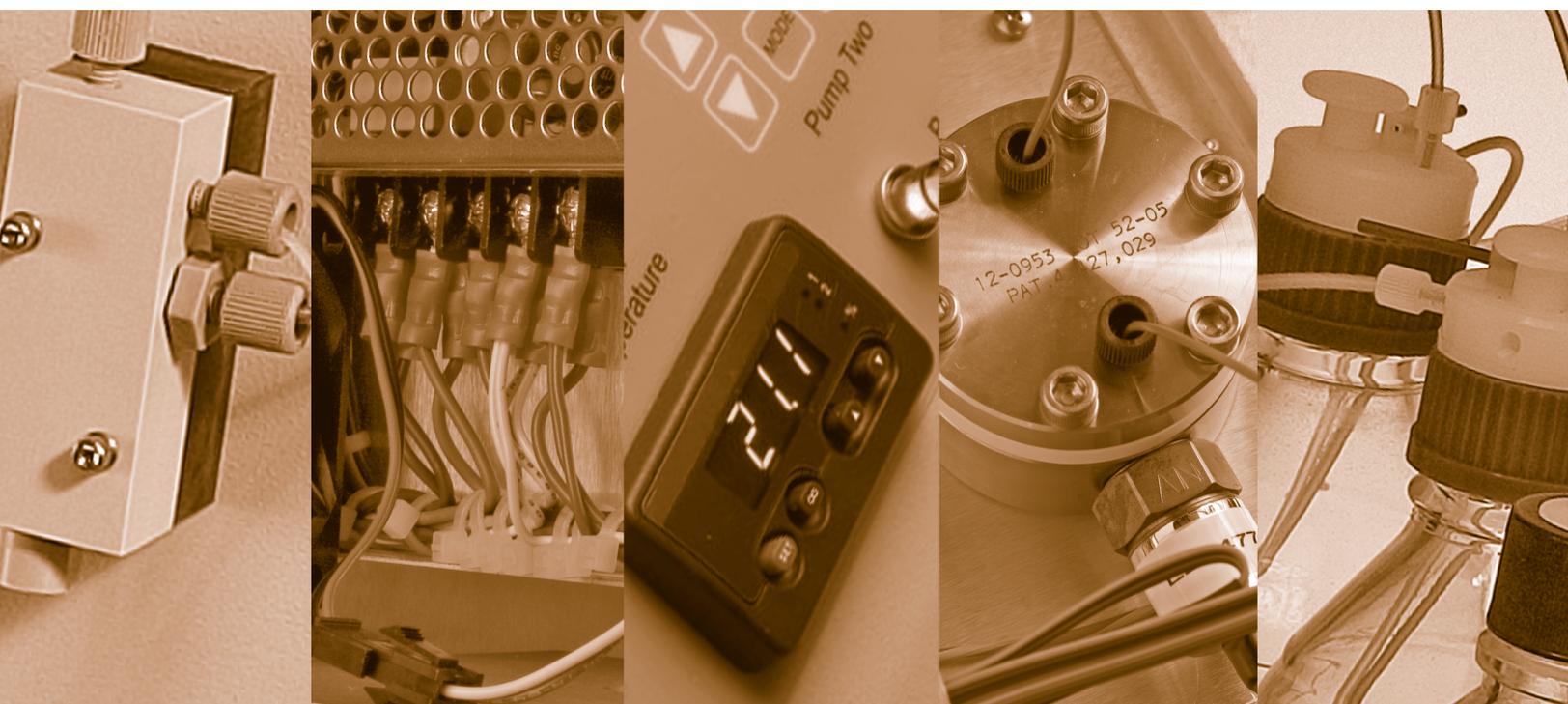


# VECTOR PCX

## OPERATORS MANUAL



**PICKERING**  
LABORATORIES



# OPERATOR MANUAL

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**Post-Column  
Derivatization  
Instrument**



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Cat. No. 0101-0009

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# GETTING STARTED

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- 1 Read this first!
- 1 Symbols and Safety Warnings
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- 4 Site Requirements

## Read this First!

Before attempting to install the Vector PCX post-column derivatization instrument, it is vitally important that you read this manual first, and attend to site, HPLC, and accessories requirements:

HPLC – Page 4

Gas Supply Requirements – Page 4

Reagent Reservoir bottles – Page 4



## Symbols and Warnings

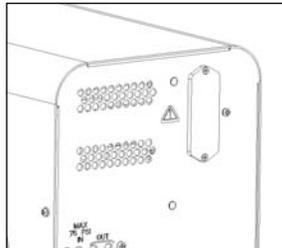
Caution – this symbol indicates that caution must be used when dealing with this part. In the Vector PCX this symbol indicates that it is possible to receive an electrical shock when dealing with this part. Use caution and always remove the source of power before performing any maintenance in these areas.

To prevent electric shock, always disconnect the power source and isolate the instrument before accessing the Vector PCX.

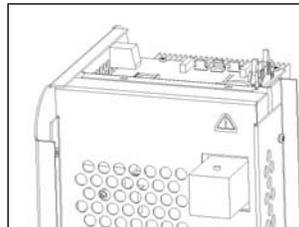
If any protective covers were removed for service, always replace them prior to use. Always ensure that all ground wires are connected properly (do not over tighten) after service before the instrument is used.

The Caution symbol can be found in 3 places on the Vector PCX:

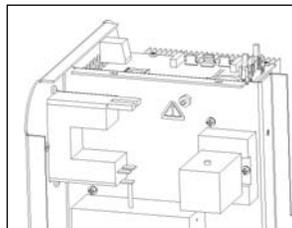
1. To the left of the power inlet module on the back of the instrument



2. On the protective barrier cover inside the instrument (covers high voltage area at rear left of chassis)



3. To the left of the grounding stud behind protective cover



Note: The user shall be made aware that, if equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

## Specifications

### INSTRUMENT

#### *Dimensions*

16.75 H x 8.75 W x 14.75 D inches (43 x 22 x 38 cm)

#### *Weight*

26 lbs (11.6 kg) for Dual-pump systems

#### *Reagent Pumps*

Max operating pressure 2000 psi (138 bar)

Flow rate range 50 $\mu$ L–2000 $\mu$ L/minute

0.5 % RSD

***Heated Reactor***

5°C above ambient to 130°C

Thermal Safety switch limits temperature to 150°C

Stability +/- 0.5°C

Accuracy +/- 1°C

***Electrical***

120 VAC +/- 10%, 240 VAC +/- 10%

2.5 A maximum at 108 VAC

50 – 60 Hz

***Fuses***

2 ea, 5mm x 20 mm, T (time lag) 3.15A

The same fuse is used for both 120V and 240V systems

***Battery***

On the control board for each reagent pump there is a replacement coin cell battery. Use a CR2032, lithium battery, 3-volt, 20 mm diameter, 3.2 mm thick

**ENVIRONMENTAL**

Indoor use only

Altitude up to 6500 ft (1981 m)

Ambient Temperature 5 – 40°C

Relative Humidity up to 80% at 31°C

This device complies with Part 15 Class B of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

**WETTED MATERIALS*****Tubing***

PEEK, Teflon FEP, SARAN, Titanium, Borosilicate Glass, Synthetic Ruby, Sapphire, Stainless Steel, Kel-F

## Site Requirements

### INSTRUMENT

#### ***Bench Space***

21 H x 13.75 W x 19 D inches (53 x 35 x 48 cm), with bottles and electrical connections in place.

Minimum 3 inches clearance at back of instrument for venting.

The total space requirement depends on the brand and model of HPLC.

#### ***Electrical Outlet***

One grounded outlet must be provided for the Vector PCX.

#### ***Gas Supply***

High purity Nitrogen, regulated to 45-75 psi.

Outlet of regulator must connect to 1/8" OD tubing.

The maximum gas inlet pressure must not exceed 75psi.

#### ***Reagent Reservoir Bottles***

The Vector PCX includes pressurized reagent reservoirs.



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

#### ***HPLC Pump***

Application Dependent; binary gradient for glyphosate, carbamate applications

#### ***Autosampler***

Min injection volume 10uL, preferably by full-loop injection

For drinking water, min injection volume 200uL

Tefzel rotor seal required for all applications using eluants with pH>10

PEEK needle seat required for all applications using eluants with pH>10

**Column Oven**

The Vector PCX does not include a column oven. Therefore a column oven is needed to regulate the column temperature to +/- 1°C of the set point.

**Detector**

Pressure rating of flow cell must be > 110 psi

**MISCELLANEOUS SUPPLIES**

If applicable, a Dead-Head Kit may be required to reduce the flush volume of the HPLC and to protect the pressure switch from corrosive eluants. This can be purchased from Pickering Laboratories.

**Chemistry**

The user must check the chemistry requirements for the specific application.

**For Carbamate Analysis**

HPLC Grade Methanol

HPLC Grade Water

Materials for calibration standards

Carbamate hydrolysis reagent (Cat. No. CB910)

Carbamate OPA diluent (Cat. No. CB130)

o-phthaldehyde (Cat. No. O120)

Thiofluor™ (Cat. No. 3700-2000)

**For Glyphosate Analysis**

5% Sodium hypochlorite solution

Materials for calibration standards

Methanol for OPA reagent preparation

Glyphosate Eluant, pH 2.0 (Cat. No. K200)

Glyphosate Regenerant (Cat. No. RG019)

Glyphosate hypochlorite diluent (Cat. No. GA116)

Glyphosate OPA diluent (Cat. No. GA104)

o-phthaldehyde (Cat. No. O120)

Thiofluor™ (Cat. No. 3700-2000)

## Section 1

# INTRODUCTION

---

- 1.1 What is Post-column Derivatization?
- 1.2 Requirements for a Successful Post-Column Method
- 1.3 Design of an HPLC System
- 1.4 Designing a Post-Column System
- 1.5 Design of the Vector PCX

### What is Post-column Derivatization?

This is a method which renders visible certain compounds that are normally invisible. Since this reaction occurs after, or post-separation, it is referred to as post-column derivatization. The analytes of interest are separated on the column first, and then reacted with a chemical that will render them detectable at a desirable wavelength, voltage, or any number of various means of detection.

Post column derivatization enhances the sensitivity of HPLC by several means:

1) Most reagents are selective for a particular class of substances, so analytes of that class are more easily seen against a complex background.

2) Since the separation is performed first, the matrix of the sample is either washed off of the column before the analytes, or is retained by the column. This leaves a very pure sample of analyte to react. This eliminates the need for extensive sample clean-up, and provides a very reproducible reaction because there are no matrix interferences.

The Vector PCX post-column derivatization instrument automatically mixes the stream of effluent flowing from the HPLC column with a stream of reagent solution. The mixture flows through a reactor to allow enough time for the chemical reactions to complete. In many cases, the reaction is very slow at room temperature. For this reason, the reactor can be heated.

There are some methods that require two or more reagents added in sequence. This is done by the addition of a second reagent pump. In many cases, the second reaction occurs at a much faster rate, and can be efficiently accomplished at room temperature.

After the reaction is complete, the derivatives flow into the detector, where the absorbance or the fluorescence (usually) is measured by the HPLC system. These two means of detection are the most common, but they are certainly not the only means of detection.

---

## Requirements for a Successful Post-Column Method

There are many things to take into consideration when developing a method and instrument for post-column derivatization. For example, many pumps have a periodic motion when drawing and dispensing that will manifest itself in the baseline of a chromatogram unless it is properly dampened. Below are the basic requirements for a successful automated post-column method.

- 1) **Reagent Stability.** The minimum reagent stability sufficient for routine work is one day. This means that the yield and signal-to-noise ratio for a given sample must remain constant for at least 8 hours.
- 2) **Reaction Speed.** The analytical separation is complete when the reagent is mixed with the column effluent. Therefore it is important that the analyte react as quickly as possible. The longer the reaction time, the larger the reactor volume required. With larger volumes, the peak shape will become distorted. To minimize band spreading, it is important to keep the overall time (and therefore volume) as low as possible between the column and detector. If the reaction is slow (in excess of one minute), an elevated temperature can be used to decrease the reaction time.
- 3) **Reproducibility.** Because the reaction is occurring “on the fly,” as the combined column and reagent stream flows toward the detector, the reproducibility is linked to the flow rate precision of the pumps and to the temperature. Accordingly, even an incomplete reaction will be as repeatable as the retention time for any given species. Therefore, it is important that the pumps maintain a constant flow rate, and that the reactor maintain a constant temperature. It is also very important that the column be maintained at constant temperature to ensure that the analytes are properly separated and identified.
- 4) **Minimal Detector Response of Reagents.** The color or background fluorescence of the reagent (or its by-products) represents a continuous noise source. Because the reagent is present in excess relative to the analyte, the analyte’s signal could be obliterated by the reagent’s strong background signal. The baseline noise is proportional to the background signal.
- 5) **Solubility.** All species must remain in solution, including the combined components of the eluants and the reagent(s), as well as the newly formed derivative(s). Precipitates can block capillary tubes, burst reactors, and foul detector flow cells.
- 6) **Uniformity of flow.** The baseline noise is a function of the flow-noise in the eluant and reagent pumps. Non-uniform flow causes non-uniform mixing leading to modulation of the background signal which appears as noise. Refractive index noise can be even more objectionable than absorbance noise. Common techniques for evening the flow of the pumps is the addition of a pulse dampener, or the use of a syringe pump. Most modern detectors are not sensitive to refractive index noise.

## Design of an HPLC System

This next section is a simplified view of an HPLC, followed by the ideas behind a post-column system. This section is intended to help novice HPLC operators.

In order to understand post-column HPLC, we need to understand the design of an HPLC. If we connect an HPLC pump directly to a detector (with nothing in between), the baseline from the detector shows a periodic noise (Figure 1-1); the time period is equivalent to the pump stroke.

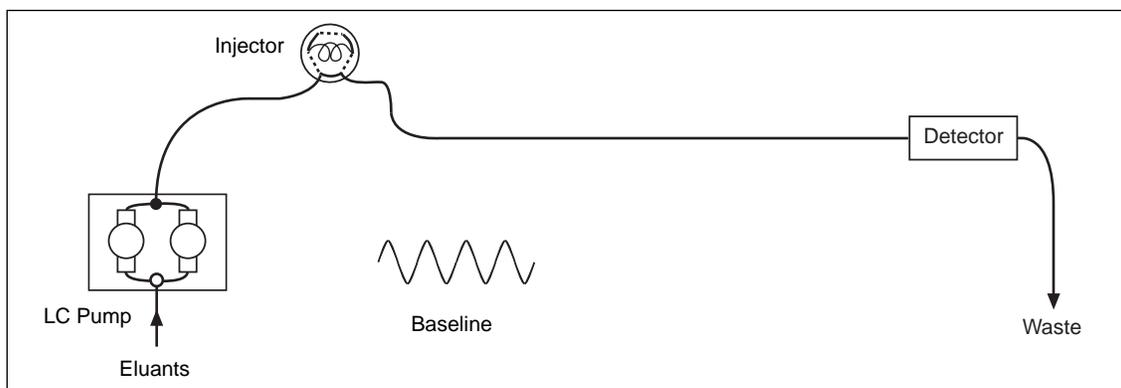


Figure 1-1

Now add a commercial pulse dampener. The baseline is still not smooth; the periodic noise is still there although less pronounced (Figure 1-2). The pulse dampener absorbs most of the pulses from the pump, but the flow requires more stabilization.

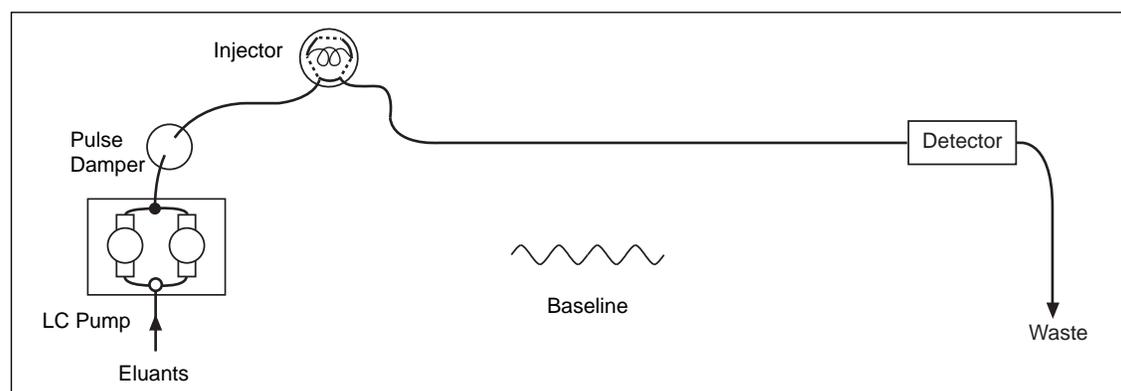


Figure 1-2

A restriction inline will cause the flow at the outlet of the restriction to be constant. In an HPLC system, this is accomplished with the analytical column. Actually, the column does more than separation; it creates a back-pressure. The combination of the pulse dampener and the column that creates a smooth baseline.

