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Appendix A

Limited Warranty

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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 (5µm C₁₈ 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.

In addition, there are a number of carbamate pesticide compounds employed worldwide which are not included in the 10 compounds mandated by USEPA Method 531.1 and AOAC Protocol 29.A05. The Pickering Laboratories 5µm C₈ column can separate as many as 23 compounds. The C₈ column can also be used as a confirmation column when using a water/acetonitrile gradient instead of a water/methanol gradient.

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 manual chapter 1

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

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

Figure 1-1. Analytes in the Pickering carbamate test mixture. (*4-Bromo-3,5-dimethylphenyl-N-methylcarbamate; an internal standard)
The general structure of the carbamate insecticides is an N-methyl substituted urethane with the variation in the ester moiety. The structural formulas for the ten analytes specified in USEPA Method 531.1 are shown in Figure 1-1 (including 1-naphthol and BDMC). They are listed in the order in which they elute from the Pickering carbamates column. All but 1-naphthol (10) contain the N-methylcarbamoyl moiety (indicated by –OR). The hydrolysis of carbaryl (9) in the post-column reactor also produces 1-naphthol. Note that 1-naphthol hydrolyzed from carbaryl and 1-naphthol in the calibration standard are at different retention time. This observation is useful for troubleshooting, see page 5-13.

Each unique R– group represents a different commercial product or its metabolite. The separation of the carbamates is achieved with the Pickering 5µm, C₁₈ or C₈ column maintained at 42°C and 37°C, respectively. The chromatographic method recommended for this column is a simple linear water/methanol binary gradient that resolves the twelve carbamate products provided in the test standard (Figure 1-2). The carbamates elute principally in relation to their relative hydrophobicity. Aldicarb sulfone, which is minimally hydrophobic, elutes early while methiocarb, which is more hydrophobic, elutes towards the end of the gradient.

Figure 1-2. 2.1 ng in 150 µL water (14 ppb); 25 cm C₁₈ column
The separated carbamates are first saponified by sodium hydroxide (NaOH) at 100°C to release an alcohol, carbonate, and methylamine. In the second post-column reaction, methylamine reacts with o-phthalaldehyde (OPA) and the nucleophilic Thiofluor™ (or 2-mercaptoethanol) to form a highly fluorescent 1-methyl-2-alkylthioisoindole derivative (Figure 1-3). This fluorescent derivative provides detection ≤ 3ng per component on-column, which meets the method requirements of the EPA. Depending on the type of fluorescence detector used, detection limits ten times better than the EPA requirements may be obtained.

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

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

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

The Pickering design (Figure 1-4) uses a single-piston reagent pump to deliver the reagent. Pulses are eliminated by the combination of a gauge followed by a packed-bed restrictor. The pulses are absorbed by the mechanical action of the Bourdon tube inside the gauge, and then released through the restrictor. The mixing device is simply a steel tee-fitting with a 0.010 inch bore. The continuous-flow reactor is a length of 0.011 inch ID Teflon capillary.

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

Safety systems have also been incorporated into the design. Two greatest hazards to post-column systems are rupture of the reactor because of the excessive pressure and the back-flow of caustic reagent onto the analytical column. The first hazard is managed by providing a relief valve that opens at about 525 psi (36 bar) and diverts flow away from the reactor. Two devices protect against reagent back-flow. To ensure flow through the column during operation, a pressure switch upstream of the analytical column must detect at least 500 psi (34 bar) or else the entire system turns itself off. Second, check-valves in the reagent delivery system prevent reagents from siphoning when the pump is off.
Figure 1-4. The Pickering post-column system is depicted inside the dotted-box.
# Chapter 2
## Installation & System Operation

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

**Site Requirements**

**Standard Analysis System**

- Pickering PCX5200 Post-column Derivatization Instrument
- Pickering Carbamate option package
- HPLC manual injector or autosampler
- Binary (or more) gradient HPLC pump
- HPLC fluorescence detector
- Integrator or data system

