

# APPLICATION MANUAL

# Glyphosate

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**Why Post-column Derivatization?** 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 the herbicide glyphosate (and its metabolite AMPA), meeting or exceeding performance requirements for precision and accuracy of USEPA Method 547.

- Post-column Analysis** A complete Post-column Analysis system for glyphosate 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

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

Glyphosate and AMPA are separated on a strong cation-exchange column (fully sulfonated, cross-linked polystyrene, mixed K<sup>+</sup>/H<sup>+</sup> form). After isocratic separation, the column is regenerated with dilute KOH, then re-equilibrated with eluant (Figure 1-1).

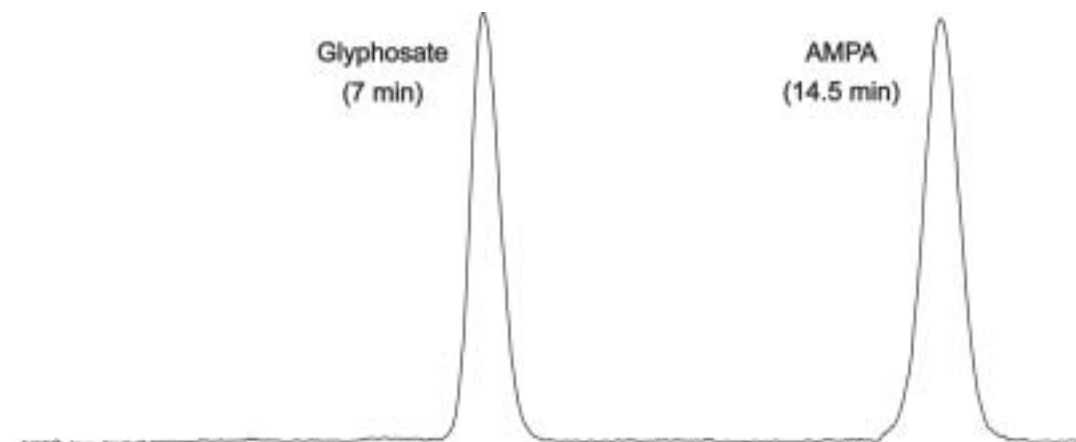


Figure 1-1

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

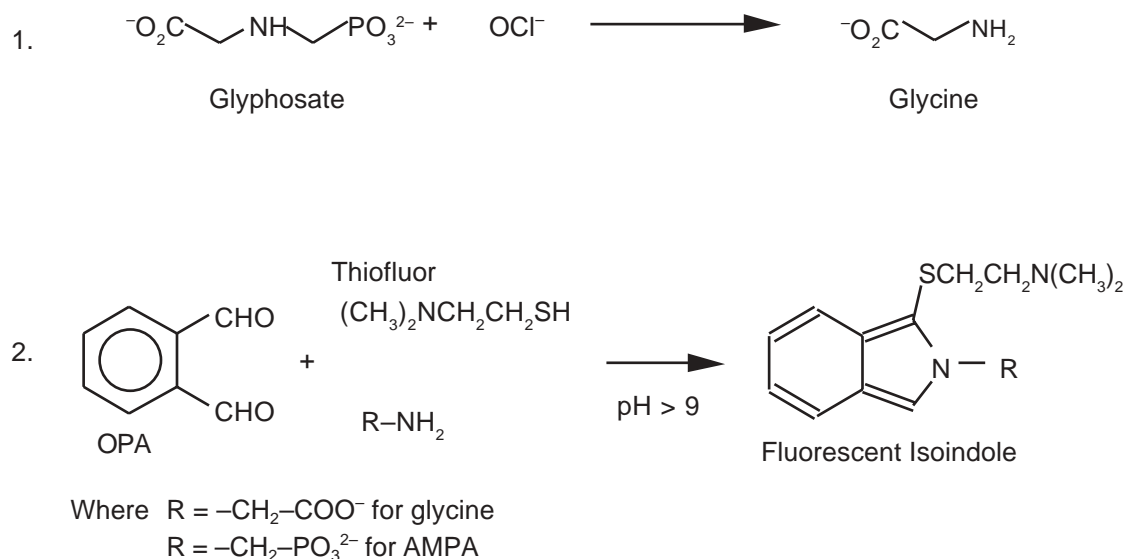


Figure 1-2

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

There are 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 hazards to post-column systems are 1) rupture of the reactor because of the excessive pressure and 2) back-flow of caustic reagent onto the analytical column. The first hazard is managed by providing a relief valve that opens at  $525 \pm 10$  psi (36 bar) and diverts flow away from the reactor.

