

Limited Warranty

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Pickering Laboratories, Inc., (Pickering) Instruments in standard configuration (see Instrument List below) are warranted to be free of defects in material and workmanship under normal installation, use, and maintenance, for a period of one year from the date of delivery to the original Customer. Pickering will replace or repair, without cost, any defective items. Expendable items such as check valves, pistons, piston seals, and filters are excluded from this warranty. In addition, physical damage, poor-quality reagent- and sample-induced damage, and instrument damage due to Customer's misuse are not covered by this warranty.

Instrument List

AO3100	AO3102	EC5100	EC5102	EC5150	PCX5111	CRX400
AT3100	AT3102	EG5100	EG5102	EC5152	PCX5112	CHX700

Analytical Columns

Pickering's Analytical Columns are warranted to be free of defects in materials and workmanship under normal installation, use, and maintenance, for a period of ninety days from the date of delivery to the original Customer. Pickering will replace the Analytical Column under warranty if found defective in material or workmanship. However, the warranty is void if the Analytical Column was damaged due to Customer's misuse.

How to Obtain Warranty Service

If there is a problem with your Instrument or Analytical Column within the Warranty period, notify Pickering immediately at **(800) 654-3330;** if calling from outside U.S.A., use **(415) 694-6700.** If the Instrument or Analytical Column was not purchased directly from Pickering, please contact the vendor where it was purchased from. Any Instrument, part of the Instrument, or Analytical Column returned to Pickering for examination or repair shall have Pickering's prior approval (call for a Returned Goods Authorization number) and be sent prepaid by the Customer. Return transportation will be at Pickering's expense if the Instrument, part of the Instrument, or Analytical Column is found to be defective and under warranty.

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