**HPLC system requirements**

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

- The HPLC pump must be capable of binary gradient elution.
- The injector should be able to inject a 10µL sample, preferably by full-loop injection.
- If drinking water is to be analyzed for carbamate insecticide residues, the injector should be able to inject at least 200µL, and preferably 400µL.
- If the system also will be used for glyphosate analysis, be aware that the column regenerant is *strongly alkaline*. Any polymers or other materials in the HPLC pump, injector, needle seat, and detector must be compatible. For example, the standard rotor seal in Rheodyne injector valves is *Vespel* polyimide, which is not recommended at pH ≥ 9; a *Tefzel* or *PEEK* rotor seal must be installed.
- The PCX5200 includes an external back-pressure regulator for the detector waste line. The pressure rating of the detector flowcell must be ≥ 110 psi (7.5 bar). If your detector flowcell is rated lower, consult Pickering Laboratories.
- Because the OPA reaction is extremely sensitive, the HPLC system must be thoroughly clean before using it with the PCX5200. Pay special attention to the cleanliness of eluant reservoirs and delivery tubings.

**Space Requirements**

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

**Electrical**

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

**Gas**

Nitrogen, helium, or argon (in order of preference), is required to pressurize the reagent reservoirs. The 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).
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).

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

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
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 hard to obtain NaOH of adequate purity.

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

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

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

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

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

**Note!** If Thiofluor is not available, pipette 1 mL of 2-mercaptoethanol. Handling of 2-mercaptoethanol should be in the hood since it is volatile and has an unpleasant odor which will permeate the laboratory. 2-Mercaptoethanol should be replaced with Thiofluor (Pickering Laboratories brand of N,N-dimethyl-2-mercaptoethylamine hydrochloride), a non-volatile thiol salt.
f. Replace the cap and turn on the gas flow. Continue sparging for another minute. Close the vent valve. Gently swirl the reagent to complete the mixing.

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

**Note!** The Hydrolysis reagent remains stable indefinitely. The OPA reagent is sensitive to air oxidation and degrades over time. The 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 two weeks without significant loss of activity.

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

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.
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 \( \downarrow \) key once; the LCD shows: “1 L Carbamate”.
   Optional: 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.

5. With the HPLC on at 1.0 mL/min, press the **ENABLE** key.
   The **POWER** LED remains green.
   The **ENABLE** LED turns green.
   The **PUMP** LED is off.
   The **STATUS** LED turns amber.

6. Once the temperatures of the heated reactor and column oven reach their set-points, press the **PUMP** key.
   The **POWER** LED remains green.
   The **ENABLE** LED remains green.
   The **PUMP** LED turns green.
   The **STATUS** LED turns green.

   The two reagent gauges should begin pulsing with a maximum of about 1,000–1,500 psig. **Note!** The pulsating pressure readings of the reagent pumps (approximately 500 psig swing) are normal. These pulsations are dampened by the liquids in the Bourdon tubes of the gauges and the flow restrictors (packed with diamond particles), located on the back of the gauge panel. The pulse dampening is very effective as indicated by post-column pressure gauge pulsations of less than 10 psig.

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

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

Pickering Laboratories recommends various gradient conditions depending on the column and type of sample. For the purposes of testing and set-up, use the 15 cm column with the 4th gradient on the next page. Note that the exact time of equilibration depends on the internal volume of your HPLC. When the column pressure is stable for at least one minute, the column has been re-equilibrated. Allow the column to equilibrate for about ten minutes under initial conditions. Inject 10 µL of Carbamate Test Mixture, and collect the first chromatogram.

There are six possible gradients: two for the C18 15 cm column, two for the C18 25 cm column, and two for the C8 25 cm column. Two programs are for aqueous samples, three programs for methanolic samples, and one program for methanolic samples using a water-acetonitrile gradient. When using a C8 25 cm column, the water-acetonitrile gradient can be used as a confirmation method. The C18 columns operate at 42°C; the C8 column at 37°C.

