

# APPLICATION MANUAL

# Carbamates

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# Chapter 1

## Introduction

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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 PCX5200 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 531.1, 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.

### Post-column Analysis

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

- HPLC binary gradient pump
- Manual injector or autosampler
- Pickering Laboratories columns
- Pickering Laboratories PCX5200 Post-column Derivatization Instrument
- Pickering Laboratories eluants, reagents, and standards
- Fluorescence detector
- Chart recorder, integrator, or data system

### Carbamates

Carbamates, a class of highly effective commercial insecticides, are used worldwide to protect crops from insect pests. Applied directly to food crops such as grains, fruit, and vegetables, carbamates may seep into drinking water sources through agricultural runoff. In addition, if food crops are harvested too soon after application, residues of carbamates and their 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 1-1.

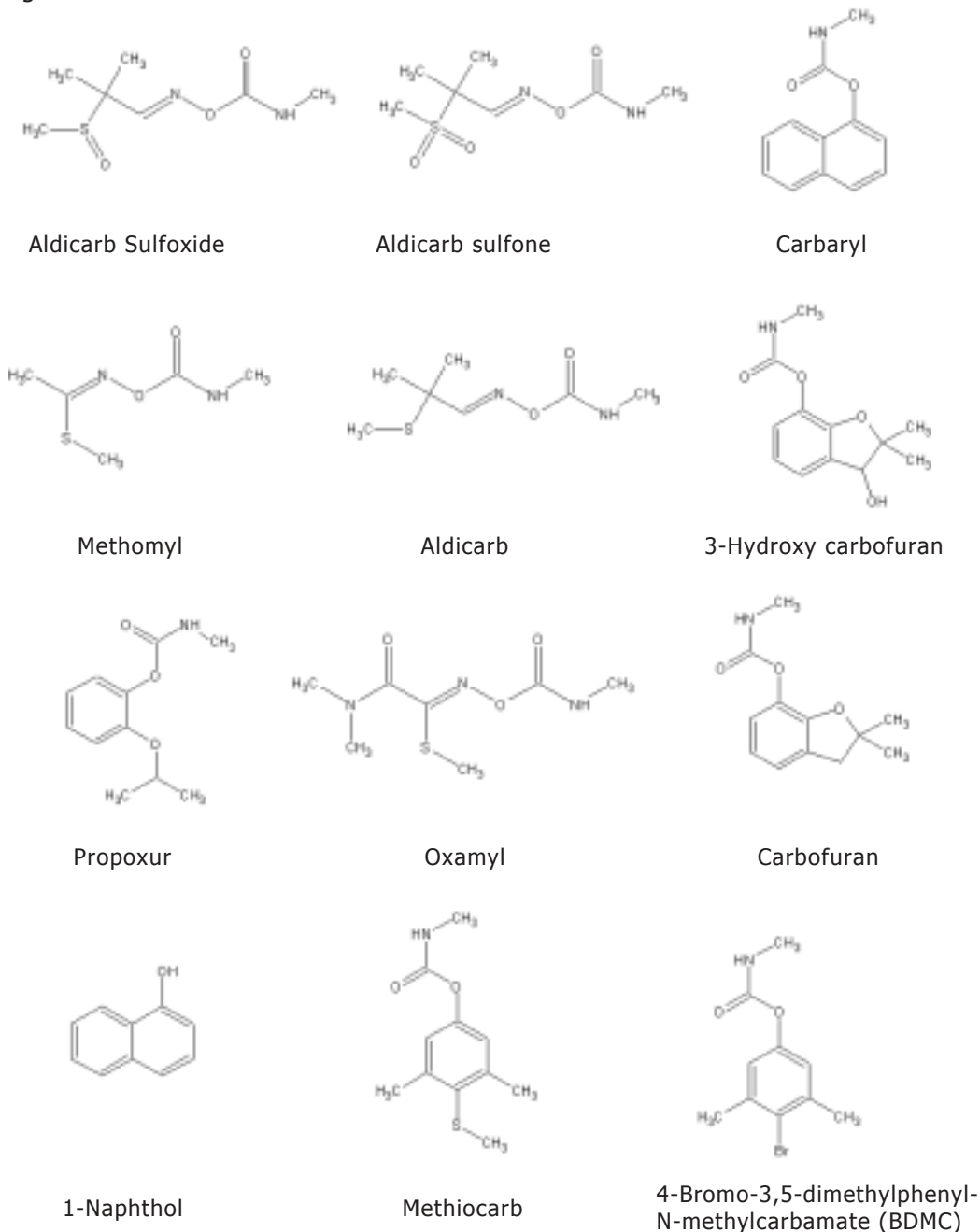
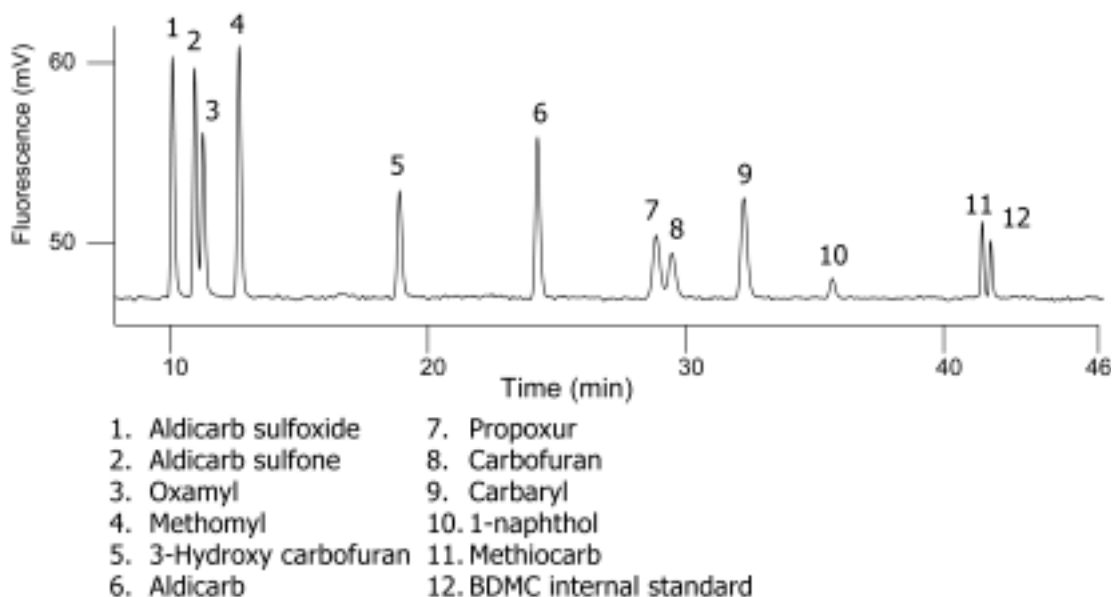


Figure 1-1. Analytes in the Pickering carbamate test mixture.