The second hazard is managed by anti-siphon valves and a pressure switch located before the column. The anti-siphon valves in the reagent delivery system prevent reagents from siphoning when the pump is off. The 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).

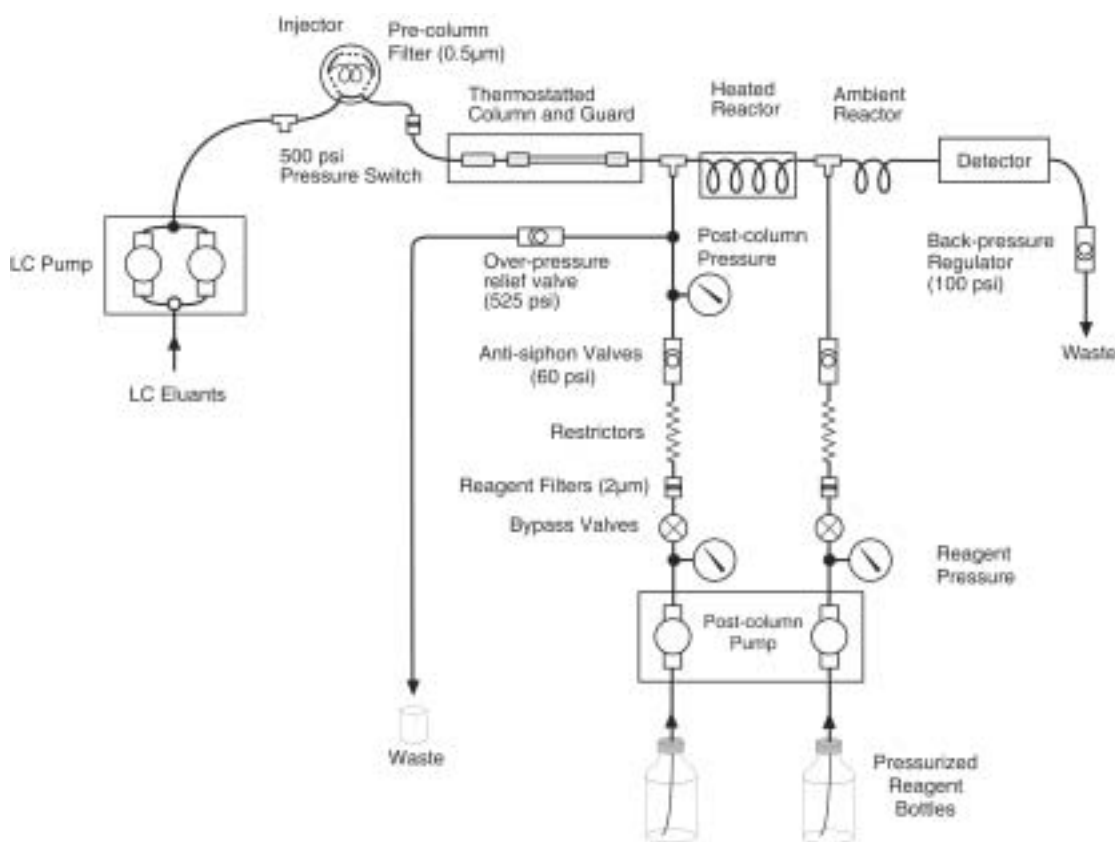


Figure 1-3. Pickering two-reagent post-column systems



# 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 Glyphosate analysis kit (P/N 0352-0010)
- 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 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.
- 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.
- 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.



**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 such as Saran or metal for the *entire* gas supply line.

**Miscellaneous Supplies** The user will need to provide adequate lengths of capillary tubing to connect HPLC pump and injector to pressure switch (0.010–0.020 inch ID), to detector inlet (0.010 inch ID), to detector outlet (0.010–0.020 inch ID), and to injector outlet (0.007–0.010 inch ID). If installation and training has been purchased, these supplies will be provided by the service personnel.