(Figure 1-3)

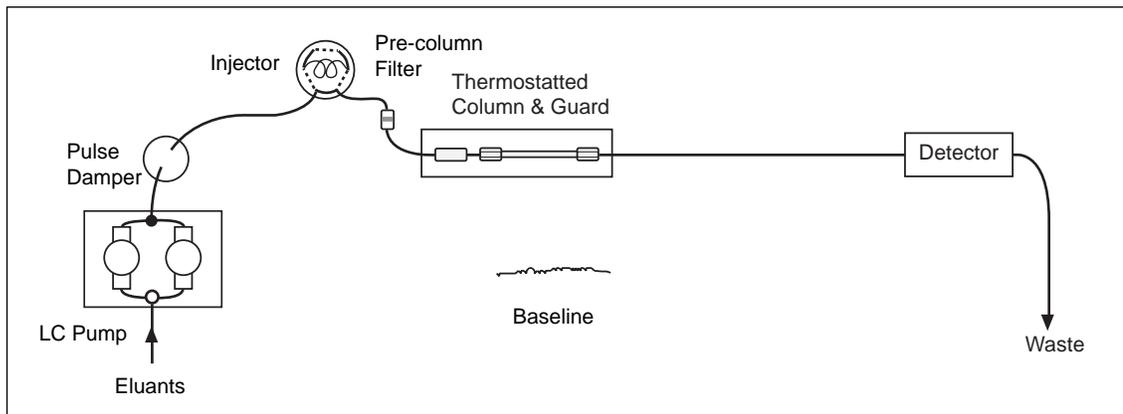


Figure 1-3

An analogy will help us understand the concept. Let us use a river as an example. If it rains; the river swells. If it stops raining; the level goes down. As the level fluctuates, it is equivalent to a periodic noise. To obtain a constant flow, we need to add a reservoir (pulse dampener) and a dam (column). The flow downstream from the dam is constant (smooth baseline).

## Designing a Post-Column System

The same principals that are used in HPLC can be applied to the post-column system. What happens if we simply add a post-column pump, a mixing tee, and a reactor? The periodic noise returns to the baseline (created by the post-column pump; Figure 1-4).

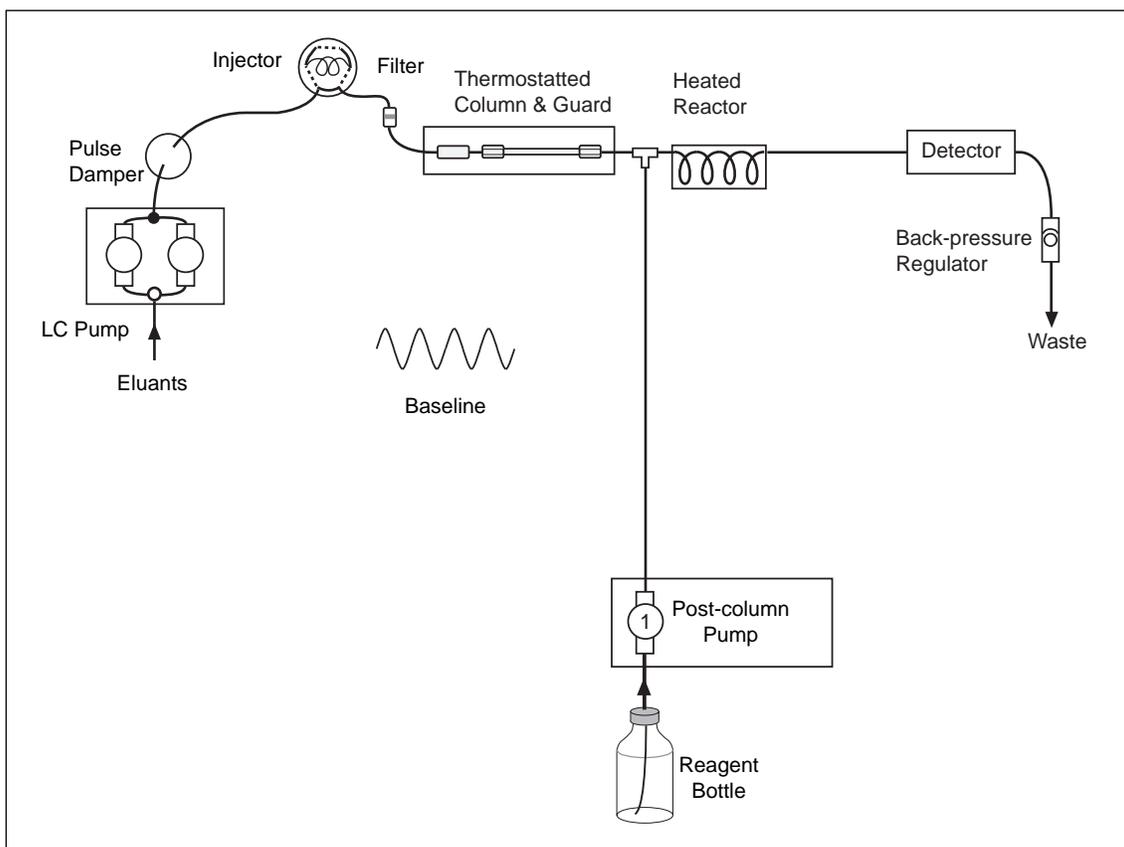


Figure 1-4

## Design of the Vector PCX

However we do not need to invent anything new; we just need a pulse dampener and a column. The Vector PCX contains a pulse dampener after the pump and the Pickering “column” is a restrictor packed with inert material. With this “flow conditioner” in place, the baseline is now acceptable.

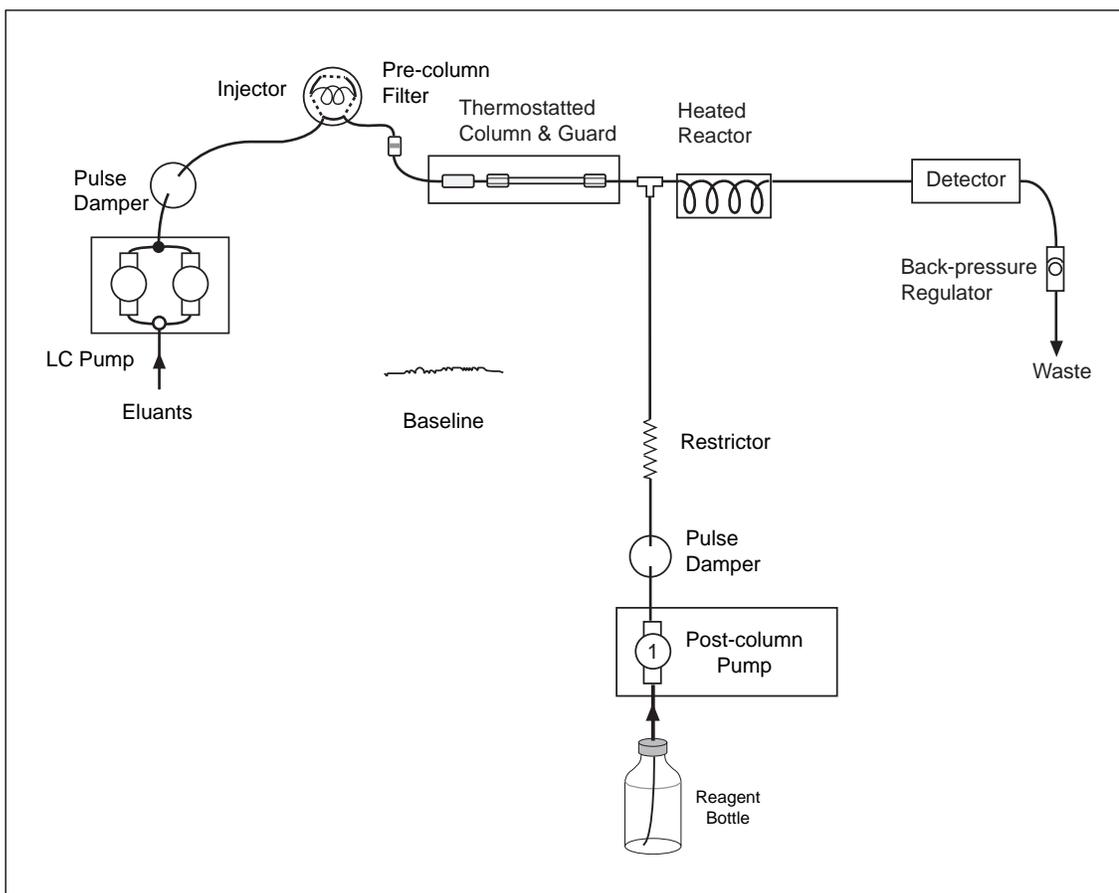


Figure 1-5

## Section 2

# OVERVIEW

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- 2.2** Parts Identification
- 2.2** Pressure Switch
- 2.4** Reagent Pumps
- 2.5** Prime/Purge (bypass) Valves
- 2.5** Mixing Manifolds
- 2.6** Reactors
- 2.6** Control/Display Panel
- 2.7** Reagent Reservoirs
- 2.7** Detector Connections
- 2.7** Internal Components
- 2.8** Power
- 2.8** Standard Configurations

This chapter is designed to familiarize you with the components, layout, and function of the Vector PCX. Here you will find descriptions of each key component of the instrument and what it does. At the most basic level, the Vector PCX performs two main tasks:

1. Delivers the reagent in a smooth and consistent manner
2. Heats the reaction

In addition to accomplishing the above two tasks, the Vector PCX has various features to make the analysis more reliable, convenient and simple. It also contains features to protect the instrument from accidental damage.

The flow path of the Vector PCX is extremely inert, rendering it very versatile. The same instrument can be used for many different applications, and will tolerate a high percentage of reagents.

## Parts Identification/Definition

The front panel is the busiest area of the instrument. Everything that you need is located on the front of the panel. Refer to Figure 2-1 for parts identification throughout the next section.

1. Pressure Switch
2. Pump 1
3. Pump 2
4. Prime/Purge Valve 1
5. Prime/Purge Valve 2
6. Mixing Manifold 1
  - Reagent Filter
  - Heated Reactor inlet
  - Heated Reactor Outlet
  - Over pressure Relief Valve
7. Mixing Manifold 2
  - Reagent Filter
  - Heated Reactor Outlet
  - Ambient Reactor Inlet
8. Heated Reactor
9. Ambient Reactor
10. Gas manifold
11. Enable Button
12. Heated Reactor Controller
13. Power Indicator
14. Pump 1 Control Pad
15. Pump 2 Control Pad

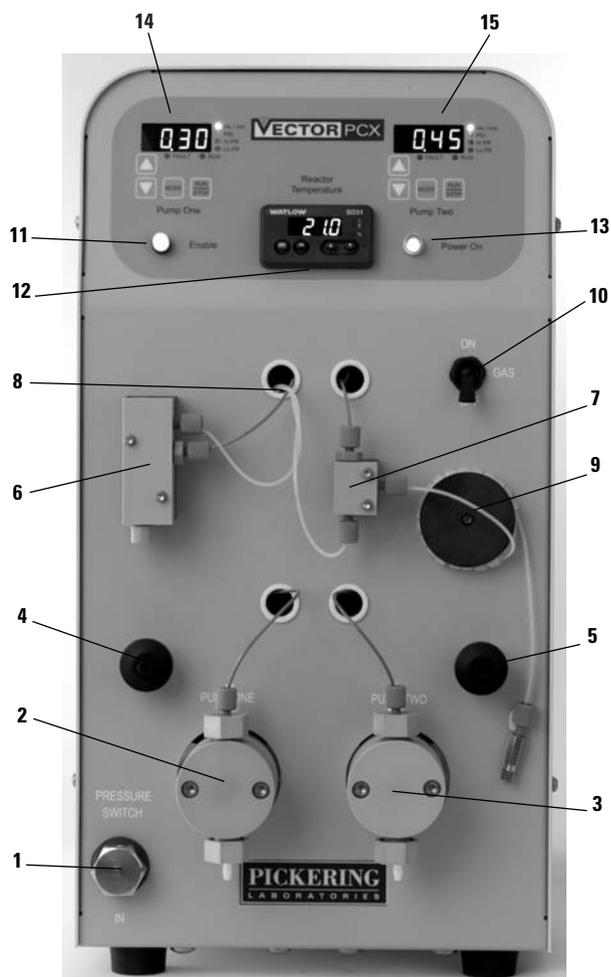
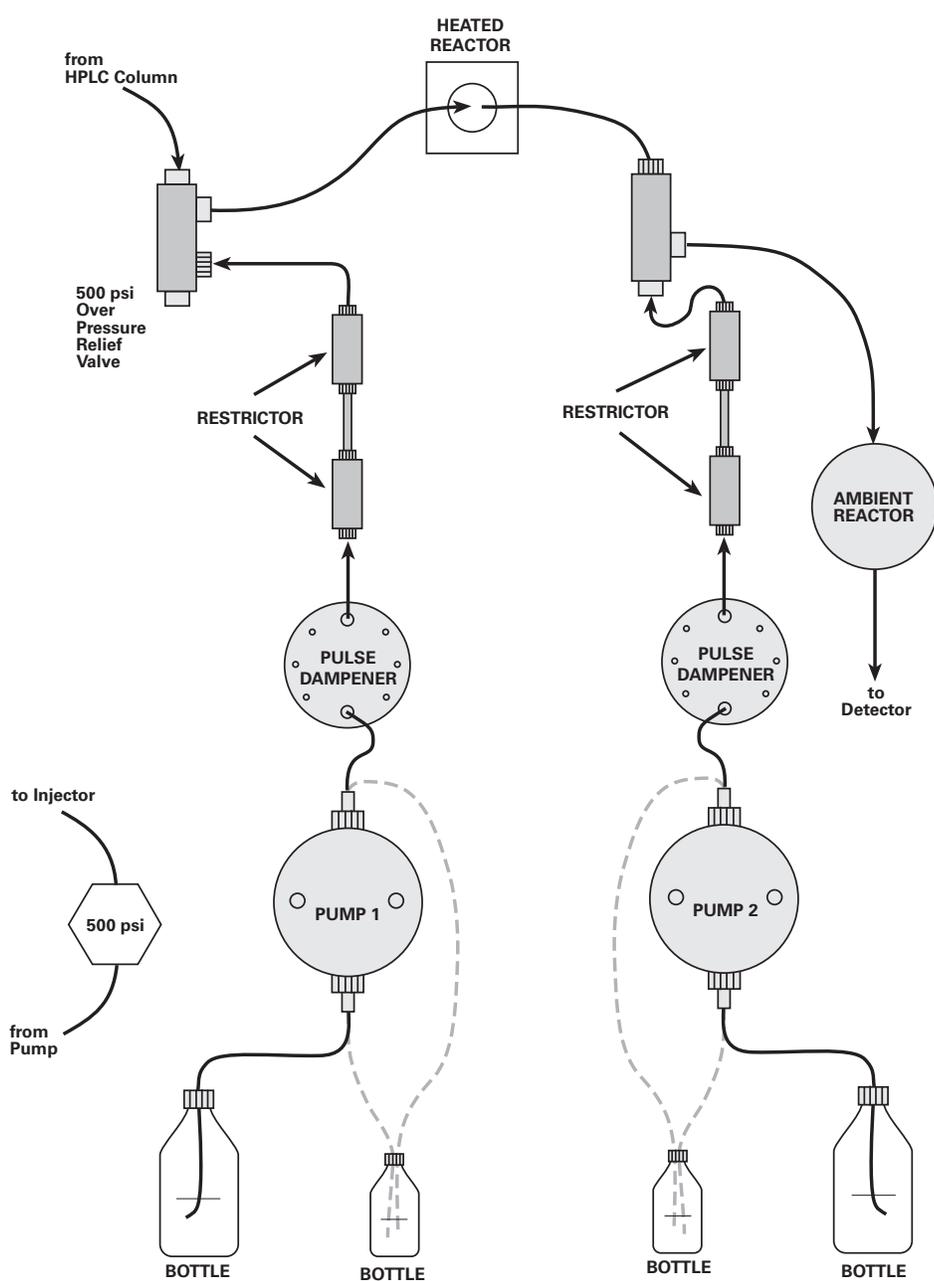


Figure 2-1

## Pressure Switch

The liquid connection (figure 2-2) from the LC Pump to the pressure sensor is at the port labeled “IN” and the connection to the Injector is from the port at the top of the pressure switch. The pressure sensor is part of the safety interlock system. The sensor requires 500 psi (35 bar) before the module can be enabled.



Flow Path

Figure 2-2

## Reagent Pumps

The liquid end of the reagent pump with a piston-wash system is at the front of the Vector PCX. In normal operation, the pump requires no adjustment. It is set at the factory to 0.30 mL/min for both channels. However, the user can adjust the flow rate at any time. That procedure is described later in this manual.