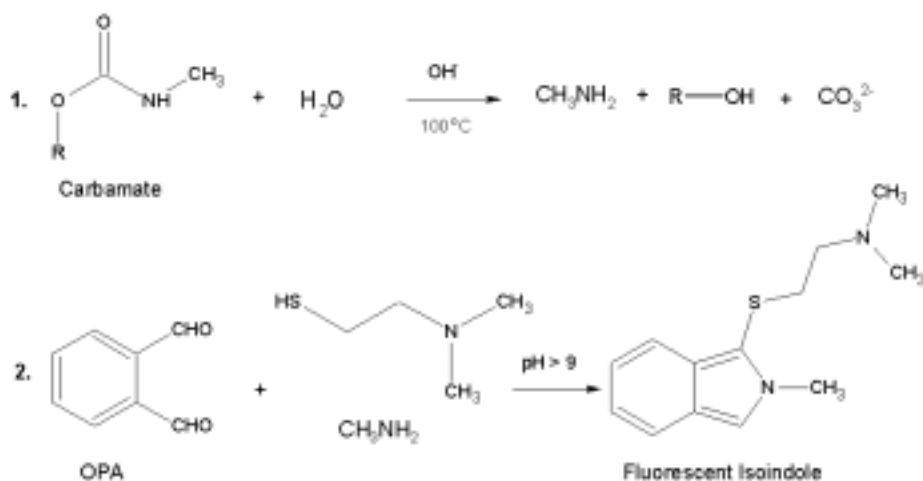
## HPLC Separation

The separation of the 12 carbamates shown in Figure 1-2 is achieved with the Pickering 5 $\mu$ m, C<sub>18</sub> column maintained at constant temperature and a water/methanol gradient. The carbamates elute principally in relation to their relative hydrophobicity. Aldicarb sulfone, which is minimally hydrophobic, elutes early while methiocarb, which is more hydrophobic, elutes towards the end of the gradient.

**Figure 1-2.** 0.4 ng carbamates in 150  $\mu$ L water 25 cm C<sub>18</sub> column

## Post-column Derivatization

The separated carbamates are first saponified by sodium hydroxide (NaOH) at 100°C to release an alcohol, carbonate, and methylamine. In the second post-column reaction, methylamine reacts with *o*-phthalaldehyde (OPA) and the nucleophilic Thiofluor™ to form a highly fluorescent 1-methyl-2-dimethyl-ethylamine thioisindole derivative (Figure 1-3).

**Figure 1-3.** Carbamate derivatization reactions

Note that 1-naphthol fluoresces without derivatization and that the hydrolysis of carbaryl in the post-column reactor also produces 1-naphthol, but at a different retention time. This observation is useful for troubleshooting, see page 3-6.

## Post-column Hardware

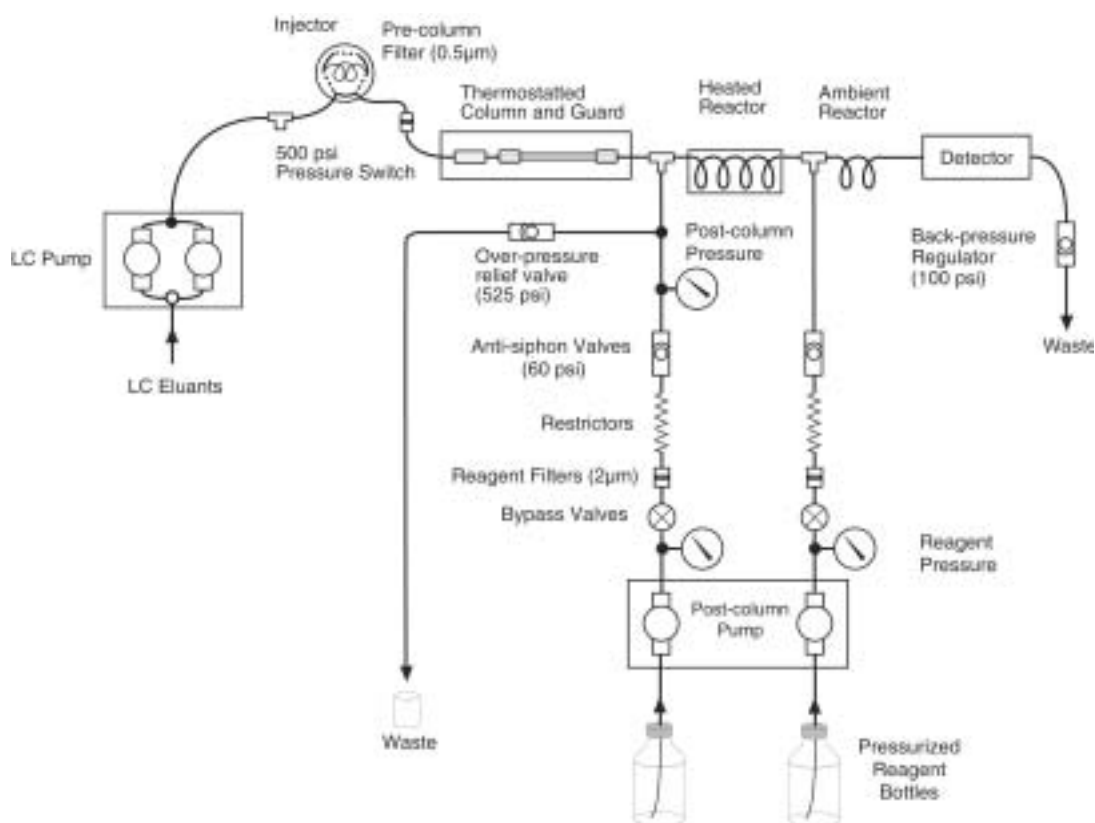
The Pickering design (Figure 1-4) uses a thermostatted column oven, single piston reagent pump, heated reactor coil, and an ambient reactor coil. However, there are many refinements to the system which increase sensitivity and ease of use:

- Pump pulses are eliminated by the mechanical action of the Bourdon tube inside the reagent gauge, and then released through a packed-bed restrictor.

- Elevated reactor temperatures require a back-pressure regulator to suppress boiling inside the heated reactor.
- A post-column gauge monitors pressure at the first mixing tee, which is also the pressure at the first reactor.
- Bypass valves are provided for priming or purging the reagent pumps.
- Pressurized reagent reservoirs allow the pump to operate more precisely at low flow rates, and also provides an inert atmosphere to protect air-sensitive reagents.

Safety systems have also been incorporated into the design to protect against 1) rupture of the reactor because of the excessive pressure and 2) back-flow of caustic reagent onto the analytical column:

- An over-relief valve opens at  $525 \pm 10$  psi (36 bar) and diverts flow away from the reactor.
- A pressure switch ensures eluant flow through the column during operation by enabling the system only when there is a column pressure  $> 500$  psi (34 bar).
- Anti-siphon valves in the reagent delivery system prevent reagents from siphoning when the pump is off.