**Chemicals Supplied by User**

- 5% Sodium hypochlorite for preparing oxidizing reagent (can be obtained from local grocery stores)

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

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


**Getting Started** **Important!** This section assumes that the PCX5200 has been installed according to the directions in PCX5200 Operation Manual. **Do not operate the instrument until it has been installed properly, and you have read and understood the instructions in this section.**

**HPLC Mobile Phase** The Pickering Laboratories glyphosate analysis requires two mobile phases: K200 eluant and RG019 column regenerant. **Do not use** water or eluant containing any organic modifiers such as methanol, acetonitrile, etc., for the glyphosate column.



**Do not exceed a flow rate of 0.4 mL/min.**

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

 **Note!** Pickering mobile phases are filtered before bottling, so it is unnecessary to filter the mobile phases before use. Filtering with marginally clean glassware has been known to introduce large amounts of contaminating fluorescent compounds to the mobile phases. Always wear gloves for this operation. Avoid touching the inside of reservoirs or handling the tubing 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. Remove any stainless steel inlet frit or sinker from the HPLC reservoirs.
2. Fill eluant reservoir "B" with RG019 column regenerant (0.005 M KOH).
3. Fill eluant reservoir "A" with K200 glyphosate eluant.

4. Place the filled eluant reservoirs on or near the HPLC pump.
5. If your HPLC requires it, sparge the eluants with helium. Do not use stainless steel frits in the sparging line. Do not use continuous sparging with buffers, because the composition will change with continuous sparging.
6. Prime the HPLC pump by withdrawing at least 30 mL of each solvent from the prime/purge port with the priming syringe that is supplied. An HPLC pump method can be configured to facilitate this step. Consult your HPLC manual.
7. Close the HPLC prime/purge valve. Do not connect the glyphosate column yet. Flush the HPLC at flow rates  $> 1\text{ mL/min}$  with 100% A and check the pH of the liquid exiting the end of the inlet tubing with pH papers. When the Eluant A tubing is thoroughly flushed, the pH should be 2.
8. Switch to 100% B and repeat step 7. When the Eluant B tubing is thoroughly flushed, the pH should be pH 12.
9. Stop the HPLC pump. Connect guard and column according to Figure 2-1.
10. Start the HPLC pump at  $0.40\text{ mL/min}$ , 100% A. Do not exceed a flow rate of  $0.4\text{ mL/min}$  for the glyphosate column and guard. The column back pressure should stabilize at approximately 1000 psi (67 bar).