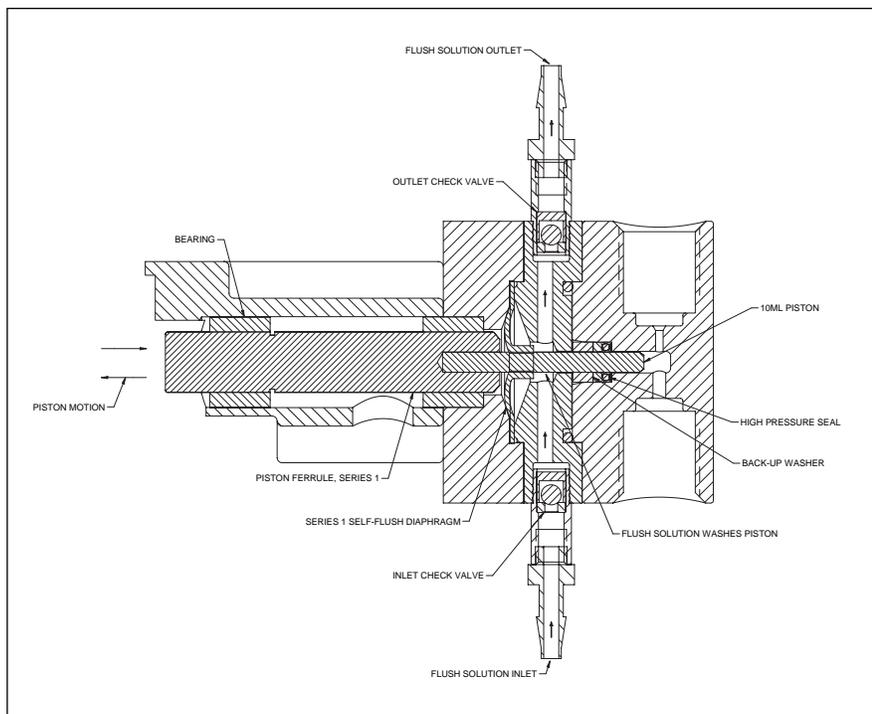
The flow can be set in 0.01 mL increments from 0.01 to 2.00 mL/min with a precision of 0.5%.

The pump is a reciprocating, single-piston pump with an advanced rapid-refill cam design.

Automatic, self-flushing pump heads provide continuous washing of the piston surface without the inconvenience of a manual flush or gravity feed arrangement. The self-flushing pump head uses a diaphragm and secondary set of check valves to create a continuous and positive flow in the area behind the high pressure pump seal. The flushing solution washes away any buffer salts that have precipitated onto the piston. If not removed, these precipitates can abrade the high pressure seal and cause premature seal failure, leakage, and can possibly damage the pump.

There are pressure maximum and minimum limits which will stop the pump if the pressure exceeds 2000psi or drops below 10psi for more than a minute.

**WARNING:** Always release pressure from the pump slowly. A rapid pressure release could cause the pulse damper diaphragm to rupture.



Self-flushing mechanism of Reagent pump

Figure 2-3

---

The self-flush piston wash requires 250-500 mL of 20% methanol in water as a flushing solution. A pH indicator that will indicate the concentration of salts in the solution is recommended as a reminder to change the solution. This flush solution should be replaced with a fresh solution weekly to avoid frequent pump maintenance.

**Important!**

Make sure the piston seals are wetted before turning on the pumps. This is easily accomplished by priming the pump as described in the Operation section of this manual.

**Prime/Purge Valves**

The Prime/Purge valves are used to purge and prime the reagent pumps. They are needed to remove air bubbles from the tubing, check valves and pulse dampener.

**Mixing Manifolds****MANIFOLD 1**

This manifold is a mixing tee designed to begin mixing the sample from the column with the first reagent. The sample and reagent are further mixed in the reactors.

In addition to the ports for sample in from column and out to the heated reactor, this manifold contains:

1. Reagent Filter: The reagent filter element is a 2  $\mu\text{m}$  frit located in each of the mixing manifolds. They are disposable and cannot be cleaned.
2. Over Pressure Relief Valve: The Over Pressure Relief valve is a safety relief valve that opens in case the post-column pressure reaches >500 psi. 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 piece of tubing from this fitting to a clean dry beaker. Any evidence of liquid in this tubing indicates a fault condition.

**MANIFOLD 2**

This manifold is a mixing tee designed to begin mixing the sample from the heated reactor with the second reagent. In addition to the ports for sample in from heated reactor and out to the ambient reactor, this manifold contains:

1. Reagent Filter: The reagent filter element is a 2  $\mu\text{m}$  frit located in each of the mixing manifolds. They are disposable and cannot be cleaned.

---

## Reactors

### HEATED REACTOR

The heated reactor is a coiled tubing that can be heated from 5°C above ambient to 130°C. It heats the reaction between Reagent one and the sample.

### AMBIENT REACTOR

The ambient reactor is a coiled tubing that is at room temperature. It is 100µL in volume. The outlet of this reactor is connected to the inlet of the detector via a zero-dead volume union.

### GAS MANIFOLD

The “Gas Inlet” fitting is where inert gas enters the external gas regulator for pressurizing the reagent reservoirs. The gas regulator requires an input pressure of 45–75 psi (3–5 bar) to function properly.

Gas is controlled by the toggle valve. Lever ON pressurizes the manifold.

The manifold 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 allows the gas to vent from the relief valve, rapidly depleting a gas cylinder.

Gas tubing for all reservoirs are 1/8” OD Saran 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.

Each tubing line from bottle to pump has its own check valve to prevent back flow of gas from the pressurized reagent reservoirs.

## Control/Display Panel

The display panel is the information panel of the Vector PCX.

It contains:

1. Displays and control pads for adjusting the flow rates and monitoring the pressure of each pump (Items 14 and 15 on Figure 2-1)
2. Displays and control pad for heated reactor temperature or set point (Item 12 on Figure 2-1)
3. Enable Button (Item 11 on Figure 2-1). An Orange LED that is illuminated when there is 500psi of pressure reading on the Pressure Switch. Used to bring up the pump and reactor displays, and also start heating the reactor prior to turning on the reagent pumps.
4. Power indicator (Item 13). A Green LED that is illuminated when power is going into the Vector PCX.

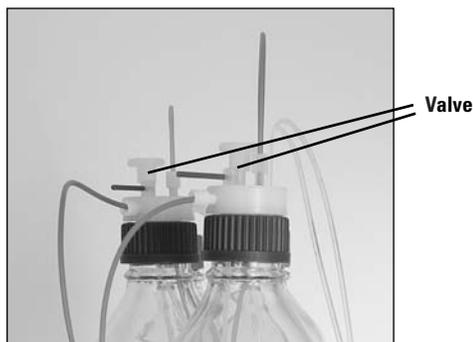
## Reagent Reservoirs

The Vector PCX includes two pressurized reagent reservoirs.

The pressurized reagent reservoir serves two purposes:

1. It protects the air-sensitive reagents from oxidation
2. It helps the reagent pump fill consistently and quickly by providing a source of pressure.

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. On the side of the cap, away from the on-off valve, there is a 1/4-28 fitting; you may optionally connect a tube here to carry vapors to an exhaust vent.



## Detector Connections (100 psi Back Pressure Regulator)

There is a 100psi (5bar) back-pressure regulator on the exit line from the detector; it suppresses boiling inside the hot reactor and prevents bubbles from forming in the detector flow cell. This in turn provides for a smooth baseline and therefore high-sensitivity.

The union at the outlet of the ambient reactor should be connected to the detector with 0.010 inch (0.25 mm) ID tubing.

## Internal Components

The removable cover gives access to the motor ends of the pumps, restrictors, heated reactor, electrical connections, gas regulation system and pulse dampeners

The only time you will need to access the inside is for restrictor replacement, heated reactor replacement, or piston replacement. The pump seals can be replaced externally without having to remove the Vector PCX cover.

On the control board for each reagent pump there is a replaceable coin cell battery.

Use a CR2032, lithium battery, 3-volt, 20 mm diameter, 3.2 mm thick.

---

## Power

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 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 as listed under Specifications.

*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 fusibile.

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

*Avvertimento.* Fare attenzione che la corda del voltaggio sia staccata prima di cambiare valvole. Usa solo valvole di capacità precisata dalla fattoria.

## Standard Configurations of Vector PCX

The Vector PCX is available as a dual pump system with a 0.5, 1.0 or 1.4 mL heated reactor volume, 120V or 240V operation, and is shipped completely assembled, calibrated, and tested.

The Vector consists of a duplex reagent pump, heated and ambient reactors, backflow and over-pressure safety devices, filters and flow conditioning components, reagent reservoirs, Saran<sup>®</sup> gas tubing, and other accessories. Note that the two-reagent Vector PCX can easily be used for one-reagent applications.

If this system was purchased to analyze for carbamates, glyphosate, or in some cases amino acid analysis, there is an appropriate column kit, chemistry, and manual available for your application. All Pickering manuals can also be downloaded from [www.pickeringlabs.com](http://www.pickeringlabs.com).

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**SAFETY FEATURES IN THE VECTOR PCX**

The Vector PCX system has features designed into the instrument and operation that will prevent reagent back-flow onto the column, and bursting of reactor tubing due to a blockage.

Post-column reagent can immediately damage the analytical column if the reagent flow is diverted in the wrong direction by a lack of HPLC flow. This is prevented by inserting a pressure switch before the column. The 500 psi switch prevents the accidental pumping of the reagents when there is no flow through the column, or in the event that the HPLC stops pumping, the reagent pumps will stop as well.

In the rare event there is a blockage in the post-column system, an integrated 500 psi over-pressure relief valve will open and divert the liquid into the drip tray. This will prevent a bursting of tubing or fittings. By relieving the pressure, this will give you the opportunity to correct the blockage rather than having to replace the heated reactor.

## Section 3

# INSTALLATION AND OPERATION

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- 3.1** Site Requirements
- 3.3** Instrument Unpacking and Preparation
- 3.3** Pump and Autosampler Connections
- 3.5** Priming of Eluants
- 3.5** Column and Guard Installation
- 3.6** Detector Connections
- 3.6** Gas Connections
- 3.8** Reagent pump Preparation
- 3.8** Basic Start-up
- 3.9** Over-pressure Relief Valve Connection
- 3.9** Chromatograms
- 3.10** Shutdown
- 3.11** Changing Reagents

Before the Vector PCX can be installed and qualified properly, the HPLC must be completely installed and in good working order (including pump, injector, detector and data collection system). Before installing the instrument, remove all organic compounds that are immiscible with the Pickering eluants as well as any hazardous chemicals.

Always wear safety glasses or goggles, laboratory coat, gloves, and other appropriate safety-clothing. Read and understand the instructions in the MSDS's shipped with the chemicals. If the MSDS's are missing, please contact Pickering Laboratories and we can fax you a copy, or you can download them from our website at [www.pickeringlabs.com](http://www.pickeringlabs.com)

Note: The user shall be made aware that, if equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

## Site Requirements

### 1.1 VECTOR PCX REQUIREMENTS

The Vector PCX weighs approximately 25 lbs. The minimum bench top space required for the Pickering system is approximately 17in (43 cm) high X 8.75in (21.6 cm) wide X 16in (41.2 cm) deep. This does not include the HPLC system. The total space requirement depends on the brand and model of the HPLC.

For most cases, it is best to place the LC pump and injector system on the left side of the Vector PCX, and the detector on the right.

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

Nitrogen, helium, or argon (in order of preference), is required to pressurize the reagent reservoir(s). The Vector PCX requires gas pressure of 75 psi (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 Trione ninhydrin or OPA reagent, use oxygen-impermeable tubing for the entire gas supply line (Saran or metal).

NOTE: If Trione is to be used for reagent 1, Nitrogen must be used to prevent out-gassing.

A waste container should be provided for the waste line from the HPLC detector.

### 1.2 HPLC REQUIREMENTS

Since every HPLC is different, the following procedure has been generalized. Before attempting to connect any tubing, examine the HPLC setup, and determine the best possible means of making the connections. Small ID tubing (0.011") should be used wherever the sample is in the flow path. PEEK ferrules must be used to make the connections on the mixing manifolds of the Vector PCX (i.e. the column outlet connection).

Ensure that the HPLC pump, injector and detector meet all of the necessary requirements for the application to be run.

For all applications, the pressure rating of the detector flow cell must be  $\geq 110$  psi (7.5 bar)

### GLYPHOSATE ANALYSIS

#### *Pump*

Minimum binary gradient elution  
Piston wash capability is preferable

#### *Injector*

Tefzel or PEEK rotor seal for injector valve  
Tefzel or PEEK needle seat if it is an autosampler  
For water samples, at least 200 $\mu$ l injection; preferably 400 $\mu$ l capability

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## CARBAMATE ANALYSIS

### *Pump*

Minimum binary gradient elution

### *Injector*

For water samples, at least 200ul injection; preferably 400ul capability

For all other applications, review the method notes for chemistry requirements.

## Instrument Unpacking and Preparation

### 2.1 UNPACKING

Unpack all cartons and review the contents using the Packing List to ensure that all of the items are present. If any items are missing, immediately contact Pickering Laboratories, Inc.

Toll Free: (800) 654-3330

International: (650) 694-6700

Unpack the instrument and place it on the bench. Place it so there is enough clearance between the PCX and the HPLC, detector, and the edge of the bench.

### 2.2 PREPARATION AND INSPECTION

Remove the cover. Check for any damage to the internal parts. Make sure that the pump is secured to the chassis.

Connect the power cord to the outlet in the back of the VECTOR PCX, just above the power switch.

Turn on the power, but do not operate the buttons. Check that the power indicator light turns green.

## Pump and Autosampler Connections

NOTE: Do not fit the analytical column and guard yet.

Wash the HPLC reservoirs if it is a new HPLC system, or if the eluants are more than 2 weeks old. Wash the reservoirs 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, lint-free cellulose tissue. Avoid touching the tubing or the interior of the reservoir with your skin and do not leave caps and lines dangling without a reservoir because this can cause contamination.

NOTE: If the HPLC reservoirs require cleaning, wash the reagent reservoirs as well and set them aside temporarily.

Fill reservoirs with 80/20 Water/Methanol

Open the prime-purge valve on the HPLC. Purge each line in the system with a water/methanol mixture to flush the system. Set the flow rate to the maximum and purge at least 25 mL through each line.

Drop the flow rate to 1 mL/min and close the prime-purge valve to flush the lines to the injector.

Connect the pressure switch using one of the two connection methods below. Use the HPLC connection kit provided with the system. Use the appropriate fittings for the HPLC. The inlet and outlet of the pressure switch should be a Parker-style fitting.

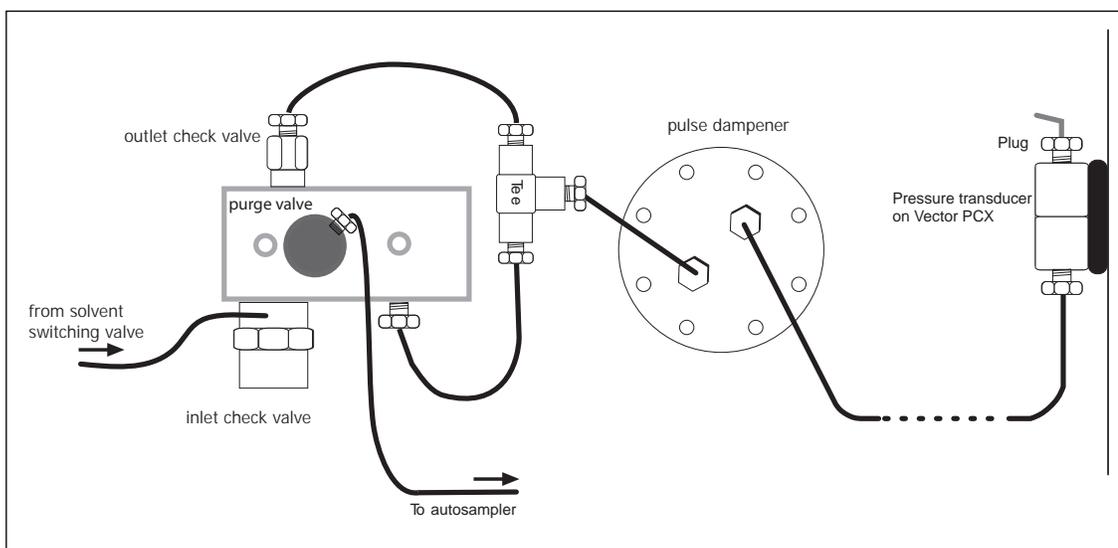
Use one of the two methods below for making the connections.

Note: For Amino Acid applications refer to the Appendix for more information.

Connect the outlet of the pump to the inlet of the pressure switch. The inlet is the bottom opening. Next connect a tubing from the outlet of the pressure switch. The outlet is the top opening on the pressure switch.

OR

Connect a tee to the inlet of the pulse dampener on the HPLC. Connect a line from the outlet of the pulse dampener to the inlet of the pressure switch and place a high-pressure plug in the outlet of the pressure switch on the Vector PCX. Connect the inlet and outlet of the pump through the tee. Refer to the diagram below for more information.



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Connect one end of the column inlet tubing to the injector. Use the appropriate fittings. Leave the other end of the tubing free to protect the column in the next step.