**Figure 1-4.** Diagram of the Pickering two-reagent post-column system.

## Chapter 2

# Installation & System Operation

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### Site Requirements

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

- Pickering PCX5200 Post-column Derivatization Instrument
- Pickering Carbamate chemical kit (P/N 0352-0002/3/4)
- HPLC manual injector or autosampler
- Binary (or more) gradient HPLC pump
- HPLC fluorescence detector
- Integrator or data system

### HPLC system

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

- The HPLC pump must be capable of binary gradient elution.
- The injector should be able to inject a 10 $\mu$ L sample, preferably by full-loop injection.
- If drinking water is to be analyzed, the injector should be able to inject at least 200 $\mu$ L, and preferably 400 $\mu$ L.
- The pressure rating of the detector flowcell must be >110 psi (7.5 bar) because of an external back pressure regulator. If your detector flowcell is rated lower, consult Pickering Laboratories.
- The HPLC system must be thoroughly clean before using it with the PCX5200. Pay special attention to the cleanliness of eluant reservoirs and delivery tubings. The OPA reaction is very sensitive and contamination is easily noticed in the baseline.
- If the system will also be used for Glyphosate, all materials in the HPLC must be able to tolerate a pH >11. Rotor seals and needle seats must be either **Tefzel** or **PEEK**. The column regenerant is **strongly alkaline**. Vespel rotor seals will NOT tolerate the high pH regenerant used in Glyphosate analysis.



### Space Requirements

Space requirements for the entire HPLC system are determined by the brand of HPLC pump and detector in use. Minimum benchtop space required for the Pickering system is approximately 17 inches (42 cm) long by 17 inches (42 cm) deep.

### Electrical

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

### Gas

Nitrogen, helium, or argon (in order of preference), is required to pressurize the reagent reservoirs. The PCX5200 requires gas pressure of 45–75 psi (3–5 bar) at the gas inlet. An *adaptor* from the gas regulator to 1/8 inch OD tubing is required. To minimize oxidation of the OPA reagent, use oxygen-impermeable tubing for the *entire* gas supply line (\*SARAN® or metal).

\*SARAN is a Registered Trademark of DOW Chemical Corporation.

**Tubing  
Connections**

Unless an HPLC connection kit was purchased with the system, the user will need to provide adequate lengths of capillary tubing to connect HPLC pump and injector to pressure switch (0.010–0.020 inch ID), to detector inlet (0.010 inch ID), to detector outlet (0.010–0.020 inch ID), and to injector outlet (0.007–0.010 inch ID).

**Chemicals  
Application Kit**

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

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

**Supplied by User**

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

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



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



**Note!** HPLC-grade mobile phases are filtered before bottling, so it is unnecessary to filter the mobile phases before use. Filtering with marginally clean glassware has been known to introduce large amounts of contaminating fluorescent compounds to the mobile phases. Degassing the mobile phases with an inert gas prior to operation of the PCX5200 system is recommended for optimum performance.

\*ChlorAC and Chromatographic Grade are Trademarks of Pickering Laboratories.

### Mobile Phase Preparation



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

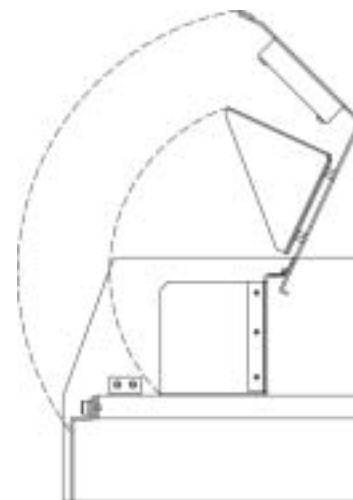
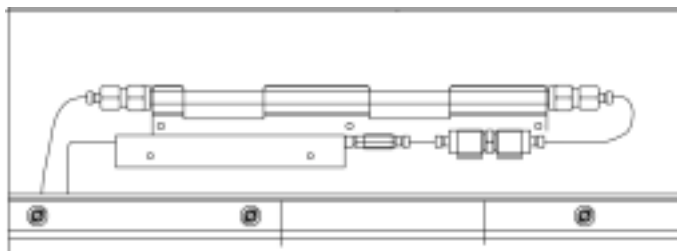
**Caution!** Always wear gloves for this operation. Avoid touching the inside of reservoirs or handling the solvent filters with bare fingers since amino acid contamination present on hands causes high fluorescence background. Do not leave caps and lines dangling without a reservoir. To fill reservoir,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

1. Fill eluant reservoir "B" with HPLC-grade methanol.
2. Fill eluant reservoir "A" with HPLC-grade water.
3. Place the filled eluant reservoirs on or near the HPLC pump.
4. If your HPLC requires it, sparge the eluants with helium.
5. Prime the HPLC pump by withdrawing at least 30 mL of each solvent from the prime/purge port with the priming syringe that is supplied. An HPLC pump method can be configured to facilitate this step. Consult your HPLC manual.
6. Close the prime/purge valve.

### Column Connection

Connect the guard column and 25 cm analytical column according to the diagrams below.

- The heater block is slotted to receive the analytical column (5, 10, 15, and 25 cm long).
  - The last part of the lead-in capillary is embedded in the heating block to preheat the eluant for a more uniform temperature within the column. The lead-in capillary is 0.007 inch (0.17 mm) ID to minimize loss of efficiency.
7. Start the HPLC pump. Pump methanol through the column and system at 0.8ml/min or 1.0 ml/min, depending on the column. Continue pumping until the entire post-column system is primed. The column back pressure should stabilize at approximately 880 psi (60 bar) for the 15 cm column or 1175 psi (80 bar) for the 25 cm column.



### Post-column Reagent Preparation

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



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

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

1. Turn off the inert gas.
2. Thoroughly wash the two reagent reservoirs and then rinse with methanol. Wipe down the dip tubes with methanol and a clean cellulose tissue.
3. The hydrolysis reagent does not require preparation. Pour the hydrolysis reagent (Cat. No. CB130) directly into the reagent reservoir labeled Hydrolysis Reagent (Hydrolysis reagent reservoir cap has TFE lines). Put the cap on the reservoir. Close the vent valve.