From time to time, Pickering may change the recommended gradient conditions as needed. This may happen because of lot changes in the columns, or other reasons. The recommended gradient for the column will always be in the package, and it supersedes the information in this manual.
### 1846250 column (4.6 mm ID x 250 mm) with aqueous samples

<table>
<thead>
<tr>
<th>Step</th>
<th>Times (min)</th>
<th>Interval</th>
<th>%Water</th>
<th>%MeOH</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equil.</td>
<td></td>
<td></td>
<td>100</td>
<td>0</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>inject up to 400 µL water</td>
</tr>
<tr>
<td>1</td>
<td>0–1.7</td>
<td>1.7</td>
<td>100</td>
<td>0</td>
<td>concentrate sample on column</td>
</tr>
<tr>
<td>2</td>
<td>1.71</td>
<td>0.01</td>
<td>80</td>
<td>20</td>
<td>step change</td>
</tr>
<tr>
<td>3</td>
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<td>44</td>
<td>25</td>
<td>75</td>
<td>linear gradient</td>
</tr>
<tr>
<td>4</td>
<td>45.71</td>
<td>0.01</td>
<td>0</td>
<td>100</td>
<td>step change</td>
</tr>
<tr>
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<td>4.29</td>
<td>0</td>
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<tr>
<td>6</td>
<td>50–</td>
<td>8–12</td>
<td>100</td>
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### 1846150 column (4.6 mm ID x 150 mm) with aqueous samples

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<th>Times (min)</th>
<th>Interval</th>
<th>%Water</th>
<th>%MeOH</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equil.</td>
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<td></td>
<td>100</td>
<td>0</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>inject up to 200 µL water</td>
</tr>
<tr>
<td>1</td>
<td>0–1</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>concentrate sample on column</td>
</tr>
<tr>
<td>2</td>
<td>1.01</td>
<td>0.01</td>
<td>82</td>
<td>18</td>
<td>step change</td>
</tr>
<tr>
<td>3</td>
<td>1.01–36</td>
<td>35</td>
<td>30</td>
<td>70</td>
<td>linear gradient</td>
</tr>
<tr>
<td>4</td>
<td>36.01</td>
<td>0.01</td>
<td>0</td>
<td>100</td>
<td>step change</td>
</tr>
<tr>
<td>5</td>
<td>36.01–38</td>
<td>2</td>
<td>0</td>
<td>100</td>
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<tr>
<td>6</td>
<td>38–</td>
<td>5–10</td>
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### 1846250 column (4.6 mm ID x 250 mm) with methanolic samples

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<th>Times (min)</th>
<th>Interval</th>
<th>%Water</th>
<th>%MeOH</th>
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<td></td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>inject up to 10 µL methanol</td>
</tr>
<tr>
<td>1</td>
<td>0–1</td>
<td>1</td>
<td>80</td>
<td>20</td>
<td>isocratic</td>
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</tr>
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<td>0</td>
<td>100</td>
<td>step change</td>
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<td>44.01–49</td>
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<td>0</td>
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<td>49–</td>
<td>5–8</td>
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<td>20</td>
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### 1846150 column (4.6 mm ID x 150 mm) with methanolic samples

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<th>Times (min)</th>
<th>Interval</th>
<th>%Water</th>
<th>%MeOH</th>
<th>Comment</th>
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<td></td>
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<td>82</td>
<td>18</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td>82</td>
<td>inject up to 10 µL methanol</td>
</tr>
<tr>
<td>1</td>
<td>0–0.5</td>
<td>0.5</td>
<td>82</td>
<td>18</td>
<td>isocratic</td>
</tr>
<tr>
<td>2</td>
<td>0.5–29</td>
<td>28.5</td>
<td>30</td>
<td>70</td>
<td>linear gradient</td>
</tr>
<tr>
<td>4</td>
<td>29.01</td>
<td>0.01</td>
<td>0</td>
<td>100</td>
<td>step change</td>
</tr>
<tr>
<td>5</td>
<td>29–31</td>
<td>2</td>
<td>0</td>
<td>100</td>
<td>Cleanout</td>
</tr>
<tr>
<td>6</td>
<td>31–</td>
<td>5–8</td>
<td>82</td>
<td>18</td>
<td>re-equilibration</td>
</tr>
</tbody>
</table>
Post-column Conditions