Flush the line from injector into a beaker.

With the HPLC pump running, carefully crack the outlet of the pressure switch to remove any air bubbles. Check that it reads accurate pressure. It should be similar to the column pressure reading on the HPLC.

### Priming of Eluants

Before proceeding, check for and repair any leaks between the pump and the outlet of the autosampler. Once you are certain there are no leaks, do not open the connections between the pump and injector.

*Important!* If any application other than carbamates is to be used, remove water/methanol and replace with water. Flush at least 25 mL through lines and flush injector line.

Fill the reservoirs with the appropriate eluants/mobile phases and again flush at least 25 mL OF EACH ELUANT with the purge valve in the open position.

Close the purge valve, and pump each eluant at 1 mL/min for 5 minutes.

If buffers will be used as the eluant, use pH paper to measure the pH of the solution coming through the tubing.

### Column and Guard Installation

Set the HPLC to 100% of the storage solution in the column

Set the flow rate to 0.2 mL/min

Before starting the flow, connect the guard using the stainless steel tubing provided with the guard, but do not tighten the fitting.

Connect one end of the “column connect” tubing provided in the application kit (1100-0450) to the guard. Swage the nut and ferrule on the other end, but do not connect to the column. Turn on the flow at the recommended rate for the column. Wait for liquid to drip at the inlet of the guard. Tighten the inlet fitting of the guard column and wait for liquid to drip from the outlet of the tubing. Connect the inlet of the column, and then connect the blue PEEK tubing from the outlet of the column to the inlet of the Mixing Manifold 1.

NOTE: The inlet is the top 10/32 opening on Mixing Manifold 1. The Vector PCX is shipped with a plug in this position. Remove the plug in order to connect the column outlet tubing. Be sure to use a PEEK Lite-touch

fitting at this port.

If the application is one that uses sodium or lithium buffers, use deionized water to clean any stainless steel fittings that have come into contact with buffer to prevent corrosion.

Monitor the pressures.

### Detector Connections

Connect a 1/16 inch x 0.020 inch ID tubing from the outlet of the flow cell to the external 100psi back-pressure regulator using a 1/4-28 nut with a 1/16 inch reversed-ferrule (PN 3102-9025). There is an arrow on the back pressure regulator indicating direction of flow. Insure that the arrow is pointing away from the detector and toward the waste line.

Connect the 0.020 inch ID PTFE tubing provided (PN 2101-0225) to the outlet of the external 100 psi back-pressure regulator. Place the other end in an appropriately labeled waste container.

Using the ZDV (zero dead-volume) union provided in the packing kit, connect a 0.011" ID tubing from the outlet of the reactor to the inlet of the detector flow cell. The outlet of the ambient reactor is the length of tubing hanging at the right side of the Vector PCX. See also, Section 2 Overview for identification of parts. Use the PEEK Lite-touch fittings in the connection kit (PN 1100-4020) and a 0.01" ID tubing.

Set the time constant on the detector to 2–4 seconds.

### Gas Connections

Set the regulator on the inert gas supply to between 50-75 psi. Using a portion of the Saran tubing (PN 2103-0463) and the 1/4-28 Nut and inverted 1/8 ferrule (PN 3101-0005 and 3101-0006) connect the "Gas In" port to the inert gas supply. Turn on the main gas supply. Switch the toggle valve to the ON position to start gas flow. Let the gas system purge for about one minute. Switch the toggle valve off. Using the remaining Saran tubing, connect the manifold to the reservoirs.

Connect one end of the Saran tubing to the manifold, and the other end to the inlet at the TOP of the reservoir cap. Use the 1/4-28 Nut and inverted ferrule (PN 3101-0005 and 3101-0006).

See also diagram in Section 2 Overview for proper location.

### Reagent Pump Preparation

Wash the reagent reservoir(s) with laboratory detergent and hot water (if not already completed in Step 4). Rinse with methanol then with deionized water. Wipe down the dip tubes on the caps with methanol and a clean, lint-free cellulose tissue. Avoid touching the tubing or the interior of the reservoir with your skin and do not leave caps and lines dangling without a reservoir because this can cause contamination.

Fill the reservoirs with about 100mL of deionized water.

If a chemical kit was ordered with the system, appropriate labels are included. Place them on the reservoirs. If no chemical kit was ordered, the reservoirs should be labeled with an appropriate label using the GLP of the laboratory.

Create the reagent lines from the front of the Vector PCX to the reservoir caps using the SARAN tubing provided. The reagent tubing should be connected to the reagent bottle cap directly facing the shut-off valve. Connect the reagent bottles to the pump using the Saran tubing. Nuts and reversed-ferrules (1/4-28 ) are provided.

Feed both lines of the 1/4" OD C-flex tubing to a bottle of 80/20 Water/Methanol.

**PRIMING THE PUMP AND FLUSH LINES**

Attach the 20 mL 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 pump, reagent supply line and pulse dampener. 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.



The 3-digit display shows the pump flow rate (mL/min), system pressure (psi), or the set upper or lower pressure limit (psi) when operating. The choice of display is selected with the MODE key.

To prime the flush lines for a self-flush head, simply place the inlet line in the flush solution and connect a syringe to the outlet line and apply suction until the line is filled with flush solution. Place the outlet line in the flush solution. Secure both flush lines in the

flush solution container so they stay immersed during pump operation.

Prime the reagent pump(s) by connecting a 20mL syringe to the bypass valve, opening the valve 2 complete

turns counter-clockwise, and pulling liquid into the syringe. Slowly let go of the plunger and close the valve after about 10 seconds. Press “Mode” once to display the pressure on each pump. It should read about 450-650 psi. If the pressure does not go up, try priming the pump again.

Flush for about 10 minutes.

Monitor the pressures for about 10 minutes, and then stop Vector PCX and the HPLC.

Replace the water in the reagent bottles with reagent.

Prime the pump.

### **Basic Start-up**

This section describes the basic start up of the Vector PCX (enable button, reagent pump, heated reactor)

For chromatographic conditions, please refer to the appropriate Application Manual or column sheet included with every Pickering column.

Always ensure that liquid is flowing through the system from the HPLC pump and that there is 500psi pressure on the column (indicating liquid flowing) before starting the reagent pump.

Start the HPLC pump at the desired flow rate for your application. Allow the pressure to build.

Push the “Enable” button once. This will cause the pump and reactor display to light up, and the heated reactor to begin to warm to its current set point.

Adjust the reactor set point by holding down on the blue “set” key while using the up/down arrows to select the desired number. When you have reached the set point, let go of the buttons and the heater will begin to warm up to the desired temperature.

Ensure that the reagent pumps are at the desired flow rate (Pickering recommends 0.3 mL/min). Use the “Mode” key to select the display function on the pump (described in further detail below).

With the display showing mL/min, use the up/down arrow keys to adjust the flow rate if needed. Turn on the reagent pump by pressing the Run/Stop key.

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Press “Mode” once to display the pressure in psi. Watch to ensure that the pressure is stable and reads about 500psi. If there is no pressure on the pumps, they need to be primed (see below).

NOTE: The over pressure relief valve is set to open at a post-column pressure of 500psi. If the relief valve opens, a partial or complete blockage is indicated.

Set the column temperature at 40°C on the HPLC.

With the HPLC pump running and the column pressure at 500psi +, press the Enable button. This will cause the reactor and pump displays to light up.

Set the reactor to the desired temperature.

When the reactor is hot, turn on the post-column pumps.

Note: It is strongly recommended that you warm the reactor prior to starting the reagent pumps to prevent over-pressure of the reactor.

### **Over Pressure Relief Valve Connection**

Using the short Teflon tubing provided (PN 1100-2731), connect the over-pressure relief valve – feed into a clean, dry beaker.

### **Chromatograms**

Look in the column box for the gradient program and conditions.

Load the appropriate temperatures and gradient method for the application and verify the set-points.

Start the HPLC, press Enable when the column pressure reaches 500 psi (35 bar). When the reactor reaches temperature, turn on the post-column pump.

Set the HPLC pump to run the starting eluant conditions, and while the system is equilibrating, set up the method.

When the method is set up and the systems pressures are acceptable, make an injection of the appropriate test mix. Collect at least 3 chromatograms.

For Carbamate and Glyphosate Analyses:

Set the detector excitation wavelength to 330 nm and the emission wavelength to 465 nm

Do at least two runs of the appropriate test mixture. Inject 10 $\mu$ L.

Compare the chromatogram with that of the QC test of the system and column.

Verify that the system is functioning by using the IQ/OQ document as reference (See Operation Manual, Appendix B)

### Shutdown

Turn off the Vector by lowering the flow rate of the HPLC pump so that the column pressure drops below 500psi (and thereby stopping the heater and reagent pumps). This can be done manually by setting the HPLC at a low flow rate, or via the Slowdown method below.

Choose an eluant that elutes contaminants from the column. For example, use methanol for a reversed-phase column and regenerant for an ion-exchange column.

Set the HPLC pump at the normal flow rate to flush the system for at least 20 minutes. Choose an eluant that elutes contaminants from the column. For example, use methanol for a reversed-phase column and regenerant for an ion-exchange column.

Set the HPLC pump to  $\leq 0.1$  mL/min.

Turn off the detector lamp.

The slowdown method can be used to accomplish all the above steps.

| Step | Time (min) | % Eluant | Flow (mL/min) |
|------|------------|----------|---------------|
| 0    | 0          | 100      | 0.02          |
| 1    | 5          | 100      | 0.02          |
| 2    | 7          | 100      | Normal flow*  |
| 3    | 27         | 100      | Normal flow*  |
| 4    | 27.1       | 100      | 0.02          |

(\*) Normal flow rates are different for various columns. Follow the instructions that came with your column.

Note: It is VERY important to allow the heated reactor to flush out and cool before stopping the HPLC pump. If the heated reactor is not cooled and flushed properly, it is very likely that a blockage will form in the heated reactor.

### LONG TERM SHUTDOWN

Remove the column and guard, and the post-column reagents. Put water/methanol (80/20) on both the HPLC pump and the reagent pump. Pump water/methanol through the entire system for 30 minutes.

---

## **Changing Reagents**

When changing reagent, first turn off the gas using the toggle valve on the manifold. 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.

## Section 4

# VECTOR MAINTENANCE

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- 4.1** Pump Maintenance
- 4.2** Replacing Piston Seals
- 4.4** Cleaning Pump Head Assembly
- 4.5** Cleaning/Replacement of Check Valves
- 4.6** Replacing Pistons
- 4.7** Replacement of Reagent Filter
- 4.7** Replacement of the Heated Reactor
- 4.8** Fuse and Battery Replacement

Caution: To avoid electrical shock and possible injury, remove the power cord from the back panel of this equipment before performing any type of service procedures. The equipment must be isolated or disconnected from the hazardous live voltage before access by the operator.

To avoid chemical or electrical hazards, always observe safe laboratory practices while operating this equipment

Always wear safety glasses or goggles, laboratory coat, gloves, and other appropriate safety-clothing. Read and understand the instructions in the MSDS's shipped with the chemicals. If the MSDS's are missing, please contact Pickering Laboratories and we can fax you a copy, or you can download them from our website at [www.pickeringlabs.com](http://www.pickeringlabs.com).

### Pump Maintenance

Lower than normal pressure, pressure variations, or leaks in the pumping system can all indicate possible problems with the piston seal, piston, or check valves. Piston seal replacement could be necessary after 1000 hours of running time.

#### REPLACING THE PISTON SEALS

See also Figure 4-1 for the following procedure.

Turn OFF the power to the Vector PCX.

Close the blue reagent valve on the Reagent reservoirs to prevent the reagent from flowing out the tubes during this procedure.

Remove the outlet line from the outlet check valve.

Using a 5/64" Hex wrench, loosen the two nuts at the front of the pump head.

**CAUTION:** Be careful not to break the piston when removing the pump head. Twisting the pump head can cause the piston to break.

Carefully separate the pump head from the pump. Move the pump head straight out from the pump and remove it from the piston. Be careful not to break or damage the piston. Also remove the seal and seal backup washer from the piston if they did not stay in the pump head. Remove the O-ring.

Carefully separate the flush housing from the pump. Move the flush housing straight out from the pump and remove it from the piston. Be careful not to break or damage the piston. Also remove the self-flush diaphragm from the piston by carefully grasping the sealing flange on two sides and sliding it straight out on the piston being careful not to exert side pressure that may break the piston.

Each replacement seal kit contains one seal, one backup washer, a seal insertion/removal tool, a diaphragm and a pad to clean the piston when changing the seal.

Insert the flanged end of the seal insertion/removal tool into the seal cavity on the pump head.

Tilt it slightly so that flange is under the seal and pull out the seal.

**CAUTION:** Using any other "tool" will scratch the finish.

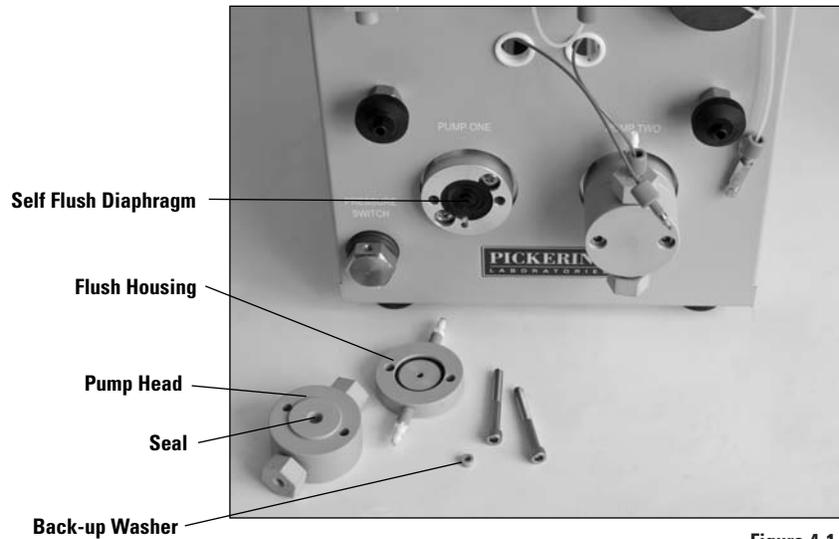


Figure 4-1

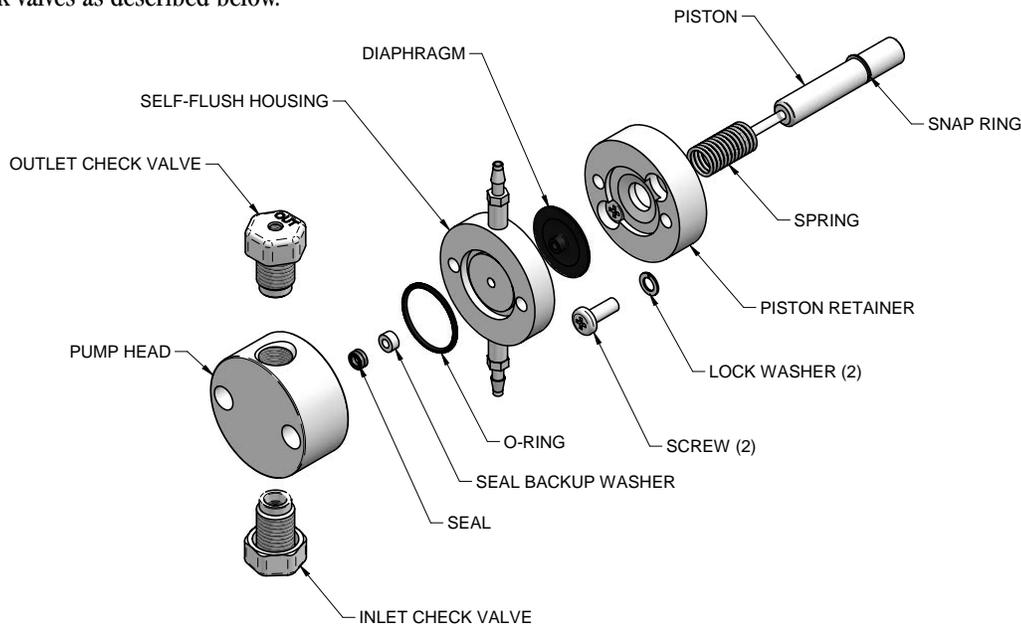
Clean the Piston to remove any salt build up or dirt. If there is a lot of build-up, clean the piston using the scouring pad as described below. Otherwise, use a lint-free cloth dampened with alcohol to wipe the piston clean.

It is not necessary to remove the piston from the housing to clean the piston.

To Clean the Piston using the Scouring pad:

Use the scouring pad included in the seal replacement kit. Gently squeeze the piston within a folded section of the pad and rub the pad along the length of the piston. Rotate the pad frequently to assure the entire surface is scrubbed. Do not exert pressure perpendicular to the length of the piston, as this may cause the piston to break. After scouring, use a lint-free cloth, dampened with alcohol, to wipe the piston clean.

Before replacing the pump head and seals, inspect, and if necessary, clean the pump head and check valves as described below.