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

### OPA Reagent Preparation

4. Preparation of the OPA Reagent:
  - a. Pour 945 mL of the OPA Diluent (Cat. No. CB910) into the reagent reservoir.
  - b. Put the cap on the bottle, open the vent valve, and turn on the gas supply. Thoroughly de-aerate the contents by sparging with inert gas. Continue bubbling for at least 10 minutes.
  - c. Dissolve 100 mg of OPA (Cat. No. O120) in approximately 10 mL of HPLC-grade methanol in a clean, dry container.
  - d. Turn off the gas supply and remove the cap from the bottle. Add the OPA solution to the deoxygenated Diluent in the reservoir.
  - e. Dissolve 2 g of Thiofluor (Cat. No. 3700-2000) in the remaining 5 mL of the OPA Diluent and add into the reservoir.
  - f. Replace the cap and turn on the gas flow. Continue sparging for another minute. Close the vent valve. Gently swirl the reagent to complete the mixing.



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

**Note!** The 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.

## Priming the Reagent Pumps

1. Ensure the reagent and gas supply tubes for the reservoirs are connected to their proper fittings on the right side of the instrument.
2. Connect a 20 mL disposable syringe to the Luer fitting in the center of one of the prime/purge valves.
3. Open the prime/purge valve 1/2 to 1 full turn (CCW) and let the flow exit into the syringe.
4. To purge air bubbles from the reservoir line, pump head, or reagent gauge, syringe suction may be applied. Draw liquid until no bubbles come through.
5. Close the valve, remove the syringe, and wash the Luer fitting with a little water.
6. Repeat the process for the other valve.

If priming the reagent pump is difficult, see page 4-13 of the PCX5200 User's Manual.

## System Set Up PCX5200

1. Turn on the HPLC pump (1 mL/min, 100% methanol) and wait until at least 500 psi (35 bar) of pressure develops.
2. Turn on main power switch in the back of the PCX5200;  
The POWER LED turns green.  
The ENABLE LED turns amber.  
The PUMP LED is off.  
The STATUS LED is off.
3. Press and hold the PRESET key; the LCD shows: "Load preset..."
4. While holding down the PRESET key, press the ▼ key once; the LCD shows: "1 L Carbamate". Let go. The Carbamate temperature settings are now loaded.
5. Check that the column temperature setting is 42°C and the reactor temperature setting is 100°C. Press the COLUMN TEMP key or REACTOR TEMP key on the keypad to view the setpoint and release it to show the actual temperature.
6. With the HPLC on at the analytical flow rate, press the ENABLE key.  
The POWER LED remains green.  
The ENABLE LED turns green.  
The PUMP LED is off.  
The STATUS LED turns amber.
7. Once the temperatures of the heated reactor and column oven reach their setpoints, press the PUMP key.  
The POWER LED remains green.  
The ENABLE LED remains green.  
The PUMP LED turns green.  
The STATUS LED turns green.



**Note!** The two reagent gauges should begin pulsing with a maximum of about 1,000–1,500 psig. The pulsating pressure readings of the reagent pumps (approximately 500 psig swing) are normal and are an essential part of the pulse dampening of the reagent pump.

### Fluorescence Detector

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

### Data Collection

Prepare the HPLC data station or integrator and set up a data handling method to accept data from the fluorescence detector. Initially, an area % method without naming peaks is adequate. This method should have a peak width of about 10 seconds and data end-time of about 35 minutes for the 15 cm column, or a data end time of about 50 minutes for the 25 cm columns. This time can be adjusted after the initial chromatograms are collected.

### HPLC Method

Pickering Laboratories recommends various gradient conditions depending on the column and type of sample. Use the conditions on the following pages for your column and sample type. Note that the exact time of equilibration depends on the internal volume of your HPLC. When the column pressure is stable for at least one minute, the column has been re-equilibrated. Allow the column to equilibrate for about ten minutes under initial conditions. Inject 10  $\mu$ L of Carbamate Test Mixture, and collect the first chromatogram.

### Gradient Conditions

There are two possible gradients for each of the Pickering carbamate columns. There are separate conditions for water and methanolic samples, as well as a water/acetonitrile gradient. When using a C<sub>8</sub> 25 cm column, this gradient is useful for confirmation. The C<sub>18</sub> columns operate at 42°C; the C<sub>8</sub> column at 37°C.



The recommended gradient conditions are subject to change without notice. This may happen because of lot changes in the columns, or improvements in the overall method. **The recommended gradient for the column will always be in the column package, and it supersedes the information in this manual.**

### Post-column Conditions

The post-column conditions apply to all of the Pickering Carbamate gradient programs:

Reagent 1: 0.05 N NaOH (CB130)

Pump 1: 0.30 mL/min

Reactor 1: 500  $\mu$ L at 100°C

Reagent 2: OPA & Thiofluor in pH 9.1 borate buffer

Pump 2: 0.30 mL/min

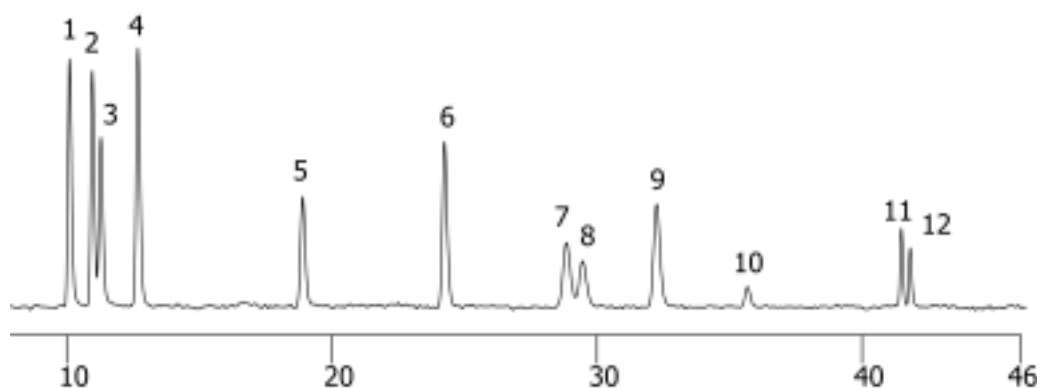
Reactor 2: 100  $\mu$ L at ambient temperature

## Peak Identification

1. Aldicarb sulfoxide
2. Aldicarb sulfone
3. Oxamyl
4. Methomyl
5. 3-Hydroxy carbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbaryl
10. 1-Naphthol
11. Methiocarb
12. BDMC internal standard

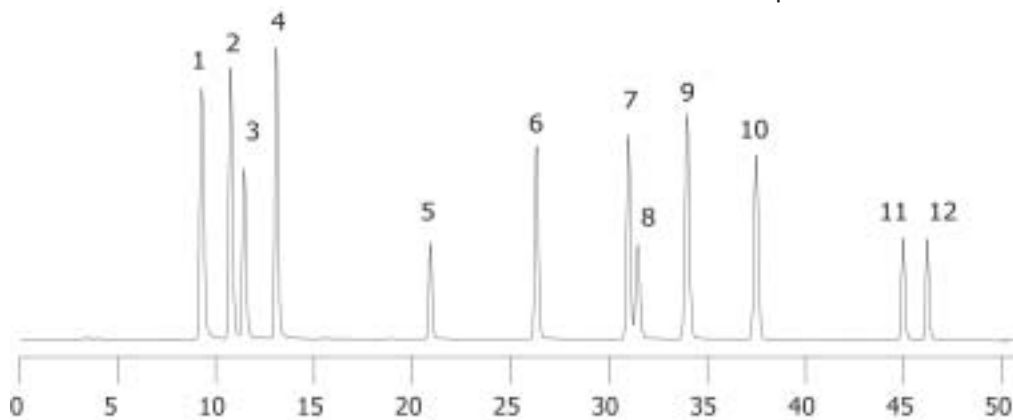
1846250 column (4.6 mm ID x 250 mm) with aqueous samples