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

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

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

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

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

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

Reagent 2: OPA & Thiofluor in pH 9.1 borate buffer
Pump 2: 0.30 mL/min
Reactor 2: 100 µL at ambient temperature
Figure 4-1 Peak Identification

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

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

Methanolic samples on a C8 column using a Water/Methanol gradient

Methanolic samples on a C8 column using a Water/Acetonitrile gradient
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.

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.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time (min)</th>
<th>%MeOH</th>
<th>Flow (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0.02</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>100</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>5.1</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>15.1</td>
<td>100</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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

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 mL/min 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 1/1).

5. Turn off the fluorescence detector and HPLC pump.

6. 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.

7. Relieve the pressure in the reagent gauges by briefly opening the bypass valves.

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. Set the HPLC to pump methanol at 1 mL/min.

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 (1/1).

7. Turn the reagent pump on and flush the system until the temperature of the reactor has fallen below 60°C or at least 30 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.

Long Term (7 days or more)

1. Set the HPLC to pump methanol at 1 mL/min.

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 (1/1).

7. Turn the reagent pump on and flush the system until the temperature of the reactor has fallen below 60°C or at least 30 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.
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.
Chapter 3
Troubleshooting

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.

• Avoid touching the interior of the mobile phase reservoirs and the dip tubes with your fingers. Amino acids in fingerprints will cause contamination. Gloves are suggested.

• Do not leave caps and lines dangling without a reservoir. To fill reservoir, transfer caps and lines into a spare bottle or an Erlenmeyer flask filled with deionized water.

• Use HPLC-grade methanol and water (Fisher Scientific, JT Baker, or Merck) for carbamate analysis to avoid problems with baseline drift, spurious peaks, and noise.

• Use bottled HPLC-grade water if possible (Fisher Scientific, JT Baker, or Merck), especially during the initial system start-up. If water from a water purification system is used, ensure the system has an activated charcoal unit to eliminate organics, and that the charcoal cartridge is placed after the ion-exchange cartridges. (Many ion-exchange resins leach out OPA-positive contaminates that cause unacceptable fluorescence background.)

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

• Avoid purging the system with 100% acetonitrile as precipitation of borate salt in the reactor might occur. Do not exceed 70% acetonitrile if it will be used as the mobile phase. (Methanol is recommended as the organic mobile phase for the Pickering Laboratories column and it is less expensive. Reagent precipitation problems rarely occur using methanol as the flushing solvent.)

• When switching a system between glyphosate and carbamate modes, be sure to flush the HPLC and injector with compatible mobile phase before connecting the column. Eluants for one analysis will damage the column for the other.

Mobile Phase
Column Maintenance and Precautions

- Always protect the analytical column by use of the pre-column filter and guard column.

- Check for leaks daily at column fittings. In particular, glyphosate eluants are corrosive.

- If the column back-pressure is high (> 2000psi), isolate the source of the high pressure—guard, analytical column, or the 0.5µm in-line filter. Replace items causing the increased back-pressure (Back-pressure from filter and guard should be < 200psi).

- During shutdown, flush the column with pure methanol. Do not store the column in water.

- The analytical column can be back-flushed with methanol at 1 mL/min to clear partial blockage. (Do not disassemble or attempt to replace column inlet frit as this will void the column warranty.) Disconnect the outlet of the column during the back flush operation.

- Organic contaminants can be washed off the column by first washing with methanol then with dichloromethane. Wash again with methanol before use.

- The column is temperature-controlled to reduce baseline shift (caused by viscosity changes during gradient formation), to reduce back-pressure, and to improve retention time reproducibility.

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

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

- Never disconnect any fittings between the HPLC pump and the column until the post-column system has been shut down and depressed 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 \(\leq 10\mu\)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 up to 500\(\mu\)L, only the 25cm column should be used, and a gradient delay time should be programmed into the analysis (0% organic) to trap the sample onto the head of the column.