**Pump Head Assembly**

**Figure 4-2**

Place a high pressure replacement seal on the rod-shaped end of the seal insertion/removal tool so that the spring is visible when the seal is fully seated on the tool. Insert the tool into the pump head so that the open side of the seal enters first, facing the high pressure cavity of the pump head. Be careful to line up the seal with the cavity while inserting. Then withdraw the tool, leaving the seal in the pump head. When you look into the pump head cavity, only the polymer portion of the seal should be visible.

Gently place the diaphragm onto piston with center hub protruding towards you. Push the diaphragm all the way back into recess and against metal base of piston. Do not exert pressure perpendicular to the length of the piston, as this may cause the piston to break.

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Carefully align the flush housing and gently slide it into place on the pump. Make sure that the Inlet valve is on the bottom and the Outlet valve is on the top.

Line up the pump head and carefully slide it into place. Be sure that the Inlet valve is on the bottom and the Outlet valve is on the top. Do not force the pump head into place.

Tighten both hex screws in the pump head using the 5/64" wrench. DO NOT over tighten! Turn each screw alternately 1/4 turn while gently wiggling the pump head to center it.

Reattach the inlet and outlet lines. Change the flushing solution.

Lastly, condition the new seal using the following procedure:

Using a restrictor coil or a suitable column, run the pump with a 50:50 solution of isopropanol (or methanol) and water for 30 minutes at a back pressure of 2000 psi.

Note: Use only organic solvents to break-in new seals. Buffer solutions and salt solutions should never be used to break-in new seals.

#### **CLEANING THE PUMP HEAD ASSEMBLY**

Note: If you choose to remove the piston seal or self-flush diaphragm, you should have a new set on hand to install after cleaning. It is not recommended that you reinstall the used piston seal or diaphragm since they are likely to be scratched and damaged during removal and would not provide a reliable seal if reused. If you decide to remove the seal, use only the flanged end of the plastic seal removal tool supplied with the seal replacement kit, and avoid scratching the sealing surface in the pump head.

Inspect the piston seal cavity in the polymer pump head. Remove any foreign material using a cotton swab or equivalent, and avoid scratching the sealing surfaces. Be sure no fibers from the cleaning swab remain in the components.

The pump head, check valves, and flushing housing may be further cleaned using a laboratory grade detergent solution in an ultrasonic bath for at least 30 minutes, followed by rinsing for at least 10 minutes in distilled water. Be sure that all particles loosened by the above procedures have been removed from the components before reassembly.

**CAUTION:** When cleaning check valves, be sure that the ball is not against the seat in the ultrasonic bath. This may destroy the precision matched sealing surface and the valve will not check.

If the check valves have been removed, tighten each check valve to 10-15 inch-pounds. This is usually done by tightening each check valve by hand, then using a wrench no more than 1/4 turn past finger tight. DO NOT over tighten the check valve assembly as this will deform the seal and it will not work properly.

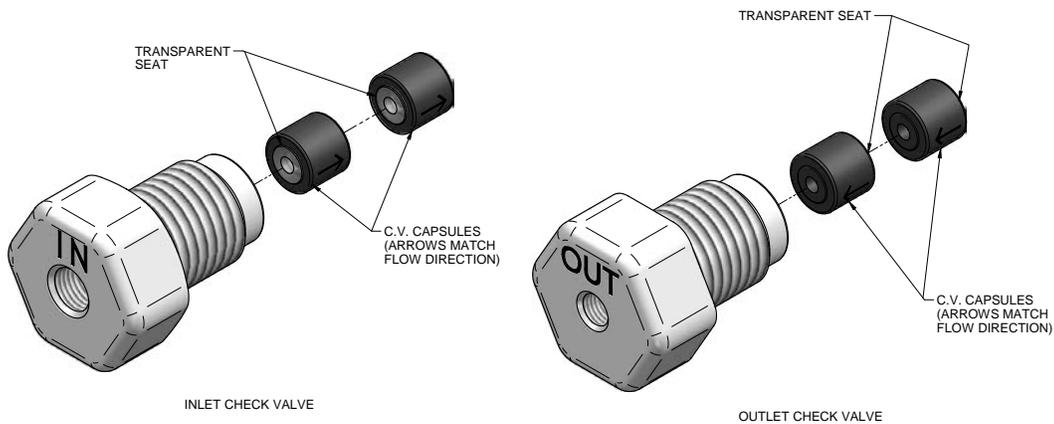
**CLEANING/REPLACEMENT OF CHECK VALVES**

Each check valve assembly contains two capsules. The sapphire seat in each capsule must be oriented downward in all cases in the final pump assembly.

Note: The inlet check valve has a larger opening (1/4"-28, flat-bottom seat) for the 1/8" inlet tubing; the outlet check valve has a smaller opening (#10-32, cone seat) for the 1/16" outlet tubing.

The inlet check valve must be connected at the larger opening in the pump head.

If the piston seal has been removed, insert a new seal before replacing the pump head.



**Check Valves**

**Figure 4-3**

Many check valve problems are the result of small particles interfering with the operation of the check valve. As a result, most problems can be solved by pumping a strong solution of liquid laboratory grade detergent through the check valves at a rate of 1 ml/min for one hour. After washing with detergent, pump distilled water through the pump for fifteen minutes. Always direct the output directly to a waste beaker during cleaning. If this does not work, the check valve should be replaced.

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If the check valves had been removed, tighten each check valve firmly by hand, then no more than 1/4 turn with a wrench. DO NOT over tighten the check valve assembly as this will deform the seal and it will not work properly.

#### **REPLACING THE PISTON**

Turn off the power to the Vector PCX and then remove the power cord.

Remove the cover from the Vector PCX by removing the 6 screws on each side of the instrument.

Remove the pump head as described above.

With your thumb pressing the piston retainer against the pump housing, remove the two phillips head screws from the retainer. Do not allow the spring pressure to force the retainer away from the housing as the screws are loosened.

After both screws have been removed, slowly allow the spring pressure to push the retainer out of the housing. Gently pull the retainer straight out and carefully remove it from the piston and threaded rods. Also, gently pull the spring straight out of the housing and remove.

Grasp the metal base of the piston assembly so that you avoid exerting any pressure perpendicular to the length of the piston, and gently pull it from the pump housing.

Remove the snap ring from the groove on the old piston and place it into the groove on the new piston.

Place a small amount of high quality grease on the back end of the metal base of the piston assembly. Grasp the metal base of the piston assembly near the front so that you avoid exerting any pressure perpendicular to the length of the piston, and gently slide it into the pump housing.

Gently slide the spring over the piston assembly and back into the pump housing. Carefully align the retainer and gently push it straight in against the spring force until the retainer is against the housing. If misalignment with the piston occurs, wiggle while pushing the retainer to align the piston & retainer.

Hold the retainer flush against the housing with your thumb. Insert and tighten the phillips head screws. Do not allow the spring pressure to force the retainer away from the housing. Insure that there are no gaps between the retainer and the housing.

Re-attach the pump head as described above.

## Replacement of Reagent Filter

The reagent filters are located in each of the mixing manifolds. When there is an increase in the reagent pressure, it is usually an indication that the reagent filter should be replaced.

The reagent filters are a 2 $\mu$ m frit inside a PEEK housing. They are disposable and cannot be cleaned.

To replace the filter, ensure that both HPLC and Reagent pumps are stopped. Turn the Blue valve on the reagent bottles to the Closed position.

Disconnect the Lite-touch fitting from the inlet of the filter that is blocked.

Using a 7/16" wrench, loosen and remove the blocked reagent filter. Replace it with a clean filter (PN 3102-9042). Tighten firmly.

Re-connect the reagent line(s).

Turn on the HPLC pump, press the Enable button, and then start the Reagent pump to check for leaks.

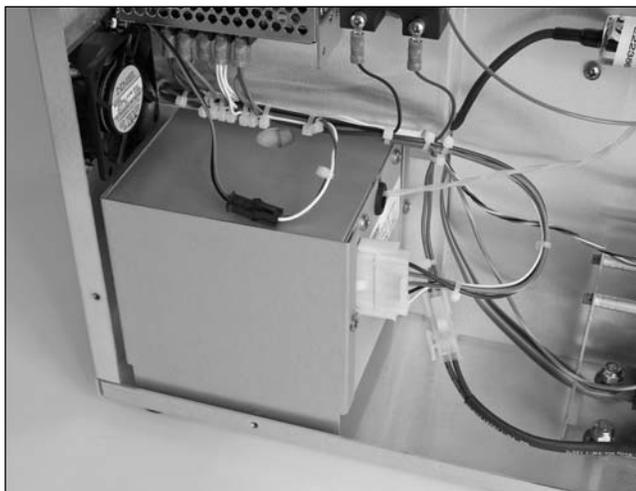
## Replacement of the Heated Reactor

Unless there is a complete blockage in the heated reactor, it can usually be cleaned. For cleaning procedures, refer to the troubleshooting section of this chapter.

If the reactor is completely blocked, it must be replaced.

To replace the reactor, turn off the power to the Vector PCX.

Unplug the power cord and remove the cover by loosening the 6 screws on each side.



The heated reactor is located at the lower left back corner. Remove the two Phillips head screws that hold it to the chassis.

Unplug the white cable harness by squeezing on the two tabs and then pulling out.

---

A replacement reactor (PN 1100-2660 for 120V 0.5mL or 1100-2661 for 240V 0.5mL) can be installed by reversing the above steps.

### Fuse and Battery Replacement

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.



#### REAGENT PUMP BATTERY REPLACEMENT

There is very little drain on this battery during normal operation. It is included on the board to store settings such as the last flow rate used and pressure max/min for the pump in the case when the Vector PCX is powered down.

It should not need to be replaced unless you see that the pump settings are no longer being stored when the Vector PCX is powered down.

On the control board for each reagent pump there is a replaceable coin cell battery. Use a CR2032, lithium battery, 3-volt, 20 mm diameter, 3.2 mm thick.

The battery provides power for the memory that holds the current pump configuration. If the pump is set at a flowrate other than 1.00 or 10.0 and the power is turned off, when the power is turned back on the flowrate should appear as it was set. If this flowrate does not appear the battery will need replaced.

Unplug the unit.

Remove the cover.

Turn the unit so that the control panel is to the right. The battery can be seen in the lower right corner of the circuit board. The battery is circular and has a positive pole mark (+) on the top. Gently pull it from its socket.

With the positive mark (+) up, gently slide the new battery into the battery socket. Be sure the battery is all the way into place. It must contact the base of the battery socket.

Replace the cover to the unit.

Plug the unit back in.

**WARNING:** Unplug power cord before removing cabinet lid.

## Section 5

# TROUBLESHOOTING

---

- 5.1** Contact Pickering Laboratories for Support
- 5.2** Instrument Parameter Log
- 5.2** Troubleshooting Advice
- 5.3** Common System Problems
- 5.4** Common Chromatography Problems
- 5.5** Common Column Problems
- 5.6** Application-specific Troubleshooting
  - Carbamate
  - Glyphosate
- 5.6** Reagent Pump Troubleshooting
- 5.8** Procedures
  - To Remove Silica deposits From the Reactor
  - To Remove Mineral Deposits In The Reactor From Hard Water
  - To Remove Grease Deposits
  - If Reagent Backflows Onto Column
  - If TRIONE Backflows Onto Column
  - If NaOH Is On Column
  - To Remove Iron Contamination From Column
  - To Pump RESTORE Through The Glyphosate Column



**CAUTION:** To avoid electrical shock and possible injury, remove the power cord from the back panel of this equipment before performing any type of service procedures.

### Contact Pickering Laboratories for Support

There are several easy ways to contact Pickering Laboratories for Technical Support:

Email: [support@pickeringlabs.com](mailto:support@pickeringlabs.com)  
Telephone: 800-654-3330 or 650-694-6700  
Fax: 650-968-0749  
Web Site: [www.pickeringlabs.com](http://www.pickeringlabs.com)  
Click on the Support tab to send us an email.

Pickering Laboratories' business hours are:

Monday thru Friday, 8 AM to 5 PM, Pacific Standard Time (GMT – 08:00)

We will ask you a set of standard questions:

What application are you running?

What are the pressures in your Vector PCX system?

What is the brand and model of your HPLC system?

What type of samples are you injecting?

Please email or fax to us a chromatogram

### **Instrument Parameter Log**

If you should have any problems with your Vector PCX, the Instrument Parameter Log is a key part to find the cause of, and solving any problems. It is a good idea to Hopefully, keep a record of the daily instrument parameters.

A daily parameter log will enable you to see any trends in pressure, and to notice something out of the ordinary before it becomes a serious problem.

Pickering Laboratories strongly recommends that you record your daily operating pressures, and any maintenance performed on the instrument. This log will be invaluable to your laboratory for troubleshooting and problem prevention.

### **General Troubleshooting 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. Repair all leaks.
- 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.

Before making any change in the gradient, temperature, or other operating conditions, get at least two chromatograms in a row with the same problem. After you make a change, get at least two chromatograms showing the same effect of the change. This is especially true when you are trying to optimize gradient conditions.

### Common System Problems

| OBSERVED PROBLEM                               | COMMON CAUSE  | ACTION TO TAKE  | NOTES   |
|--|---|---|---|
| Low Reagent pressure                           | <ol style="list-style-type: none"> <li>1. Air in reagent pump</li> <li>2. Reagent flow rate too low</li> <li>3. Leaking fittings</li> </ol>   | <ol style="list-style-type: none"> <li>1. Check for bubbles</li> <li>2. Check for leaks</li> <li>3. Pressure-test the pump to check for seal integrity</li> </ol>                       | Tighten leaking fittings  |
| High Reagent pressure                          | <ol style="list-style-type: none"> <li>1. Obstruction of flow path by deposits</li> <li>2. Over-tightened fittings</li> <li>3. Pinched tubing</li> <li>4. Obstruction of detector flow-cell</li> <li>5. Faulty back-pressure regulator</li> </ol> | First, determine the exact location of the blockage. Next, disconnect one fitting at a time, moving backward from the back-pressure regulator end, until the pressure drops             | A: For partial blockage, clean tubing with solvent/water<br>B: For total blockage, replace appropriate part |
| Reagent pump stops or delivers wrong flow rate |   | <ol style="list-style-type: none"> <li>1. Check pump setting</li> <li>2. Check reagent pressurization</li> <li>3. Check pump seal for leakage</li> <li>4. Clean check valves</li> </ol> |   |
| Over-pressure relief valve is opened           | There is a blockage in the system   | First, determine the exact location of the blockage. Next, disconnect one fitting at a time, moving backward from the back-pressure regulator end, until the pressure drops             | Flush the system with solvent/water until pressure drops, or replace appropriate part                       |
| Blocked Heated Reactor reactor                 | <ol style="list-style-type: none"> <li>1. Improper Shutdown</li> <li>2. Deposits in the reactor</li> <li>3. Contaminated reagents</li> <li>4. Use of CaOCl vs NaOCl</li> </ol>  | Follow the procedure for removing mineral or silica deposits  |   |

## Common Chromatography Problems

| OBSERVED PROBLEM   | COMMON CAUSE   | ACTION TO TAKE   | NOTES  |
|--|--|--|--|
| High Background Signal   | <ol style="list-style-type: none"> <li>1. Contaminated Eluant</li> <li>2. Bacterial Growth</li> <li>3. Fingerprints</li> <li>4. Contaminated Reagent(s)</li> <li>5. Defective chemicals</li> </ol>   | Wash all reservoirs and flush lines  | The post column reactions are very sensitive to even minute contaminations   |
| Noisy Baseline   | <ol style="list-style-type: none"> <li>1. Worn pump seal</li> <li>2. Faulty check valves</li> <li>3. Detector noise</li> <li>4. Chemical contamination</li> <li>5. Reagent too old</li> </ol>  | <ol style="list-style-type: none"> <li>1. Check for pattern in the noise.</li> <li>2. If the background signal is also elevated, check for chemical contamination, or an error in formulation</li> </ol>   | <p>Match the frequency of the noise to one of the pumps.</p> <p>If the noise is random, check the detector</p>   |
| Peaks disappear or diminish  | <ol style="list-style-type: none"> <li>1. OPA reagent expired</li> <li>2. Improper Reagent preparation</li> <li>3. Out of Hydrolysis Reagent</li> <li>4. Reactor at wrong temperature</li> <li>5. Reagent flow rate improper</li> <li>6. Dirty flow cell</li> <li>7. Dirty auto sampler</li> <li>8. Deteriorated samples</li> <li>9. Metal contamination of column</li> <li>10. Oxidized TRIONE or OPA</li> <li>11. Reagent pump mis-adjusted</li> </ol> | <ol style="list-style-type: none"> <li>1. Prepare fresh reagent</li> <li>2. Prepare fresh standards from neat reference material.</li> <li>3. Test with a second fluorescent detector.</li> <li>4. Change the rotor seal of the auto sampler or use a manual injector.</li> <li>5. Flush amino acid column with Li220 or Na220</li> <li>6. Check reactor temperature</li> <li>7. Check for oxidized reagent</li> <li>8. Clean the flow cell</li> <li>9. Follow the procedure for Iron removal</li> <li>10. Remove all stainless steel frits from reservoirs</li> <li>11. Clean or replace any corroded parts.</li> </ol> | <p>A: All disappear except 1-naphthol and carbaryl = OPA reagent expired</p> <p>B: All disappear except 1-naphthol = Out of Hydrolysis Reagent</p> <p>C: Varied peak size, some missing = Reactor at wrong temperature</p> <p>D: All peaks diminish = dirty flow cell, auto sampler, or deteriorated samples</p> <p>E: Iron contamination can be caused from samples, long column storage, stainless steel frits in the eluant reservoirs, corrosion in system</p> |
| Retention times not stable, especially in early part of chromatogram | <ol style="list-style-type: none"> <li>1. Equilibration time too short</li> <li>2. Too much internal volume</li> <li>3. Leaking proportioning valve</li> <li>4. Auto sampler problems</li> </ol>   | <ol style="list-style-type: none"> <li>1. Increase equilibration time by 2 minute increments</li> <li>2. Re-plumb system with ZDV* connections or 0.011" ID tubing</li> </ol>  |  |
| Artifacts in Baseline  | <ol style="list-style-type: none"> <li>1. Contamination in Eluant reservoir</li> <li>2. Corrosion of spargers/filters</li> <li>3. Volatile amines used and worn in laboratory</li> </ol>   | <ol style="list-style-type: none"> <li>1. Replace eluants</li> <li>2. Clean reservoir with soap and water</li> <li>3. Remove spargers/ or eluant filters</li> </ol>  |  |
| Retention Times drift over a long time                               | <ol style="list-style-type: none"> <li>1. Buildup of contaminants</li> <li>2. Room temperature changes greatly with the seasons</li> </ol>   | <ol style="list-style-type: none"> <li>1. Flush the column</li> <li>2. Air condition the room</li> </ol>   |  |