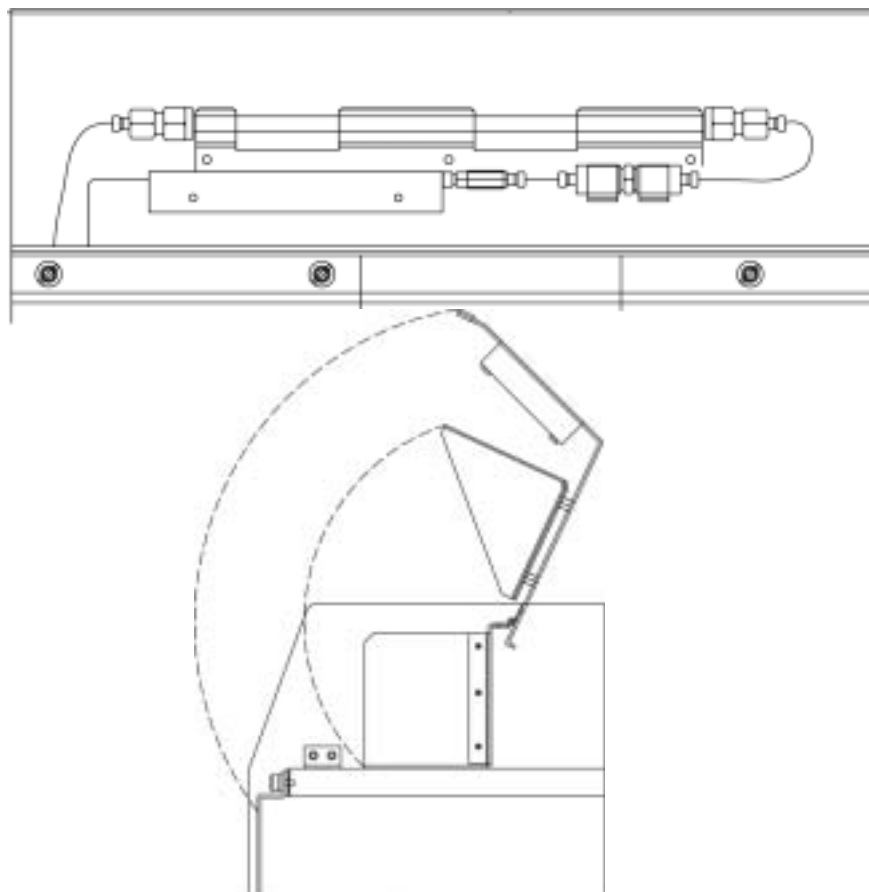


Figure 2-1

### Post-column Reagent Preparation

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



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

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

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. Preparation of the Oxidizing Reagent.
  - a. Pour 945 mL of the Hypochlorite Diluent (GA116) directly into the reagent reservoir with the TFE (clear) line from the pump to the cap. This should be labeled Oxidizing Reagent.
  - b. Add 100  $\mu\text{L}$  of 5% *sodium* hypochlorite solution to the diluent. The exact amount will depend on the actual hypochlorite concentration of the stock solution. When you get your first chromatograms, you will be able to adjust the amount to optimize the relative peak areas of glyphosate versus AMPA. Figure 4-2 shows a typical response curve.
  - c. Cap the reservoir, close the vent valve, and swirl the solution to mix it thoroughly.



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

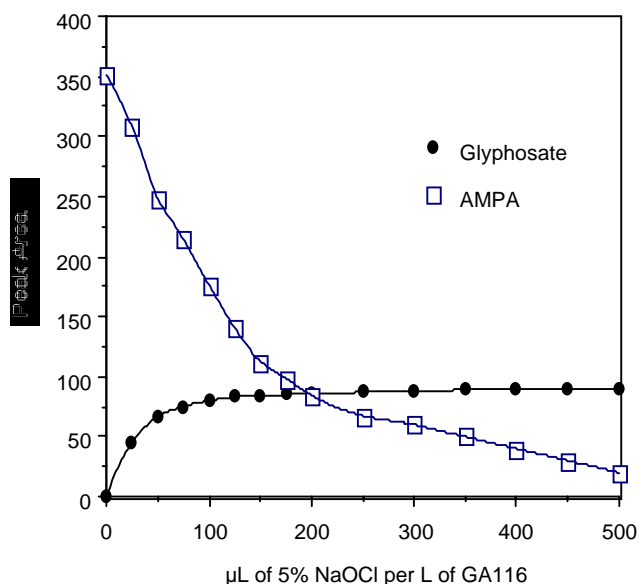


Figure 2-2



**Caution!** Do **NOT** use calcium hypochlorite in the oxidizing reagent. This will cause plugging of the post-column reactor. **The one year warranty does not cover damage caused by calcium hypochlorite-based reagents.** The EPA Draft Method 547 is wrong on this point;  $\text{Ca}_3(\text{PO}_4)_2$  is insoluble in water.

4. Preparation of the OPA Reagent:

- a. Pour 945 mL of the OPA Diluent (Cat. No. GA104) into the reagent reservoir. (Save approximately 5 mL for step e.)
- b. Put the cap on the bottle, open the vent valve, and turn on the gas supply. Thoroughly de-aerate the contents by sparging with inert gas. Continue bubbling for at least 10 minutes.
- c. Dissolve 100 mg of OPA (Cat. No. O120) in approximately 10 mL of HPLC-grade methanol in a clean, dry container.
- d. Turn off the gas supply and remove the cap from the bottle. Add the OPA solution to the deoxygenated Diluent in the reservoir.
- e. Dissolve 2 g of Thiofluor (Cat. No. 3700-2000) in the reserved 5 mL of the OPA Diluent and add into the reservoir.
- f. Replace the cap and turn on the gas flow. Continue sparging for another minute. Close the vent valve. Gently swirl the reagent to complete the mixing.



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



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

Turning On the  
PCX5200

1. The HPLC pump should be on and pumping K200 at this time. If not, turn on the pump and wait until at least 500 psi (35 bar) of pressure develops.
2. Turn on main power switch in the back of the PCX5200;
  - The **POWER** LED light 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 twice; the LCD shows: "2 L Glyphosate".  
Optional: Check that the column temperature setting is 55°C and the reactor temperature setting is 36°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 0.4 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 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.