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			100	0	1.0 mL/min
0	0	0	100	0	inject up to 400 $\mu$ L water
1	1	1	100	0	concentrate sample on column
2	1.1	0.1	82	18	step change
3	36.0	34.9	30	70	linear gradient
3	39.0	3.0	30	70	isocratic
4	39.1	0.1	0	100	step change
5	41.0	1.9	0	100	cleanout
6	41.1	0.1	100	0	step change
7	55.0	13.9	100	0	re-equilibration



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

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			85	15	1.0 mL/min
0	0	0	85	15	inject up to 10 $\mu$ L methanol
1	1	1	85	15	isocratic
2	44.0	43	25	75	linear gradient
4	44.1	0.1	0	100	step change
5	49.0	5	0	100	cleanout
5	49.1	0.1	85	15	step change
6	57.0	8	85	15	re-equilibration

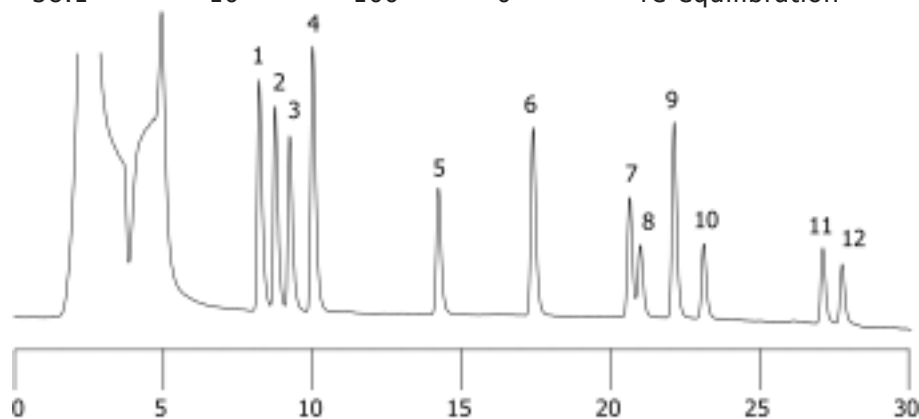


## Peak Identification

1. Aldicarb sulfoxide
2. Aldicarb sulfone
3. Oxamyl
4. Methomyl
5. 3-Hydroxy carbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbaryl
10. 1-Naphthol
11. Methiocarb
12. BDMC internal standard

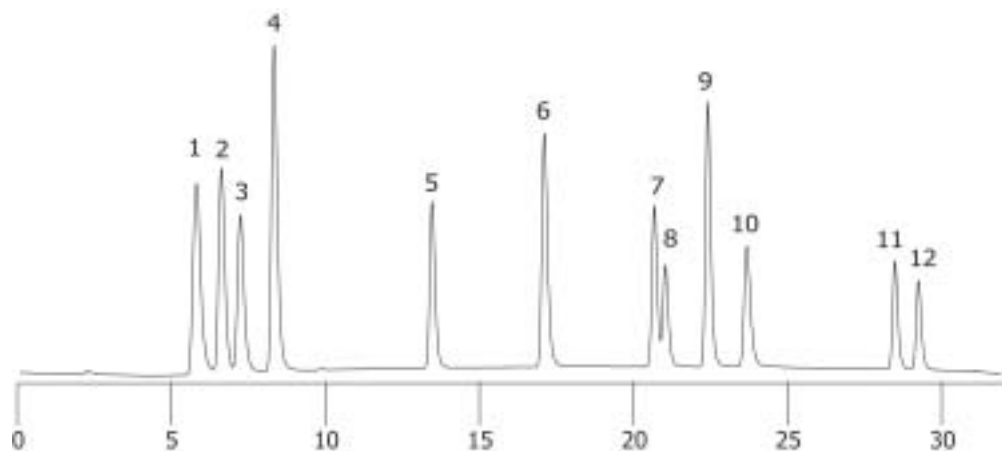
1846150 column (4.6 mm ID x 150 mm) with aqueous samples

Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			100	0	1.0 mL/min
0	0	0	100	0	inject up to 200 $\mu$ L water
1	1.0	1	100	0	concentrate sample on column
2	1.1	0.1	88	12	step change
3	26.0	24.9	30	70	linear gradient
4	26.1	0.1	0	100	step change
5	28.0	1.9	0	100	cleanout
6	28.1	0.1	100	0	step change
6	38.1	10	100	0	re-equilibration



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

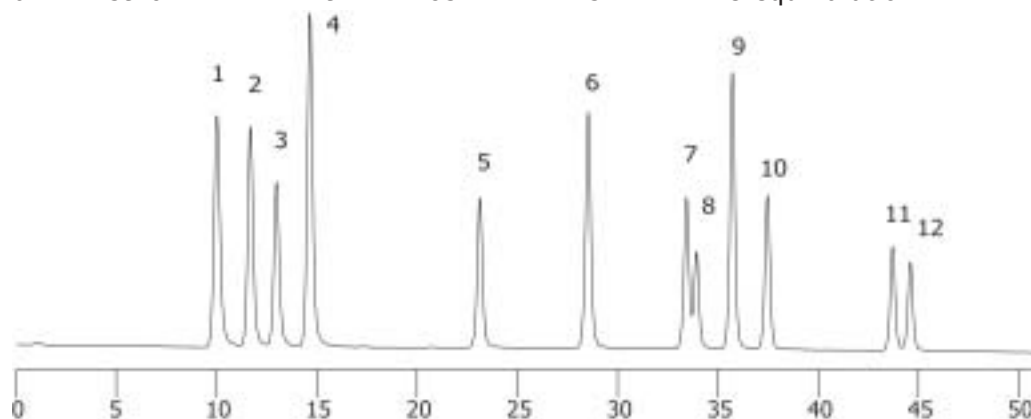
Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			85	15	1.0 mL/min
0	0	0	85	15	inject up to 10 $\mu$ L methanol
1	0.5	0.5	85	15	isocratic
2	28.5	28	30	70	linear gradient
4	28.6	0.1	0	100	step change
5	33.5	5	0	100	cleanout
5	33.6	0.1	85	15	step change
6	41.0	7.4	85	15	re-equilibration