\* ZDV = Zero Dead Volume

## Common Column Problems

| OBSERVED PROBLEM              | COMMON CAUSE   | ACTION TO TAKE  | NOTES  |
|-------------------------------|--|---|--|
| Loss of Resolution            | <ol style="list-style-type: none"> <li>1. Column worn out</li> <li>2. Guard column dirty</li> <li>3. Pre-column filter dirty</li> <li>4. Bad tubing connection: wrong style nut, too large tubing, wrong type union</li> </ol>   | <ol style="list-style-type: none"> <li>1. Replace any pre-column filters</li> <li>2. Flush or replace guard column</li> <li>3. Flush column</li> <li>4. Replace column</li> </ol>   | <p>A: Usually only the guard column is dirty</p> <p>B: Always use ZDV when making any connections</p>  |
| Poor Peak Shape - General     | <ol style="list-style-type: none"> <li>1. Column worn out</li> <li>2. Guard column dirty</li> <li>3. Pre-column filter dirty</li> <li>4. Deposits in post-column flow path</li> <li>5. Partial obstruction of flowcell</li> <li>6. Too strong a solvent Too large a sample injected.</li> <li>7. Reagent flow rate too high.</li> <li>8. Improper tubing connection.</li> <li>9. Protein contamination on amino acid column</li> </ol> | <ol style="list-style-type: none"> <li>1. Start by replacing filter, then guard.</li> <li>2. Replace column as last resort.</li> <li>3. If new tubing connections have been made, check connections.</li> <li>4. Ensure that any protein is completely removed from column</li> </ol> | <p>A: Send a chromatogram to Pickering Laboratories' Technical Support Department</p> <p>B: Improper tubing connections are: wrong style nut, too large tubing, wrong type union, improper swaging of ferrule.</p> <p>C: Reverse and flush ion-exchange column at elevated temperature</p> |
| Reagent backflows into column | <ol style="list-style-type: none"> <li>1. Improper Shutdown procedures</li> <li>2. Improper maintenance procedures</li> <li>3. Leaking fittings between column and HPLC pump</li> </ol>  | <p>If NaOH is on column, follow the cleaning procedure</p> <p>If organic solvent is on the Glyphosate column, follow cleaning the procedure</p> <p>If Reagent backflows onto column, follow the procedure below</p>   |  |
| High Column pressure          | <ol style="list-style-type: none"> <li>1. Blocked pre-column filter or column inlet tubing</li> <li>2. Guard column is blocked.</li> <li>3. Worn HPLC seal or rotor seal.</li> <li>4. Particulate matter in eluant reservoirs</li> <li>5. Column is damaged</li> <li>6. Organic solvent in ion exchange column</li> <li>7. NaOH on Carbamate column</li> <li>8. Excessive eluant flow rate through column</li> </ol>                   | <ol style="list-style-type: none"> <li>1. For reagent back-flow onto column, see below.</li> <li>2. If the column back-pressure is high (&gt; 2000psi), isolate the source of the high pressure and replace appropriate part</li> </ol>   | <p>Unfiltered samples<br/>Pressure from filter and guard should be &lt; 200psi).</p> <p>Organic contaminants can be washed off carbamate column by first washing with methanol then with dichloromethane. Wash again with methanol before use</p>  |

## Application Specific Troubleshooting

### CARBAMATES

|                                       |   |                               |  |
|---------------------------------------|---|-------------------------------|--|
| Grease deposits in the heated reactor | Fatty samples used in carbamate analysis. | Follow the cleaning procedure |  |
|---------------------------------------|---|-------------------------------|--|

### GLYPHOSATE

|   |   |   |  |
|---|---|---|--|
| Glyphosate peak is a doublet                        | Improperly buffered samples   | Add 2–4 µL Glyphosate RESTORE to the sample                 | RESTORE Cat. No. 1700-0140                   |
| Glyphosate and AMPA peaks are late and broad        | 1. Iron contamination of Column<br>2. Extremely large ID injection loop                                       | Follow the procedure for pumping RESTORE through the column | Replace the large ID loop with a smaller ID. |
| Glyphosate peak too small or gone, but AMPA present | 1. Oxidizing reagent too weak, too old,<br>2. NaOCl stock solution too old<br>3. Reactor at wrong temperature | Make fresh Oxidizing reagent                                |  |
| AMPA peak disappears, but Glyphosate present        | Oxidizing reagent too strong  | Make fresh Oxidizing reagent                                |  |

## Reagent Pump Troubleshooting

| YOU NOTICE  | THIS MAY MEAN  | POSSIBLE CAUSE   | YOU SHOULD  |
|---|--|--|---|
| 1. Uneven pressure trace.<br>2. Pressure drops.<br>3. No flow out the outlet check valve.           | 1. Bubble in check valve.<br>2. Leaks in system.<br>3. Dirty check valve.<br>4. Bad check valve. | 1. Solvent not properly degassed.<br>2. Fittings are not tight.<br>3. Mobile phase not properly filtered.<br>4. Particles from worn piston seal caught in check valve.<br>5. Plugged inlet filter. | 1. Check to be certain that mobile phase is properly degassed.<br>2. Check connections for leaks by tightening fittings.<br>3. Prime the system directly from the outlet check valve.<br>4. Clean or replace the check valves. See Section 5.4.<br>5. Clean or replace inlet filter. See Section 5.1.1. |
| 1. Uneven pressure trace.<br>2. Pressure drops.<br>3. Fluid between the pump head and the retainer. | 1. Leaks in system.<br>2. The piston seal or diaphragm is worn.                                  | 1. Fittings not tight.<br>2. Long usage time since last seal / diaphragm change.<br>3. Salt deposits on seal or diaphragm (especially if buffered aqueous mobile phases are used).                 | 1. Check all connections for leaks.<br>2. Replace piston seal & diaphragm. See Sections 5.2 and 5.3.<br>3. Check the piston for salt deposits. Clean as necessary. See Section 5.2.4.   |
| Pump makes a loud clanging or slapping noise (intermittent contact with cam).                       | Piston carrier is catching in piston guide.  | 1. Cap nut screws on the pump head are loose.<br>2. Seal(s) are worn.<br>3. Piston guide is worn   | 1. Check cap nut screws on pump head. Tighten if necessary.<br>2. Replace seals.<br>3. Replace piston guide and seals. See Sections 5.2 and 5.3.  |

**Reagent Pump Troubleshooting** *continued*

| <b>YOU NOTICE</b>   | <b>THIS MAY MEAN</b>   | <b>POSSIBLE CAUSE</b>  | <b>YOU SHOULD</b>  |
|---|--|--|--|
| No power when pump turned ON.   | Blown fuses in the power entry module.   | 1. Power surge.<br>2. Internal short.  | 1. Replace only with the appropriate fuses (1A for 100/110 Vac or 1/2A for 220/240 Vac).<br>2. Contact service technician if problem persists.   |
| Blue dye in mobile phase.   | Pulse damper diaphragm has burst.  | Sudden pressure drop when purging system.  | Replace pulse damper. See Section 5.5.   |
| Pump runs for 50 pump strokes, then shuts down.   | Lower pressure limit is activating.  | 1. Mobile phase is not properly filtered.<br>2. Particles from worn seal trapped in the system (e.g., tubing, filters, injection valve, column inlet). | 1. Check to be certain the low pressure limit is set to 0 psi.<br>2. Only increase the low pressure limit after the pump attains operating pressure.<br>3. Contact service technician.   |
| 1. Pump shuts down after run is called even with no column connected.<br>2. Pump runs to maximum pressure and shuts down. | Clog in fluid system.  |  | 1. Remove and clean both the inlet and bulkhead filters. See Section 5.2.<br>2. If the problem persists, remove tubing from system one piece at a time until you find the clogged piece. Most clogs occur outside the pump itself. |
| No power when pump turned ON. Fan does not run.   | Blown fuses in the power entry module.   | 1. Power surge.<br>2. Internal short.  | 1. Replace only with the appropriate fuses (1A for 100-120 Vac or 1/2A for 220-240 Vac).<br>2. Contact service technician if problem persists.   |
| Front panel appears OK but pump motor does not run.   | Blown fuse on the motor power circuit board.                                     | 1. Power surge.<br>2. Internal short.  | 1. Replace only with the appropriate fuse.<br>2. Contact service technician if problem persists.   |
| PEEK fittings or components leak.   | You cannot force PEEK parts with interference to seal by brute force tightening. | 1. Film of fluid between surfaces.<br>2. Salt crystals between surfaces.<br>3. Scratches in mating surfaces.   | 1. Clean and dry mating surfaces.<br>2. If scratched, replace defective part.  |

---

## Procedures

To clean the reactor, start by setting the HPLC to a flow rate that is low enough to ensure that the over-pressure relief valve remains closed.

Depending on what you suspect may be causing the blockage, you should use either a methanol solution, or a 20% nitric acid solution. See below for more details.

Usually, however a methanol solution is enough to clean the reactor.

### **TO REMOVE SILICA DEPOSITS FROM REACTOR**

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.

If NaOH backflows onto a carbamate column, it can dissolve the silica and cause it to flow into the post-column reactor. 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.

### **TO REMOVE MINERAL DEPOSITS IN THE REACTOR FROM HARD WATER**

The Pickering pumps and most (but not all) HPLC pumps will tolerate this. Columns and autosamplers probably will not tolerate this.

- 1) Start HPLC pump at < 0.5 mL/min (100% H<sub>2</sub>O).
- 2) Replace both post-column reagents with deionized water. Run post-column pumps for 5–10 min.
- 3) Stop post-column pumps. Replace deionized water with 20% nitric acid and run post-column pumps for 10–15 min.
- 4) Reverse the order of washing with water and then replace with the post-column reagents.

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.

Start HPLC pump at < 0.5 mL/min (100% H<sub>2</sub>O).

Replace both post-column reagents with deionized water. Run post-column pumps for 5–10 min.

Stop post-column pumps. Replace deionized water with 20% nitric acid and run post-column pumps for 10–15 min.

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.

#### **TO REMOVE GREASE DEPOSITS**

Grease deposits can be dissolved by turning off the post-column pumps and pumping methanol through the HPLC system. Stronger solvents such as acetone, methylene chloride, or tetrahydrofuran (THF) may be needed. If methylene chloride is used, be certain to flush the system thoroughly with methanol before and after because methylene chloride is not miscible with water. There is no need to disconnect the carbamate column.

Grease deposits (as can be found in vegetation samples) can be dissolved by turning off the post-column pumps and pumping methanol through the HPLC system. Stronger solvents such as acetone, methylene chloride, or tetrahydrofuran (THF) may be needed. If methylene chloride is used, be certain to flush the system thoroughly with methanol before and after because methylene chloride is not miscible with water. There is no need to disconnect the carbamate column.

#### **IF REAGENT BACKFLOWS ONTO COLUMN**

This procedure usually works but may not work every time.

- 1) Shut down the PCX5200 and remove the analytical and guard columns.
- 2) Flush out all organic solvents from the LC and injector.
- 3) Flush both columns with regenerant. Use a very slow flow rate so that the back pressure does not exceed 2000 psi.
- 4) Keep flushing until the pressure drops. Keep raising the flow rate until the pressure is normal at 0.40 mL/min and 55°C.
- 5) Reinstall the analytical (in reversed-direction) and guard column (normal direction) and test them.

#### **IF TRIONE BACKFLOWS ONTO COLUMN**

Pressure drop at head of column prior to release of pressure post-column. Flush analytical column with regenerant into a waste beaker.

#### **IF NAOH IS ON COLUMN**

- 1) Do not restart the system. Dissolved silica or C18 phase will re-precipitate in the post-column reactors, or flowcell. These additional complications then require replacement of both reactor coils as well as your column.

- 2) Immediately depressurize the post-column system by loosening the “To Detector” fitting.
- 3) Disconnect the outlet of the column.
- 4) Restart the HPLC pump to flush the column with 100% MeOH for 20 minutes. Complete steps 2–4 as quickly as possible because the longer the hydroxide stays inside the column, the less chance that the column will survive.
- 5) Catch the effluent from the column with paper towels. Alternatively, connect the outlet of the column to a piece of spare tubing directing the effluent to waste.
- 6) Turn off the HPLC pump and reconnect the outlet of the column and the “To Detector” fitting.
- 7) Turn on the HPLC and post-column system and run a calibration standard. Pay special attention to the first four peaks. If these four peaks are not resolved, the column needs to be replaced.

**TO REMOVE IRON CONTAMINATION FROM COLUMN**

Flush guard and column with the Glyphosate Restore solution.

**TO PUMP RESTORE THROUGH THE GLYPHOSATE COLUMNS**

Usually only the guard column is contaminated. We suggest you buy a spare guard column to minimize downtime.

- 1) Remove the analytical column after ensuring no residual post-column pressure.
- 2) 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.
- 3) Pump K200 eluant through the guard long enough to displace RESTORE.
- 4) Reconnect the column and guard in the normal directions and restart the HPLC and post-column systems.

*Section 6*  
**APPLICATIONS**

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**6.2 CARBAMATES**

**6.3 GLYPHOSATE**

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## CARBAMATES

- 6.2 Introduction
- 6.2 Background
- 6.2-1 Basic Sample Preparation
- 6.2-2 Reagent Preparation
- 6.3-5 Post-column Conditions
- 6.3-5 Procedure
- 6.3-5 Sample Chromatograms and Gradient Programs
- 6.3-10 Precautions

### 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 Vector PCX was developed to facilitate the determination of carbamate insecticides (5 $\mu$ m C<sub>18</sub> column), meeting or exceeding performance requirements for precision and accuracy of U.S. Environmental Protection Agency (USEPA) Method 531.2, and the AOAC International Protocol 29.A05:

- 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.

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 5 $\mu$ m C<sub>8</sub> column can separate as many as 23 compounds.

### Background

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 by-products 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.

The general structure of the carbamate insecticides is an N-methyl substituted urethane with the variation in the ester moiety. The structural formulas are shown in Figure 6.3-A.