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. 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.

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

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

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

Setting up the HPLC Method These are the recommended conditions for glyphosate analysis using the 1954150 column and 1953020 guard column. The column temperature is 55°C.

| Step   | Times(min) | Interval | %K200 | %RG019 | Comment               |
|--------|------------|----------|-------|--------|-----------------------|
| Equil. |            |          | 100   | 0      | 0.40 mL/min (Maximum) |
| 0      | 0          | 0        | 100   | 0      | inject - 50 µL        |
| 1      | 15.0       | 15       | 100   | 0      | isocratic             |
| 2      | 15.1-17    | 2        | 0     | 100    | step change           |
| 3      | 17.1-27    | 8-12     | 100   | 0      | re-equilibration      |

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

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

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

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

Figure 2-3 shows a typical Glyphosate and AMPA chromatogram. In a standard with Glyphosate and AMPA at equal concentration, the peak heights should be equal. The peak heights are influenced by the amount of hypochlorite in Reagent 1.

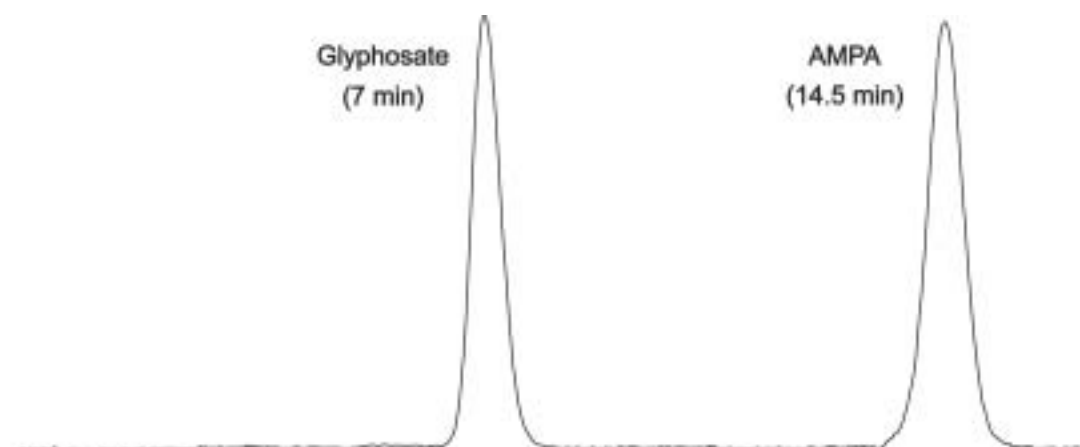



Figure 2-3

**Shutdown Procedures** Upon completion of the analyses, use one of the following three procedures to shut down the PCX5200 system properly. These procedures can prevent potential column damage, reaction coil blockage, high background fluorescence, reagent precipitation, or other problems.

- Short Term (Up to 3 days)**
- Turn off the PCX5200 either manually by pressing the ENABLE key or via the "Slowdown" program (see below).
  - Continue the HPLC pump at 0.40 mL/min of K200 to flush the system for at least 20 minutes.
  - Stop the HPLC pump.
  - Turn off the detector lamp.

• You may also program a slowdown method to accomplish all the above steps.

| Step | Time (min) | %K200 | Flow (mL/min) |
|------|------------|-------|---------------|
| 0    | 0          | 100   | 0.02          |
| 1    | 5          | 100   | 0.02          |
| 2    | 5.5        | 100   | 0.40          |
| 3    | 15         | 100   | 0.40          |
| 4    | 20         | 100   | 0.02          |
| 5    | 20.1       | 100   | 0.00          |

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

- Medium Term (Up to 6 days)**
- Turn off the PCX5200 either manually by pressing the ENABLE key or via the "Slowdown" program.
  - Set the HPLC pump to 0.40 mL/min of RG019 for 10 min. Excessive flushing will require an equally excessive re-equilibration when you start up again.
  - Replace both reagents with water and draw 10 mL through each prime/purge valve.
  - Replace the water in the reagent reservoir with water / methanol (approximately 1/1).
  - Turn off the fluorescence detector and HPLC pump.