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

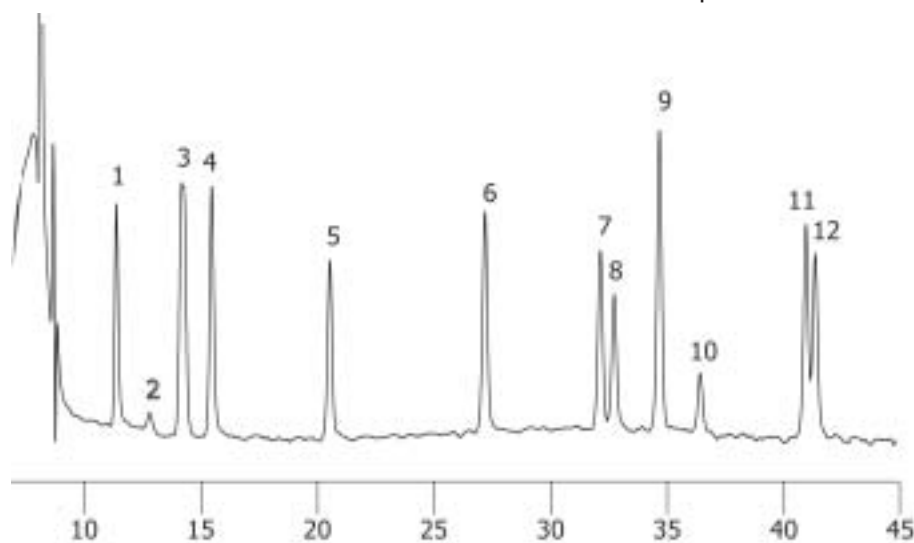
Step	Times(min)	Interval	%Water	%MeOH	Comment
Equil.			85	15	0.80 mL/min
0	2.0	2	85	15	inject up to 10 µL methanol
1	42.0	40	30	70	linear gradient
2	46.0	4	30	70	isocratic
3	46.1	0.1	0	100	step change
4	51.0	4.9	0	100	cleanout
5	51.1	0.1	85	15	step change
6	59.0	12.9	85	15	re-equilibration

1. Aldicarb sulfoxide  
2. Aldicarb sulfone  
3. Oxamyl  
4. Methomyl  
5. 3-Hydroxy carbofuran  
6. Aldicarb  
7. Propoxur  
8. Carbofuran  
9. Carbaryl  
10. 1-Naphthol  
11. Methiocarb  
12. BDMC internal standard



0840250 column (4.0 mm ID x 250 mm) with a water / acetonitrile gradient

Step	Times(min)	Interval	%Water	%MeCN	Comment
Equil.			90	10	0.80 mL/min
0	2.0	2	90	10	inject up to 10 µL methanol
1	46.0	44	49	51	linear gradient
2	46.1	0.1	30	70	step change
4	49.0	2.9	30	70	cleanout
4	49.1	0.1	90	10	step change
5	59.1	10	90	10	re-equilibration





## Shutdown Procedures

**Important!** If the system will not be used immediately after the installation, the system must be shut down properly. Upon completion of the analyses, use one of the following three procedures to shut down the PCX5200 system. These procedures can prevent potential column damage, reaction coil blockage, high background fluorescence, reagent precipitation, or other problems.

### Short Term (up to 3 days)

1. Disable the PCX5200 either manually by pressing the ENABLE key, via the computer interface, or via the "Slowdown" program (see below).
2. Set the HPLC pump at 1 mL/min of methanol to flush the system for at least 10 minutes.
3. Set the HPLC pump to - 0.1 mL/min methanol.
4. Turn off the detector lamp.
5. You may also program a slowdown method to accomplish all the above steps.

Step	Time (min)	%MeOH	Flow (mL/min)
0	0.0	100	0.02
1	5.0	100	0.02
2	7.0	100	1.0 for C <sub>18</sub> , 0.8 for C <sub>8</sub>
3	27.0	100	1.0 for C <sub>18</sub> , 0.8 for C <sub>8</sub>
4	27.1	100	0.02



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

### Medium Term (up to 6 days)

**CAUTION !** The medium term shutdown should be performed prior to any work on the HPLC or PCX5200. Failure to do so could defeat the safety systems.

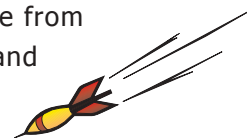
1. Disable the PCX5200 either manually by pressing the ENABLE key, via the computer interface, or via the "Slowdown" program.
2. Set the HPLC pump at 1.0 mL/min (or 0.8 mL/min for C<sub>8</sub> column) of methanol to flush the system for 30 minutes.
3. Replace both reagents with water and draw 10 mL through each prime/purge valve.
4. Replace the water in the reagent reservoir with water / methanol (approximately 80/20).
5. Turn off the fluorescence detector and HPLC pump.
6. Loosen the "To Detector" fitting to relieve pressure in the post-column system. Place paper towels under the outlet to absorb any escaping liquid.
7. Relieve the pressure in the reagent gauges by briefly opening the bypass valves.

Long Term  
(7 days or more)

1. Set the HPLC to pump methanol at 1 mL/min (or 0.8 mL/min for C<sub>8</sub> column).
2. Turn off the reagent pump by pressing the PUMP key.
3. Set the reactor temperature to < 60°C.
4. Turn off the gas at the toggle valve and vent the reservoirs.
5. Replace both reagents with water and draw 10 mL through each prime/purge valve.
6. Replace the water with water / methanol (80/20).
7. Turn the reagent pump on and flush the system until the temperature of the reactor has fallen below 60°C or at least 60 minutes.
8. Turn off the main power of the PCX5200.
9. Relieve the pressure in the reagent gauges by briefly opening the bypass valves.
10. Let the system drain for 1–2 minutes.
11. Turn off the inert gas source.
12. Turn off the HPLC system.
13. Loosen the fitting at the inlet of the 100 psi external back-pressure regulator, relieving pressure on the post-column system. Place paper towels under the back-pressure regulator to absorb any escaping liquid.
14. Remove the column and guard column and plug them (**when removing the column, disconnect the outlet fitting first**). Replace them with a tubing and unions so there are no open lines.

Carbamate/  
Glyphosate  
Systems  
Changing  
from  
carbamate to  
glyphosate

The PCX5200 can be used for carbamate or glyphosate analysis. To change from one to the other, you will need to change the reagents, column, eluants, and temperatures. Refer to the instructions above for the details.