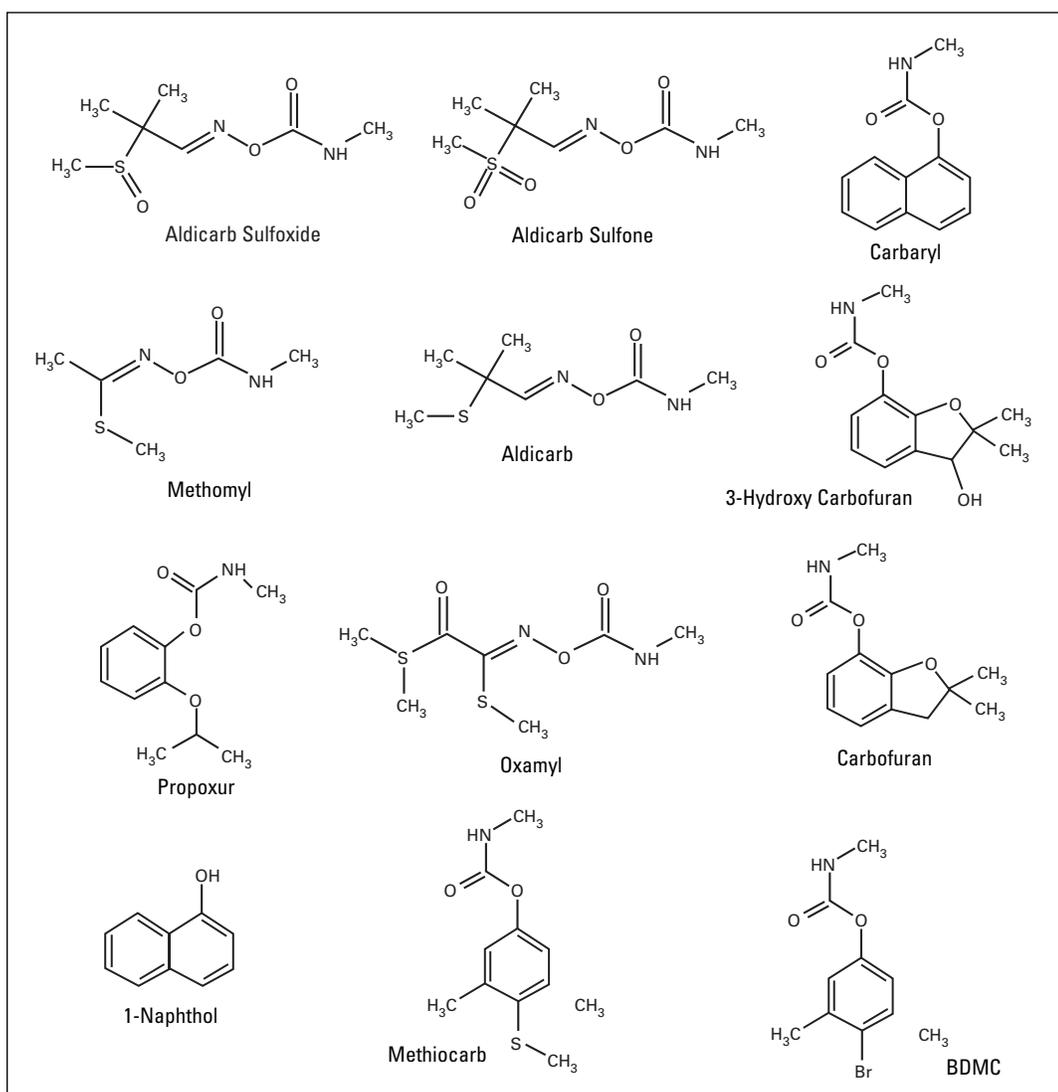


Figure 6.3-A



**FOR VEGETABLE SAMPLES**

Extract the carbamates in ACN  
Reconstitute in methanol  
Filter through a 0.45µm membrane filter

**FOR WATER SAMPLES*****Sampling Protocol***

To preserve the Carbamates in water, this procedure should be carried out in the field.

**EPA SAMPLING PROTOCOL**

1. Add 1.8 ml of ChlorAC Buffer to each pre-cleaned 60 ml sample vial (see note about well and river waters!)
2. If the water sample is chlorinated, dechlorinate with 5 mg of Sodium thiosulfate per 60 ml sample.
3. Fill the sample vials with the dechlorinated water, seal, and mix well.
4. Maintain the samples at 4 °C for transportation, and at -10 °C during storage for up to 28 days.

***Sample Preparation***

Filter 2ml of sample through a 0.45µm filter.  
Inject 200-400µl.

**FOR STANDARDS AND BLANKS**

Use 10 ml ChlorAC Buffer diluted to 1000 ml with HPLC-grade water.

*Note:* Well and river waters contain colloidal iron which would dissolve if samples are preserved prior to filtration only to precipitate out again as the hydroxide in the reactor. For well and river waters, it is recommended to filter the water first through a 0.45µm filter, and then preserve with ChlorAC™.

**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.

**REAGENT 1, HYDROLYSIS REAGENT**

Turn off the inert gas

---

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

The hydrolysis reagent does not require preparation. Pour the hydrolysis reagent (Cat. No. CB130) directly into the reagent reservoir for Reagent 1. It should be labeled Hydrolysis Reagent. Put the cap on the reservoir. Close the vent valve.

The Hydrolysis reagent remains stable indefinitely.

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

#### **REAGENT 2, OPA REAGENT**

Pour 945ml of the OPA Diluent (Cat. No. CB910) into the reagent reservoir. Save approximately 5ml for step 5.

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

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

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

Dissolve 2 g of Thiofluor™ (Cat. No. 3700-2000) in the reserved 5 ml of the OPA diluent from Step 1 and add into the reservoir.

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.

The OPA reagent is sensitive to air oxidation and degrades over time. 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.

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## Post-column Conditions

These are the recommended post-column conditions for carbamate analysis. For the HPLC conditions, refer to the section titled Sample Chromatograms and Gradient programs.

Reagent 1: CB130, Hydrolysis Reagent (NaOH)

Reagent 2: *o*-Phthalaldehyde and Thiofluor™ in CB910 Diluent

Pump 1 Flow Rate: 0.30 ml/min

Pump 2 Flow Rate: 0.30 ml/min

Reactor 1 Volume: 500 µl

Reactor 2 Volume: 100 µl

Reactor 1 Temp: 100°C

Reactor 2 Temp: Ambient

## Analytical Procedure

Allow the column to equilibrate for about 20 minutes under initial conditions.

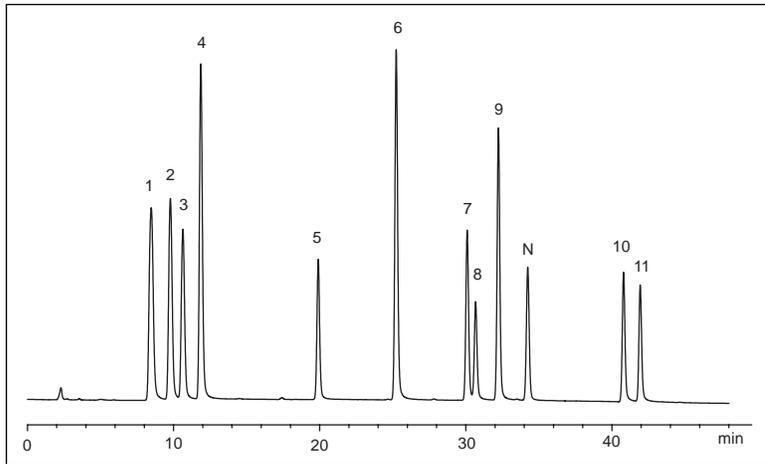
Inject 10µl of Carbamate Test Mixture (or the appropriate volume of your standard), and collect the first chromatogram.

## Sample Chromatograms and Gradient Programs

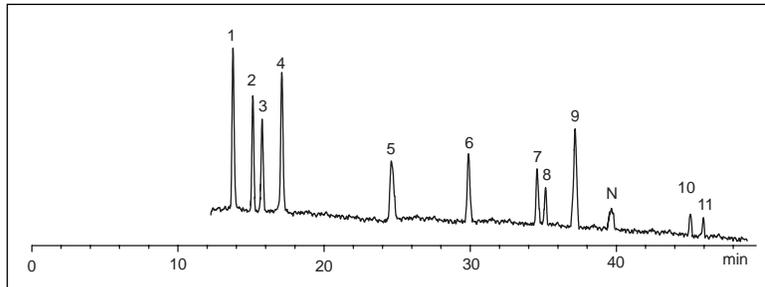
The Peak Names apply to all chromatograms in this section.

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

Carbamate Test Mix, 10ul injection, 25cm, C<sub>8</sub> column (0840250)



Carbamate Test Mix, 0.25ppb, 10ul injection, 25cm, C<sub>8</sub> column (0840250)

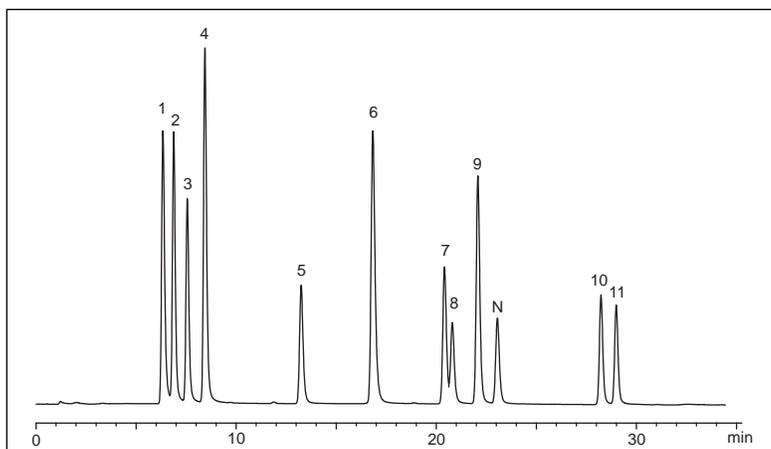


0840250 COLUMN (4.0 MM ID X 250 MM) WITH METHANOLIC SAMPLES

HPLC Flow Rate: 0.8ml/min Column Temperature: 37° C

| 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 methanolic sample |
| 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                     |

Carbamate Test Mix, 10ul injection, 15cm, C<sub>18</sub> column (1846150)

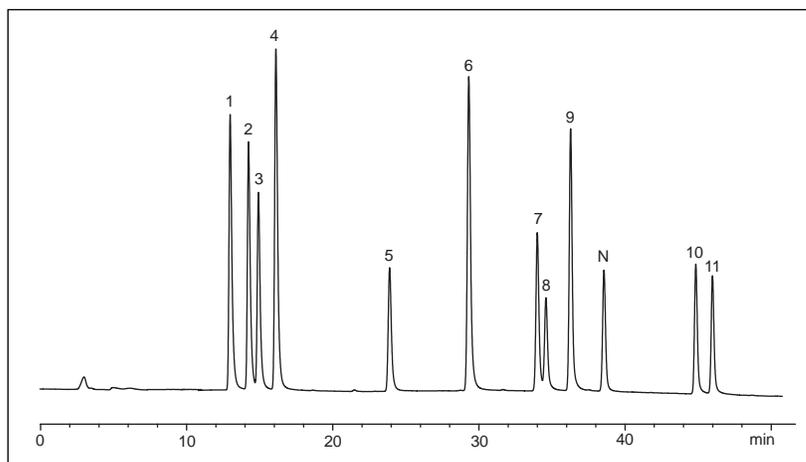


1846150 COLUMN (4.6 MM ID X 150 MM) WITH METHANOLIC SAMPLES

HPLC Flow Rate: 1.0µl/min Column Temperature: 42° C

| Step   | Times(min) | Interval | %Water | %MeOH | Comment                              |
|--------|------------|----------|--------|-------|--------------------------------------|
| Equil. |            |          | 82     | 18    | 1.0 ml/min                           |
| 0      | 0          | 0        | 82     | 18    | inject up to 10 µl methanolic sample |
| 1      | 0–0.5      | 0.5      | 82     | 18    | isocratic                            |
| 2      | 0.5–29     | 28.5     | 30     | 70    | linear gradient                      |
| 4      | 29.1       | 0.1      | 0      | 100   | step change                          |
| 5      | 29–31      | 2        | 0      | 100   | Cleanout                             |
| 6      | 31–        | 5–8      | 82     | 18    | re-equilibration                     |

Carbamate Test Mix, 10µl injection, 25cm, C<sub>18</sub> column (1846250)



1846250 COLUMN (4.6 MM ID X 250 MM) WITH METHANOLIC SAMPLES

HPLC Flow Rate: 1.0ml/min Column Temperature: 42° C

| Step   | Times(min) | Interval | %Water | %MeOH | Comment                              |
|--------|------------|----------|--------|-------|--------------------------------------|
| Equil. |            |          | 85     | 15    | 1.0 ml/min                           |
| 0      | 0          | 0        | 85     | 15    | inject up to 10 µl methanolic sample |
| 1      | 0-1        | 1        | 80     | 20    | isocratic                            |
| 2      | 1-44       | 43       | 25     | 75    | linear gradient                      |
| 4      | 44.1       | 0.1      | 0      | 100   | step change                          |
| 5      | 44.1-49    | 5        | 0      | 100   | cleanout                             |
| 6      | 49-        | 5-8      | 85     | 15    | re-equilibration                     |

Upon completion of the analysis, follow the shutdown procedure described in Section 4. Store the carbamate column in 100% Methanol

*Note:* The inert gas should be left on to preserve the OPA reagent. Close the blue reagent valve on the reservoir to prevent siphoning of the reagent when not in use.

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## Precautions for Carbamate Analysis

Always wear gloves during the preparation of the reagents. The OPA and Thiofluor™ can cause skin irritation. The OPA reagent is sensitive to air oxidation, degrades over time, and should be prepared fresh for optimum sensitivity. OPA reagent maintains its activity for up to two weeks when 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.

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 contaminants that cause unacceptable fluorescence background.)

The water in the solvent reservoir should be changed every 3 to 4 days to prevent possible bacterial growth.

The test mixture for carbamate is for qualitative use only. It is not recommended for calibration purposes.

Filter all samples 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 Method 531.2 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 greater than 300µl, use only the 25cm column. A gradient delay time should be programmed into the analysis (0% organic) to trap the sample onto the head of the column.

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.

Do not store the column in water.

Use the Pickering Laboratories carbamate analysis column, which is specifically designed and tested for the separation of carbamates in the EPA Methods.

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## GLYPHOSATE

- 6.4** Introduction
- 6.4-1** Background
- 6.4-1** Basic Sample Preparation
- 6.4-2** Reagent Preparation
- 6.4-4** Analytical and Post-column Conditions
- 6.4-4** Procedure
- 6.4-5** Sample Chromatograms
- 6.4-6** Precautions

### 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 Vector PCX was developed to facilitate the determination of the herbicide glyphosate (and its metabolite AMPA), meeting or exceeding performance requirements for precision and accuracy of USEPA Method 547.

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.

The Pickering Post-column method can also be used for the determination of Glyphosate and AMPA in plants and soils. Pickering has developed an improved sample preparation procedure for vegetable samples. It is a simple extraction followed by clean-up on a strong cation-exchange cartridge. The procedure is listed later on in this chapter.

## Background

Glyphosate and AMPA are separated on a strong cation-exchange column (fully sulfonated, cross-linked polystyrene, mixed  $K^+/H^+$  form). After isocratic separation, the column is regenerated with dilute KOH, then re-equilibrated with eluant.

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™ (a mercaptan) at pH 9–10 to produce a highly fluorescent isoindole. AMPA does not need the initial oxidation to react with OPA (Figure 6.4-A); indeed oxidation reduces its fluorescent yield.

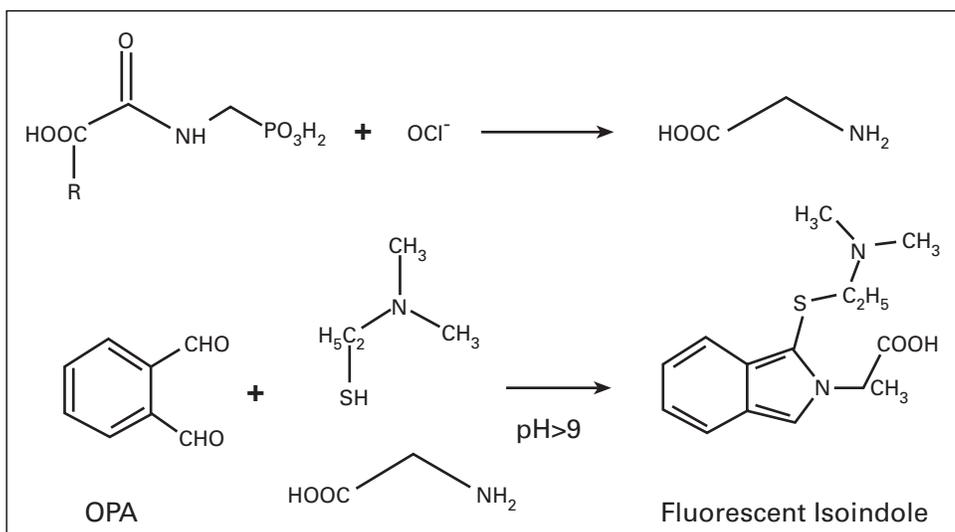


Figure 6.4-A

## Basic Sample Preparation

The following is a suggested basic sample preparation for Vegetable and Water samples containing glyphosate. The method for Vegetables is different from the procedure called out by the AOAC. We have developed ion-exchange cartridges, which we have fully qualified in our lab, and which greatly improve the ease and reproducibility of the extraction while at the same time reducing many of the trouble aspects of the original published method (e.g. iron contamination).

### FOR VEGETABLE SAMPLES

#### Extraction

To 25g of a homogenous sample add enough water (after estimation of moisture content) to make the total volume of water 125 ml. Blend at high speed for 3-5 min. and centrifuge for 10 min. Transfer 20 mL of the aqueous extract into a centrifuge tube and add 15 mL of methylene chloride (to remove nonpolar co-

extractives). Shake for 2-3 min. and centrifuge for 10 min. Transfer 4.5 mL of the aqueous layer into a vial and add 0.50 mL acidic modifier solution (16g  $\text{KH}_2\text{PO}_4$ , 160 ml  $\text{H}_2\text{O}$ , 40 ml Methanol, 13.4 ml HCl). Shake and centrifuge for 10 min.