- Loosen the "To Detector" fitting. Place paper towels under the outlet of the PCX 5200 to absorb any escaping liquid.
- Relieve the pressure in the reagent gauges by briefly opening the bypass valves.

Long Term **Caution!** The medium term shutdown should be performed prior to any work on (7 days or more) the HPLC or PCX5200. Failure to do so could defeat the safety systems.

- Set the HPLC to pump 100% RG019 at 0.4 mL/min
- Turn off the reagent pumps by pressing the PUMP key.
- Turn off the gas at the toggle valve and vent the reservoirs.
- Replace both reagents with water and draw 10 mL through each prime/purge valve.
- Replace the water in the reagent reservoir with water / methanol (approximately 1/1).
- Turn on the reagent pumps and flush for 10 minutes
- Turn off the PCX5200 and the HPLC pump.
- Relieve the pressure in the reagent gauges by briefly opening the bypass valves.
- Turn off the inert gas source.
- 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.
- Remove the column and guard column and plug them. (When removing the column, disconnect the **outlet fitting first.**) Replace them with a tubing and unions so there are no open lines.
- Replace the eluants with water and draw 10 mL through the HPLC prime/purge valve.
- Replace the water with water / methanol (approximately 1/1). Turn on the HPLC pump for 15 minutes (you need to retighten the fitting at the external 100 psi back pressure regulator for the 15 min wash).
- Turn off the HPLC system.



## Carbamate/ Glyphosate Systems

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 appropriate application manual for the details.

### Changing from carbamate to glyphosate

Before converting the instrument from Carbamates to Glyphosate, read page 2-1 for the HPLC System Requirements for *glyphosate analysis!* The HPLC components must be compatible with *high pH regenerant*.



Because the reactor is so slow to cool, this is best performed first thing in the morning after the system has been cooling off overnight.

1. Perform the medium-term shutdown at the end of the day before the conversion.
2. Remove the carbamate column and guard column and plug them. When removing the column, disconnect the outlet fitting first.
3. Remove any stainless steel inlet frits or sinkers from the HPLC reservoirs.
4. Change the HPLC eluants from water and methanol to K200 and RG019.
5. 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.
6. 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.
7. Turn off the HPLC pump
8. Install the glyphosate column and guard.
9. Change HPLC program and start the HPLC pump to a *maximum* of 0.4 mL/min of K200.
10. Change the preset program in the PCX5200 to "2 L Glyphosate".
11. Press the ENABLE key.
12. Prime the reagent pumps by drawing 10–20 mL through the bypass valves.
13. Once the temperatures of the heated reactor and column oven reach their set-points, press the PUMP key.
14. 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.
4. Flush the HPLC pump, injector, and the inlet lines of the PCX5200 without the carbamate column and guard attached for at least 15 min.
5. Replace the water in Line 2 with Methanol. Flush the HPLC system for at least another 15 minutes.



Do not allow either of the glyphosate eluants onto the carbamate column.

6. Turn off the HPLC pump
7. Install the carbamate column and guard.
8. Choose a carbamate HPLC program and start the HPLC pump.
9. Change the preset program in the PCX5200 to "1 L Carbamates".
10. Press the ENABLE key.
11. Change the reagents from GA116 and GA104 to CB130 and CB910.
12. Prime the reagent pumps by drawing 10–20 mL through the bypass valves.
13. Once the temperatures of the heated reactor and column oven reach their set-points, press the PUMP key.
14. Allow the system to equilibrate and flush itself for at least one hour before using it to collect data.



## Chapter 3 Troubleshooting

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

Set up the HPLC and the PCX5200 as recommended in Chapter 2. These conditions are close approximations to those used by Pickering for column and instrument testing.

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

**Parameter Log** Make copies of the blank forms in Appendix A of the PCX5200 Operation Manual and complete the parameter log on the photocopy. Your system should have come with a similar log from factory testing. Use the same conditions as for the test chromatogram above. Report the pressures for the system equilibrated under initial conditions. The pressures reported for Reagent 1 and Reagent 2 should be the maximum swings of the pointers. Although the parameters will not be identical to the factory, they should be similar. Keep a daily log of the four pressures for diagnostic use. See page 3-8: Interpretation of Pressures.