Reagent Precautions

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

Reactor Precautions

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

Electrical Precautions

- Always use the correct fuse.
Most Common Problems with Post-column

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

High background signal—caused by
- Contaminated eluant
- Bacterial growth
- Fingerprints
- Water purifier needs service
- Contaminated reagent(s)
- Defective chemicals

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

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

Poor peak shape—caused by
- Column worn out
- Guard column dirty
- Bad column
- Deposits in post-column flow path
- Partial obstruction of flowcell
- Too strong a solvent or too large a sample injected
- Bad tubing connection: wrong style nut, too large tubing, wrong type union
- Reagent flow rate(s) too high
- Strange injector problems
Deposits in reactor—caused by
- Dissolved silica reprecipitating (carbamate column)
  - NaOH backflow into column
  - Corrosive samples
  - Backflushing a dirty column into the system
- Contaminated reagents
- Hard water samples
- Degradation of Teflon tubing
- Greasy samples
- Using calcium hypochlorite as the oxidant in glyphosate determination
- Preparing your own reagents with poor quality chemicals

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

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

Reagent pump stops or delivers wrong flow rate
- Check pump setting
- Check reagent pressurization
- 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 User's 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% H2O).
  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 C18 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.
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.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Column</th>
<th>Post-Column</th>
<th>Reagent 1</th>
<th>Reagent 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1200</td>
<td>250</td>
<td>1500</td>
<td>1500</td>
</tr>
<tr>
<td>Pre-column filter blocked</td>
<td>↑</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Heated reactor obstructed</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Ambient reactor obstructed</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Reagent 1 not pumping</td>
<td>—</td>
<td>↓</td>
<td>↓</td>
<td>—</td>
</tr>
<tr>
<td>Reagent 2 not pumping</td>
<td>—</td>
<td>↓</td>
<td>—</td>
<td>↓</td>
</tr>
<tr>
<td>Restrictor 1 blocked</td>
<td>—</td>
<td>—</td>
<td>↑</td>
<td>—</td>
</tr>
<tr>
<td>Restrictor 2 blocked</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>↑</td>
</tr>
</tbody>
</table>
Carbamates


Glyphosate


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

Instrumentation


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


† Reprints available from Pickering Laboratories
Recommended Consumables

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

**Carbamate Reagents**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>O120</td>
<td>o-Phthalaldehyde, Chromatographic Grade crystals, 5 g</td>
</tr>
<tr>
<td>3700-2000</td>
<td>Thiofluor, Chromatographic Grade crystals, 10 g</td>
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<tr>
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<td>Carbamate Test Mixture, qualitative sample, 12 components, 1.5 mL, 2.5 µg/mL</td>
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<tr>
<td>1700-0132</td>
<td>ChlorAC™ Buffer for preservation of aqueous carbamate samples, 250 mL</td>
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**Columns & Guards**

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<tr>
<th>Cat. No.</th>
<th>Description</th>
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<td>C₃ Carbamate column, 4.0 mm ID x 250 mm</td>
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<tr>
<td>1846150</td>
<td>C₁₈ Carbamate column, 4.6 mm ID x 150 mm</td>
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<td>1846250</td>
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<td>18ECG002</td>
<td>Replacement Carbamate Guard Cartridges - (Qty. 2)</td>
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**Limited 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 a period of ninety days from the date of delivery to the original Customer. Pickering will replace the Analytical Column under warranty if found defective in material or workmanship. However, the warranty is void if the Analytical Column was damaged due to Customer’s misuse.

**How to Obtain Warranty Service**
If there is a problem with your Analytical Column within the Warranty period, notify Pickering immediately at (800) 654-3330; if calling from outside U.S.A., use (650) 694-6700. If the Analytical Column was not purchased directly from Pickering, please contact the vendor where it was purchased from. Any Analytical Column returned to Pickering for examination, repacked, or replaced, 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 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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