#### ***Matrix specific modification***

Plants with high: 1) Water 2) Protein 3) Fat Content

- 1) For crops that absorb large amounts of water, reduce test portion to 12.5g keeping water volume the same.
- 2) For crops that have high protein content add 100  $\mu\text{l}$  HCl to 20 ml aliquot of crude extract. Cap, shake and centrifuge for 10 min.
- 3) For crops that have high oil content, do the methylene chloride partition twice.

#### ***Cation-exchange cleanup***

Transfer 1 mL of extract (representing 0.18g normal crop or 0.09g dry crop) to the column reservoir and elute to the top of the resin bed. Add 0.70 mL of the elution solution (160 mL  $\text{H}_2\text{O}$ , 2.7 mL HCl, 40 mL Methanol) and discard the effluent. Repeat with a second 0.70 mL portion and discard effluent. Elute with 12 mL of the elution solution and collect in a round-bottomed flask. Evaporate to dryness in a water bath set at  $40^\circ\text{C}$  using a rotary evaporator. Or collect in a centrifuge tube and evaporate using a vacuum vortex evaporator. Dissolve residue in 2.0 mL of the elution solution (use 1.5 ml for dry crops). Extracts before evaporation can be stored refrigerated for up to 7 days.

#### **FOR WATER SAMPLES**

Filter water through a  $0.45\mu\text{m}$  membrane filter, and inject 200-400  $\mu\text{l}$ .

If the glyphosate comes out as a doublet, add 2 drops of Restore directly to the sample vial.

## Reagent Preparation

### **HYPOCHLORITE REAGENT**

*Note:* 5% Sodium hypochlorite must be used for preparing oxidizing reagent (can be obtained from local grocery stores)

Pour 945 ml of the Hypochlorite Diluent (GA116) directly into the reagent reservoir with the TFE (clear) line from the pump to the cap. This should be labeled Oxidizing Reagent.

Add 100  $\mu\text{l}$  of 5% sodium hypochlorite solution 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

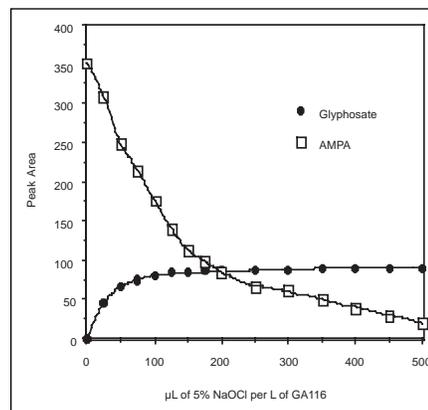


Figure 6.4-B

optimize the relative peak areas of glyphosate versus AMPA. Figure 6.4-B shows a typical response curve. 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;  $\text{Ca}_3(\text{PO}_4)_2$  is insoluble in water.

#### **OPA REAGENT**

Pour 945 ml of the OPA Diluent (Cat. No. GA104) into the reagent reservoir. Save approximately 5 ml for step 5.

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.

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

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

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

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 PCX5200 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 one week without significant loss of activity.

## Analytical and Post-column Conditions

These are the recommended conditions for glyphosate analysis using the 1954150 column and 1953020 guard column.

Column Temperature: 55°C

HPLC Flow Rate: 0.4 ml/min

HPLC Program:

| Times(min) | %K200 | %RG019             |
|------------|-------|--------------------|
| 0          | 100   | 0                  |
| 15.0       | 100   | 0                  |
| 15.1       | 0     | 100 Regeneration   |
| 17         | 0     | 100                |
| 17.1       | 100   | 0                  |
| 27.0       | 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 in GA116 Diluent

Reagent 2: *o*-Phthalaldehyde and Thiofluor™ in GA104 Diluent

Pump 1 Flow Rate: 0.30 ml/min

Pump 2 Flow Rate: 0.30 ml/min

Reactor 1 Volume: 500 µl

Reactor 2 Volume: 100 µl

Reactor 1 Temp: 36°C

Reactor 2 Temp: Ambient

## Analytical Procedure

Allow the column to equilibrate for about 20 minutes under initial conditions.

Inject 10µl of Glyphosate Text Mixture (or the appropriate volume of your standard), and collect the first chromatogram.

Figure 6.4-C shows a typical Glyphosate and AMPA chromatogram. In a standard with Glyphosate and AMPA at equal concentration, the peak heights should be equal. The peak heights are influenced by the amount of hypochlorite in Reagent 1.

### Sample Chromatograms

Glyphosate Test Mix, 10 $\mu$ l injection

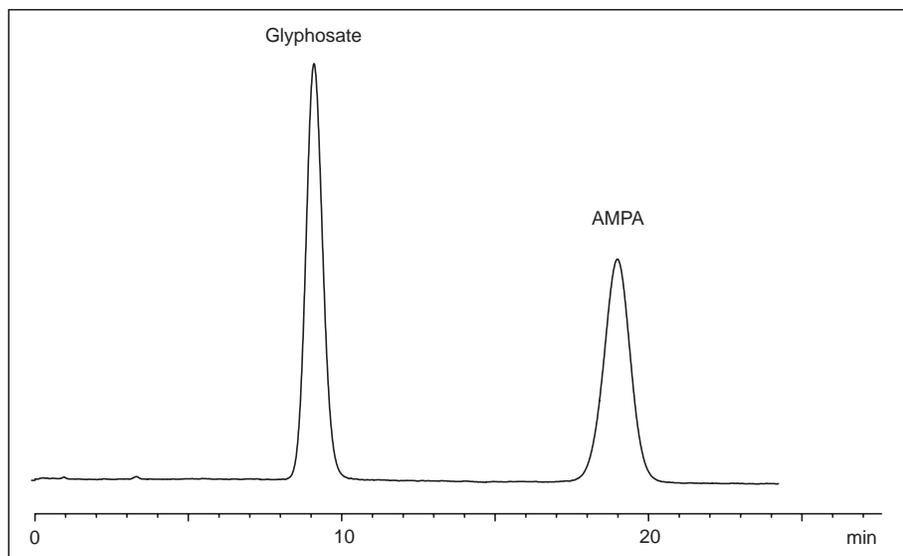


Figure 6.4-C

Glyphosate and AMPA, 13ppb in K200, 100 $\mu$ l injection

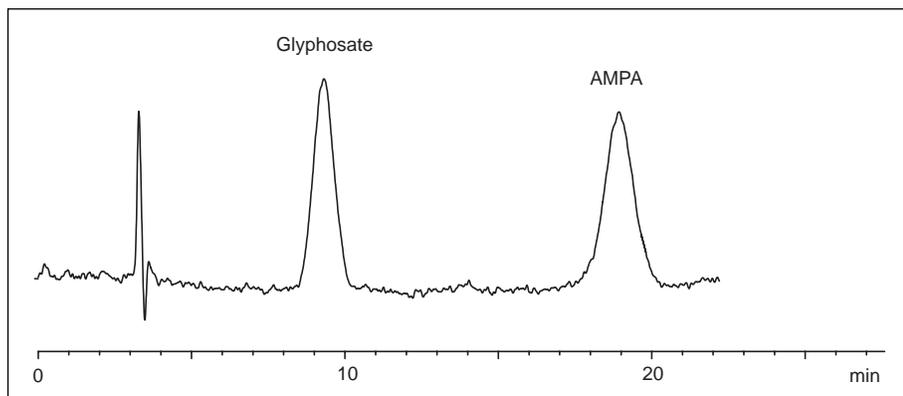


Figure 6.4-D

Broccoli sample spiked with Glyphosate and AMPA, 50 ppb, 100 µl injection

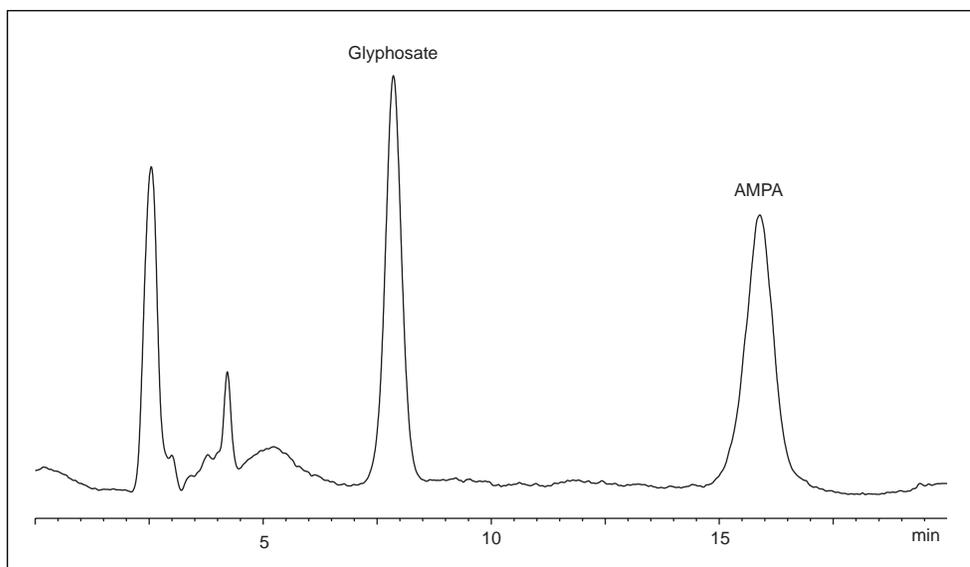


Figure 6.4-E

Upon completion of the analysis, follow the shutdown procedure described in Section 4. Store the column in RG019.

Excessive flushing will require an equally excessive re-equilibration when you start up again.

*Note:* The inert gas should be left on to preserve the OPA reagent. Close the blue reagent valve on the reservoir to prevent siphoning of the reagent when not in use.

### Precautions to be Aware of in Glyphosate Analysis

Always wear gloves during the preparation of the reagents. The OPA and Thiofluor™ can cause skin irritation. 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 one week when 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.

Thiofluor™ is extremely hygroscopic. Always keep in a tightly closed container

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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 test mixture for glyphosate is for qualitative use only. It is not recommended for calibration purposes.

Filter all samples 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 Method 547 for details.

*Section 7*  
**APPENDICES**

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- 7.2** Vector PCX Installation Checklist
- 7.3** IO/OQ Procedure and Checklist
- 7.5** Parts List for Vector PCX
- 7.6** References

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**PICKERING VECTOR PCX INSTALLATION CHECKLIST****COMPLETE AND FAX THIS FORM TO PICKERING LABORATORIES, INC: 650-968-0749**

Vector PCX Serial No: \_\_\_\_\_

User and Company: \_\_\_\_\_

Installation Completed By: \_\_\_\_\_

Date: \_\_\_\_\_

- Unpack the instrument and application kits and ensure all parts and accessories listed are present
- Place the instrument and accessories on bench and ensure there is enough clearance for ventilation
- Open the Vector PCX and ensure none of the components are damaged and that all cables are securely connected.
- Ensure a waste bottle is provided by the user to collect the instrument's waste
- Ensure the system is correctly deadheaded if necessary.
- Ensure that eluant and reagent priming procedure has been carried out correctly before fitting columns
- Ensure the piston wash is filled with 80/20 water/methanol and is connected and the reagent pump seals are wetted
- Ensure there are no leaks anywhere in the system (from HPLC to detector, including the flowcell)
- Ensure power LED indicators function properly on the Vector PCX
- Ensure the pressure transducer on Vector has no bubbles and reads accurate pressure
- Ensure standard runs produce results comparable to the final runs obtained at time of quality control testing
- Ensure the user is trained on Vector PCX and their application and user's maintenance procedures

Please report any problems encountered during the installation and training on a separate sheet.

## Supplement: Installation/Operation Qualification Checklist

### INSTALLATION QUALIFICATION:

Vector PCX Serial No: \_\_\_\_\_

Completed By: \_\_\_\_\_

Date: \_\_\_\_\_

- |  |                              |                             |
|--|------------------------------|-----------------------------|
| Turn on the power to the PCX. Does the power light come on?                                | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Inside the PCX, visually inspect for damage. Is there any?                                 | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| At the gas manifold assembly, is the gas inlet at a pressure of 75 psi?                    | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| At the gas manifold assembly, does the gas flow freely at a low rate into the reservoirs?  | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Check for leaks at the gas connections on the manifold and reservoir. Are there any?       | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Does the Enable light turn orange when there is 500psi of pressure on the Pressure switch? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Press the Enable light when it turns orange. Do the Pump and Reactor Displays appear?      | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Are there any liquid leaks in the system?  | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

### OPERATION QUALIFICATION:

- |  |                              |                             |
|--|------------------------------|-----------------------------|
| With the HPLC pump running, push the PRES key. Is the pressure stable? ( $\pm 10$ bar)   | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Does the reactor temperature heat to the setpoint and remain stable? ( $\pm 1^\circ\text{C}$ )   | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Select Pumps On. Do the pumps start at the flow rate specified?  | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Does the Vector PCX shutdown when the last method of a sequences is completed?   | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Is/are the post column reagent pressure(s) acceptable? (400-700 psi for R1)<br>(400-600 psi for R2)                                    | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Is the Retention Time %CV $\leq 0.5\%$ ?   | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Is the Peak Area %CV $\leq 1.5\%$ ?  | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Is the baseline noise acceptable for the application?<br>(short-term $\leq 500\mu\text{AU}$ for Trione; $\leq 300\mu\text{V}$ for OPA) | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Does the instrument shutdown properly?   | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

Please report any problems encountered during the installation and user's training on aseparate sheet.

## Parts List for Vector PCX

### VECTOR PCX REAGENT PUMP COMPONENTS

| CATALOG NO. | DESCRIPTION  |
|-------------|--|
| 3106-1254   | Pump Check Valve Kit – PEEK (Inlet & Outlet)   |
| 3106-1255   | Seal Kit, 10 mL (Piston seal, Back-up O-ring, Diaphragm, Tool)                           |
| 3106-1256   | Pulse Damper Rebuild Kit (Diaphragm, O-rings, Diaphragm seal, Seal tool, 4 Hex wrenches) |
| 3106-1258   | Prime/Purge Valve - PEEK   |
| 3106-1259   | Head Kit, Vector Pump, (head, check valves, piston and seal)                             |
| 3106-1257   | Piston, Vector Pump, 10 mL   |
| 1452-0176   | Ferrule, PEEK 1/16 x 10-32 S/T, 5 each   |
| 1452-0177   | Nut, PEEK Long 1/16 x 10-32 S/T, 5 each  |
| 3106-1260   | Prime Purge Seal Kit – PEEK  |

### VECTOR PCX SUBSTITUTE REACTOR VOLUMES OR REPLACEMENT REACTORS

| CATALOG NO. | DESCRIPTION   |
|-------------|---|
| 1452-0162   | Reactor Heater & Coil Assembly, 0.5mL 150°C max, 120V               |
| 1452-0163   | Reactor Heater & Coil Assembly, 0.5mL 150°C max, 240V               |
| 1452-0164   | Reactor Heater & Coil Assembly, 1.0mL 150°C max, 120V               |
| 1452-0165   | Reactor Heater & Coil Assembly, 1.0mL 150°C max, 240V               |
| 1452-0166   | Reactor Heater & Coil Assembly, 1.4mL 150°C max, 120V               |
| 1452-0167   | Reactor Heater & Coil Assembly, 1.4mL 150°C max, 240V               |
| 1452-0174   | Reactor Heater & Knitted Coil Assembly, 1.2 & 1.6mL 150°C max, 120V |
| 1452-0175   | Reactor Heater & Knitted Coil Assembly, 1.2 & 1.6mL 150°C max, 240V |
| 1100-2927   | OPA Ambient Reactor, 0.011 in ID TFE Tubing                         |
| 1100-0282   | Coil Assembly, 0.15 mL without Electronics                          |
| 1100-0281   | Coil Assembly, 0.5 mL without Electronics                           |
| 1100-0283   | Coil Assembly, 1.0 mL without Electronics                           |
| 1100-0284   | Coil Assembly, 1.4 mL without Electronics                           |

*Note: Custom volumes are available*

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## Limited Warranty

### **INSTRUMENTS**

Pickering Laboratories, Inc., (Pickering) Instruments 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 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.

### **ANALYTICAL COLUMNS**

Pickering's Analytical Columns are warranted to be free of defects in materials and workmanship under normal installation, use, and maintenance, for the warranted time beginning 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. Columns are warranted for 90 days.

### **HOW TO OBTAIN WARRANTY SERVICE**

If there is a problem with your Instrument or Analytical Column within the Warranty period, do not attempt to repair. Immediately notify Pickering at (800) 654-3330; if calling from outside U.S.A., use (650) 694-6700. If the Instrument or Analytical Column was not purchased directly from Pickering, please contact the vendor where it was purchased. 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.

Pickering Laboratories, Inc.  
1280 Space Park Way  
Mountain View, CA 94043  
U.S.A.

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