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

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

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

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

## Precautions & Problem-prevention

### General



- Use Pickering Laboratories reagents and eluants. The quality of the chemicals is excellent and the cost is low relative to the worth of your analytical results. *The one year warranty does not cover damage caused by poor-quality reagents and eluants not purchased from Pickering Laboratories.*

- Use the proper start-up and shutdown procedures consistently (see Section 2).
- 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.
- 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

- 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 0.5 $\mu$ m 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 RG019 for 5 minutes but no more than 10 minutes. Do not store the column in the eluant.
- Contamination usually occurs on the guard column. Wash it separately from the analytical column. This will save much time in the washing and re-equilibration.
- Contaminants of special concern: iron and other polyvalent cations, organic dyes, surfactants, detergents, and lipids. They may cause irreversible damage.
- Organic solvents will cause the resin in the column to swell. This leads to high back-pressure and broadened peaks. The column sometimes can be regenerated.
- Use Pickering eluants with the Pickering column, as they are designed to work together.

The 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 **depressurized**.
  - Any leaky 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 & Standard Precautions
- The test mixtures for carbamates and glyphosate are for **qualitative use only**. They are not recommended for calibration purposes.

- Filter all samples 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 or 547 for details.
- 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 up to 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.**



- Reagent 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 with a post-column pressure above 500 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 Iron contamination of column from samples, long storage of the column, stainless steel inlet frits in the eluant reservoirs, or corrosion in system
  - Flush guard and column with the Glyphosate Restore solution.
  - Remove stainless steel frits from the eluant reservoirs.
  - Clean or replace any corroded parts.
- 2 Glyphosate peak too small or gone, but AMPA present
  - Oxidizing reagent too weak, too old, NaOCl stock solution too old
  - Reactor at wrong temperature
- 3 Oxidizing reagent too strong (AMPA vanishes)
- 4 Reagent pump mis-adjusted
- 5 Using CB910 instead of GA104 for OPA Diluent (CB910 does not have enough buffering capacity)

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 0.4 mL/min (K200).
  - 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.

*Organic solvent in a glyphosate column*

- This procedure usually works but may not work every time.
  - a. Shut down the PCX5200 and remove the analytical and guard columns.
  - b. Flush out all organic solvents from the LC and injector.
  - c. Backflush both columns with K200 glyphosate eluant. Use a very slow flow rate so that the back pressure does not exceed 2000 psi.
  - d. Keep flushing until the pressure drops. Keep raising the flow rate until the pressure is normal at 0.40 mL/min and 55½C.
  - e. Reinstall the analytical (in reversed-direction) and guard column (normal direction) and test them.

*Glyphosate peak is a doublet.*

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

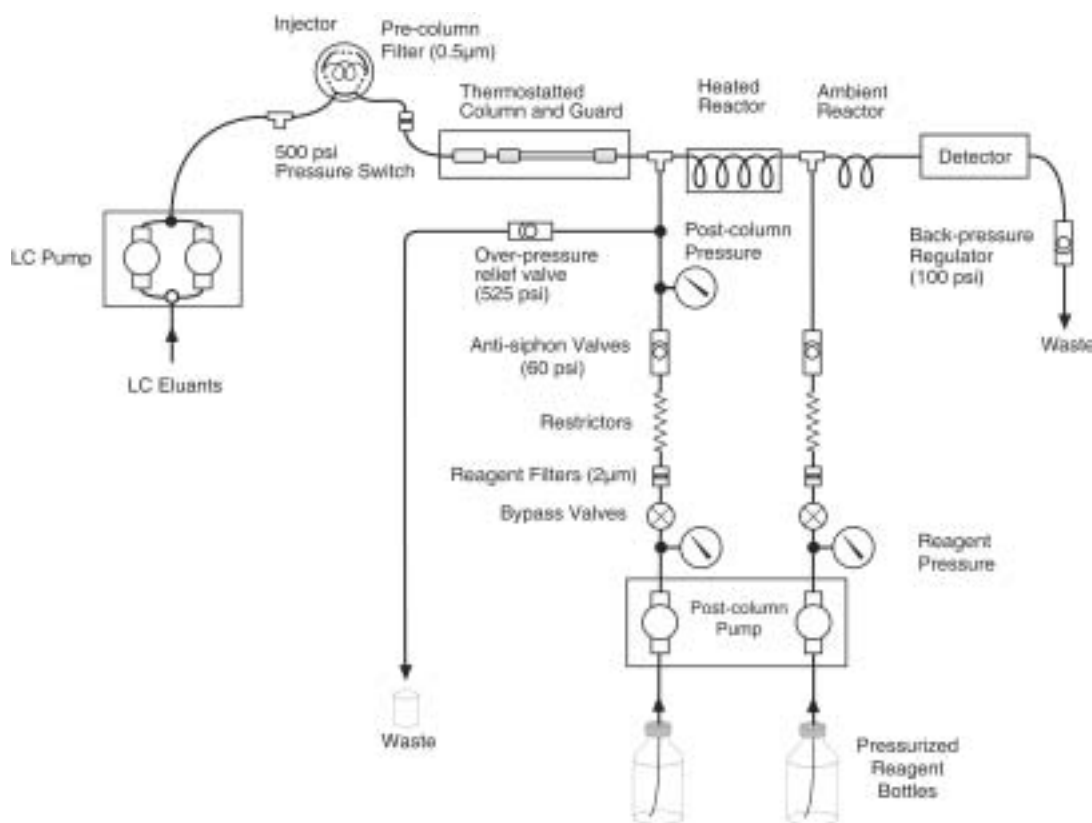
*Glyphosate and AMPA peaks are late and broad due to contamination*