1. Please read page 2-1 for the HPLC System Requirements for **glyphosate analysis!** The HPLC components must be compatible with **high pH regenerant**.
2. Because the reactor is so slow to cool, this is best performed first thing in the morning after the system has been cooling off overnight.
3. Perform the medium-term shutdown at the end of the day before the conversion.
4. Remove the carbamate column and guard column and plug them. When removing the column, disconnect the outlet fitting first.
5. Remove any stainless steel inlet frits or sinkers from the HPLC reservoirs.
6. Change the HPLC eluants from water and methanol to K200 and RG019.
7. Flush the HPLC pump, injector, and the inlet lines of the PCX5200 with K200 and RG019 for at least 30 min at > 1 mL/min **without** the glyphosate column and guard attached. Do not allow methanol into the glyphosate column. Use a pH paper to test the pH of the effluent to determine if the lines are thoroughly flushed. For example, if the HPLC is pumping 100% K200, the pH should be 2; for RG019, the pH is 12.
8. Change the reagents from CB130 and CB910 to GA116 and GA104. The buffering capacity of CB910 is inadequate to neutralize K200, so you must use GA104.
9. Turn off the HPLC pump
10. Install the glyphosate column and guard.
11. Change HPLC program and start the HPLC pump to a **maximum** of 0.4 mL/min of K200.
12. Change the preset program in the PCX5200 to "2 L Glyphosate".
13. Press the ENABLE key.
14. Prime the reagent pumps by drawing 10–20 mL through the bypass valves.
15. Once the temperatures of the heated reactor and column oven reach their set-points, press the PUMP key.
16. Allow the system to equilibrate and flush itself for at least one hour before using it to collect data.



Changing  
from  
glyphosate to  
carbamate

1. Perform the medium-term shutdown.
2. Remove the glyphosate column and guard column and plug them. When removing the column, disconnect the outlet fitting first.
3. Change the HPLC eluants from K200 and RG019 to water and methanol.
4. Flush the HPLC pump, injector, and the inlet lines of the PCX5200 without the carbamate column and guard attached for at least 30 min. Do not allow either of the glyphosate eluants onto the carbamate column. You may also use the pH paper test as described earlier to determine if the eluant lines are thoroughly flushed; in this case the pH should not be pH 2 and pH 12 for eluant lines A and B, respectively.
5. Turn off the HPLC pump
6. Install the carbamate column and guard.
7. Choose a carbamate HPLC program and start the HPLC pump.
8. Change the preset program in the PCX5200 to "1 L Carbamates".
9. Press the ENABLE key.
10. Change the reagents from GA116 and GA104 to CB130 and CB910.
11. Prime the reagent pumps by drawing 10–20 mL through the bypass valves.
12. Once the temperatures of the heated reactor and column oven reach their set-points, press the PUMP key.
13. Allow the system to equilibrate and flush itself for at least one hour before using it to collect data.

**NOTES:**

### Precautions & Problem- prevention General



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

### Mobile Phase

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

### Column Maintenance and Precautions

- Always protect the analytical column by use of the pre-column filter and guard column.
- Check for leaks daily at column fittings. In particular, glyphosate eluants are corrosive.
- If the column back-pressure is high ( $> 2000\text{psi}$ ), isolate the source of the high pressure—guard, analytical column, or the  $0.5\mu\text{m}$  in-line filter. Replace items causing the increased back-pressure (Back-pressure from filter and guard should be  $< 200\text{psi}$ ).
- During shutdown, flush the column with pure methanol. Do not store the column in water.
- The analytical column can be back-flushed with methanol at  $1\text{ mL/min}$  to clear partial blockage. (Do not disassemble or attempt to replace column inlet frit as this will void the column warranty.) Disconnect the outlet of the column during the back flush operation.
- Organic contaminants can be washed off the column by first washing with methanol then with dichloromethane. Wash again with methanol before use.
- The column is temperature-controlled to reduce baseline shift (caused by viscosity changes during gradient formation), to reduce back-pressure, and to improve retention time reproducibility.
- Use the Pickering Laboratories carbamate analysis column, which is specifically designed and tested for the separation of carbamates in the EPA Methods.

The PCX5200 has two safety systems to prevent accidental backflow of reagents onto the column. The pressure interlock requires that the HPLC pump deliver at least  $500\text{psi}$  before the reagent pump can be engaged. The second is a pair of automatic valves that prevent gas pressure from pumping reagents back through the column during extended shutdowns. However, there are ways that the safety systems can be bypassed accidentally. For example, residual pressure in the gauges immediately after shutdown will take some time to leak down to zero. Follow these procedures to avoid such accidents:



- Never disconnect any fittings between the HPLC pump and the column until the post-column system has been shut down and **depressurized** by loosening the fitting at the "To Detector" fitting.
- Any leaking-fittings between the HPLC pump and the column can permit backflow in the event of an unattended shutdown.
- When removing the column, remove the **outlet** fitting first.
- Always follow the proper shutdown procedures. See Chapter 2.

### Sample and Standard Precautions

- The test mixture for carbamates is for qualitative use only. It is not recommended for calibration purposes.
- Filter all sample through a 0.45 $\mu$ m membrane filter. Some samples may require even more stringent filtration, especially if colloids are present.
- Aqueous samples must always be properly buffered. Consult EPA Methods 531.1 for details.
- For carbamate analysis with methanolic samples, inject - 10 $\mu$ L. Large amount of organic solvents can cause peak distortion. For small aqueous sample volumes (< 20 $\mu$ L) either of the two Pickering columns can be used. For volumes greater than 300 $\mu$ L, 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.

### Reagent Precautions

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



### Reactor Precautions

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

### Electrical Precautions

- Always use the correct fuse.

## Most Common Problems with Post-column

### *High post-column pressure—caused by*

- Obstruction of flow path by deposits
- Over-tightened fittings pinching a tube closed
- Obstruction of detector flowcell
- Heat exchanger in detector is too restrictive
- Defective back-pressure regulator

### *High background signal—caused by*

- Contaminated eluant
- Bacterial growth
- Fingerprints
- Water purifier needs service
- Contaminated reagent(s)
- Defective chemicals

### *Reagent backflows into column—caused by*

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

### *Air in reagent pump or flow conditioners—check for*

- Reagent pressure is low
- Some peaks disappear or change relative intensity
- Noisy baseline with 2 second period
- Reagent pressure is low
- Pump takes too long to come up to pressure

### *Poor peak shape—caused by*

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

*Deposits in reactor—caused by*

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

*High column pressure—caused by*

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

*Noisy baseline—check for*

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

*Reagent pump stops or delivers wrong flow rate*

- Check pump setting
- Check reagent pressurization
- Check pump seal for leakage
- Do not open the restrictor. It is supposed to be full of gray-green powder
- Test or clean check-valves

*Peaks disappear or diminish*

1. All disappear except 1-naphthol and carbaryl
  - OPA reagent expired
  - Error in preparing OPA reagent (no thiol, no OPA, wrong pH)
  - Reagent 2 pump air-locked
2. All disappear except 1-naphthol
  - Out of Hydrolysis Reagent
  - Reagent 1 pump air-locked
3. Some peaks small or missing, others normal size
  - Reactor at wrong temperature
  - Mis-adjusted reagent pumps
  - Error in preparing a reagent
4. All peaks diminish, caused by a dirty flowcell, autosampler, or deteriorated samples
  - Test with a second fluorescent detector. If a second fluorescent detector is not available, use an UV-Vis detector set at 330nm absorbance.
  - Change the rotor seal of the autosampler or use a manual injector.
  - Prepare fresh standards from neat reference material. Solution standards, even stored in ampoules, are not reliable (especially when dissolved in acetonitrile!)