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

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.

### Glyphosate Reagents

| Cat. No.  | Description   |
|-----------|---|
| O120      | <i>o</i> -Phthalaldehyde, Chromatographic Grade crystals, 5 g                           |
| 3700-2000 | Thiofluor, Chromatographic Grade crystals, 10 g   |
| GA104     | OPA Diluent for glyphosate analysis, 4 x 950 mL   |
| GA116     | Hypochlorite Diluent for glyphosate analysis, 4 x 950 mL                                |
| K200      | Eluent for glyphosate analysis, 4 x 950 mL  |
| RG019     | Column Regenerant for glyphosate analysis, 4 x 950 mL                                   |
| 1700-0080 | Test mixture, 2.5 µg/mL each glyphosate and AMPA, 1.5 mL                                |
| 1700-0140 | RESTORE for removal of metal ion contamination from glyphosate column and guard, 250 mL |

### Columns & Guards

| Cat. No. | Description                                |
|----------|--|
| 1954150  | Glyphosate column, 4.0 mm ID x 150 mm      |
| 1953020  | Glyphosate guard column, 3.0 mm ID x 20 mm |

# References

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## Carbamates

- "Measurement of N-methyl carbamoyloximes and N-methyl carbamates in drinking water by direct aqueous injection LC with post-column derivatization," EPA Method 531 by D.L. Foerst, EPA/600/4-851054 (1986); Method 5, revised by T. Engels, National Pesticide Survey, Battelle Columbus Lab (1987); Method 531.1, revised by R.L. Graves, EPA, Environmental Monitoring and Support Laboratory, Cincinnati (1989).
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- K.M. Hill, R.H. Hollowell, and L. D. Dal Cortivo, "Determination of N-methylcarbamate pesticides in well water by liquid chromatography with post-column fluorescence derivatization," *Anal. Chem.*, 56 (1984) 2465–2475.
- H. Frister, H. Meisel, and E. Schlimme, "OPA Method Modified by Use of N,N-Dimethyl-2-mercaptoethylammonium Chloride," *Fresenius Z. Anal. Chem.*, 330 (1988) 631–633.

## Glyphosate

- J.E. Cowell, "Analytical Residue Method for N-Phosphono-methylglycine and Aminomethylphosphonic acid in Environmental Water," *Monsanto Method Number 86-63-1*, 1987
- Environmental Protection Agency Draft Method 597: "Analysis of Glyphosate in Drinking Water by Direct Aqueous Injection LC with Post-Column Derivatization."

## Instrumentation

- M.V. Pickering, "Assembling an HPLC post-column system: practical considerations," *LC•GC*, 6, 11 (1988) 994–997.†
- M.V. Pickering, "Modifying HPLC equipment to tolerate corrosive solutions," *LC•GC*, 6, 9 (1988) 800–809.†
- J.W. Dolan and L.R. Snyder, "Troubleshooting LC Systems," Humana Press, Clifton, NJ (1989).

† Reprints available from Pickering Laboratories

# Limited Warranty

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## **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.

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