*High Reagent Pressure— caused by*

- Dirty reagent filter. Change the frit
- Dirty restrictor. Try cleaning it with either methanol (to remove organic contaminants) or with 3M Nitric acid (to remove inorganics).
- Restrictor has packed down. Replace it.



**NOTE:** If the reagent pressure exceeds 2500 psi for an extended time *and* you have the piston seal wash design of reagent pump, you will need to replace the piston seal after correcting the high pressure. Consult your hardware Operation Manual for details.

**What to do if...** *Reactors or mixing tees have deposits*

- Mineral deposits from hard-water samples or reagents can usually be dissolved by pumping 20% nitric acid through the reactor. The Pickering pumps and most (but not all) HPLC pumps will tolerate this. Columns and autosamplers probably will not tolerate this.
  - a. Start HPLC pump at < 0.5 mL/min (100% H<sub>2</sub>O).
  - b. Replace both post-column reagents with deionized water. Run post-column pumps for 5–10 min.
  - c. Stop post-column pumps. Replace deionized water with 20% nitric acid and run post-column pumps for 10–15 min.
  - d. Reverse the order of washing with water and then replace with the post-column reagents.

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



- Grease deposits can be dissolved by turning off the 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.
- Silica deposits are too hard to remove. Replace the reactor(s). Carefully clean or replace other components in the flow path. You must remove all the silica before the system will work again. This will probably entail major repair.

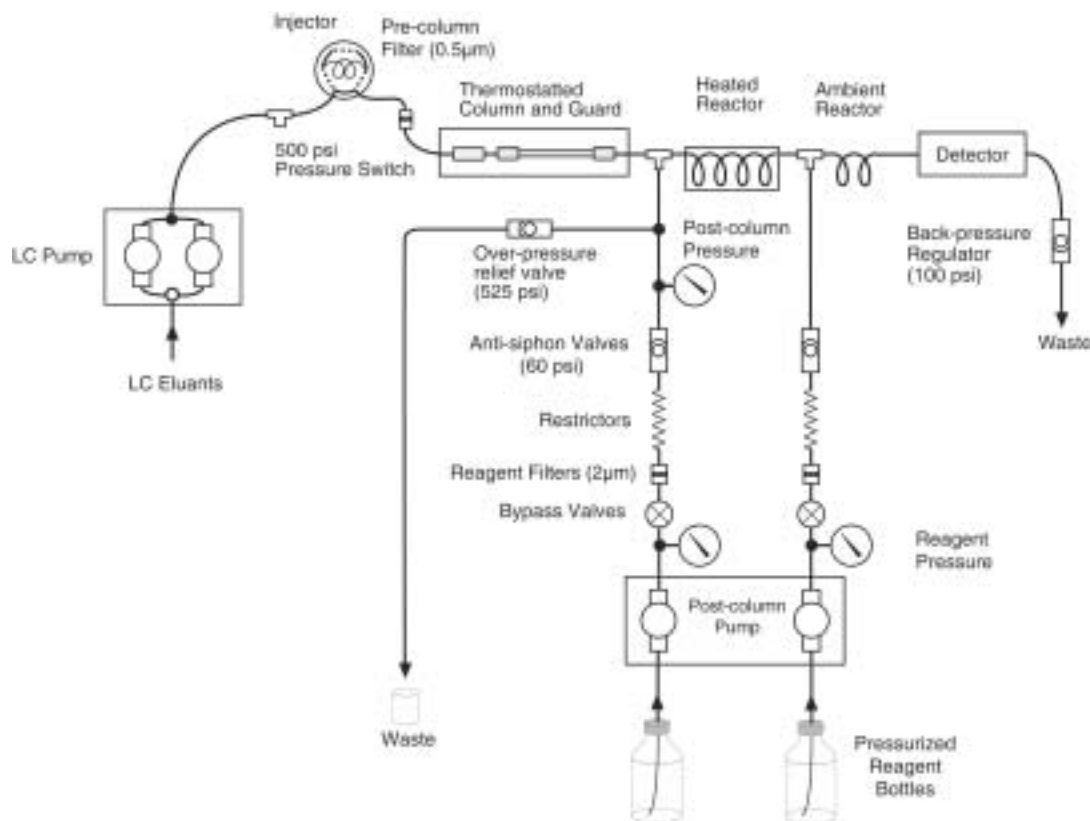
*NaOH backflows onto a carbamate column*

- a. **Do not restart the system.** Dissolved silica or C<sub>18</sub> phase will reprecipitate in the post-column reactors, or flowcell. These additional complications then require replacement of both reactor coils as well as your column.
- b. Immediately depressurize the post-column system by loosening the “To Detector” fitting.
- c. Disconnect the outlet of the column.
- d. Restart the HPLC pump to flush the column with 100% MeOH for 20 minutes. Complete steps b–d as **quickly** as possible because the longer the hydroxide stays inside the column, the less chance that the column will survive.
- e. 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.
- f. Turn off the HPLC pump and reconnect the outlet of the column and the “To Detector” fitting.
- g. 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.

## Interpretation of Pressures

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

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



## Recommended Consumables

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

### Carbamate Reagents

Cat. No.	Description
O120	<i>o</i> -Phthalaldehyde, Chromatographic Grade™ crystals, 5 g
3700-2000	Thiofluor™, Chromatographic Grade™ crystals, 10 g
CB910	OPA Diluent for Carbamate Pesticide Analysis, 4 x 950 mL
CB130	Hydrolysis Reagent for Carbamate Pesticide Analysis, 4 x 950 mL
1700-0063	Carbamate Test Mixture, qualitative sample, 12 components, 1.5 mL, 2.5 µg/mL
1700-0132	ChlorAC™ Buffer for preservation of aqueous carbamate samples, 250 mL

### Columns & Guards

Cat. No.	Description
0840250	C <sub>8</sub> Carbamate column, 4.0 mm ID x 250 mm
1846150	C <sub>18</sub> Carbamate column, 4.6 mm ID x 150 mm
1846250	C <sub>18</sub> Carbamate column, 4.6 mm ID x 250 mm
18ECG002	Replacement Carbamate Guard Cartridges - (Qty. 2)

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† Reprints available from Pickering Laboratories

# Limited Warranty

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## **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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