

# Glyphosate Analysis Hamilton™ PRP-X400 HPLC Column

**Glyphosate Analysis** using the Hamilton **PRP-X400** Cation Exchange HPLC Column.

Hamilton Company recommends the polymeric PRP-X400 column for analysis of the herbicide glyphosate, N-phosphonomethyl glycine (active ingredient in Roundup) and its metabolite aminomethylphosphonic acid in drinking water (EPA Method #547).



## Separation Mechanism

The PRP-X400, a 7µm poly(styrene-divinyl-benzene) sulfonate cation exchange support (2.5meq/gm) column, separates glyphosate and aminomethylphosphonic acid according to charge in less than 10 minutes. This separation requires post-column oxidation and derivatization.

## Elution Order

Glyphosate elutes first, followed by the metabolite aminomethylphosphonic acid.

## Typical Operating Conditions

Using a PRP-X400 column to separate glyphosate and its metabolite is more expedient than using the column specified in EPA Method #547:

- You do not have to heat PRP-X400 columns to 65°C.
- PRP-X400 columns do not require the use of methanol in the column mobile phase.
- With a PRP-X400 column, the separation of glyphosate is completed five minutes faster.

## Mobile Phase Preparation

To prepare 0.005 M monobasic potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) pH 1.9, dissolve 0.68g of monobasic potassium phosphate in 1L of deionized water. Adjust the pH to 1.9 using concentrated phosphoric acid. Prior to using this preparation, filter it through a 0.45µm nylon filter and degas.

## Detection

Post-column reaction (oxidation) with calcium hypochlorite followed by derivatization with o-phthaldehyde solution provides sensitive (6ppb or less), selective (primary and secondary amine) detection. Instructions for the preparation of all the solutions needed (oxidation and derivatization) are outlined here. To achieve low-level detection (6ppb) of glyphosate and its metabolite precisely follow these instructions.

## Oxidation Solution Preparation (15ppm calcium hypochlorite)

### Stock Oxidation Solution Concentrate Preparation

To prepare the 150ppm concentrate solution, add 0.23g of tech grade calcium hypochlorite  $\text{Ca}(\text{OCl}_2)$  to 100ml of deionized water. With a 2µm nylon filter, remove any insoluble calcium carbonate (as it produces a cloudy solution). Store the solution in the freezer. Shelf life is several freeze/thaw cycles.

### Working Oxidation Solution Preparation

Dissolve 1.36g monobasic potassium phosphate, 11.60g sodium chloride, and 0.40g sodium hydroxide (or use 0.50ml 50% w/w sodium hydroxide solution) in 950ml deionized water. Add 10.00ml of 150ppm calcium hypochlorite stock concentrate solution, and dilute to 1L. Filter through a 0.45µm nylon filter. Prepare this solution fresh daily. Use and store this solution in an inert atmosphere (helium or nitrogen). Degas before use.

### Derivatization Solution Preparation (o-Phthalaldehyde)

Dissolve 19.1g of disodium tetraborate decahydrate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) in 950ml deionized water. Heat the solution to approximately 50°C for about one hour to dissolve the disodium tetraborate decahydrate (or prepare the solution one day in advance to allow the borate to dissolve). Cool the solution to room temperature, and adjust the pH to 10.9 with 1N sodium hydroxide. Now dissolve 0.80g phthalic dicarboxyaldehyde (Aldrich Part #P3,940-0) in 10ml methanol. Add all 10ml to the disodium tetraborate decahydrate solution. Then add 50µl of 2-mercaptoethanol. (Caution: Use adequate ventilation and/or a hood when handling concentrated 2-mercaptoethanol, as the fumes are noxious.) Dilute the concentrate to 1L with deionized water, mixing well. Filter the mixed solution through a 0.45µm nylon filter, and degas before using. Store the mixed solution in an inert atmosphere (Helium or Nitrogen). Refrigerate the unused OPA derivatization solution. Shelf life is one to two weeks.

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## Troubleshooting

- Problem:** A loss in sensitivity for the glyphosate peak, relative to aminomethylphosphonic acid exists.
- Solution:** Prepare a new calcium hypochlorite stock solution. Oxidation reaction is required for detection of glyphosate, not aminomethylphosphonic acid.
- Problem:** A loss in sensitivity for both glyphosate and aminomethylphosphonic acid exists.
- Solution:** Prepare new o-Phthalaldehyde derivatization solution.
- Problem:** There is poor resolution between glyphosate and aminomethylphosphonic acid, or their retention times differ by more than 10% from the test chromatogram provided with the column.
- Solution:** Ensure the pH of the mobile phase is 1.9. You may need to regenerate the column.
- Problem:** Neither compound is detected.
- Solution:** Ensure the pH of the effluent coming out of the detector is higher than 9.5. If it is not, increase the pH of the ophthalaldehyde solution with 1 N sodium hydroxide until the effluent pH is greater than 9.5.

Separation Conditions	
<b>Column Mobile Phase</b>	
Flow Rate:	0.5mL/min
Isocratic. Ambient	
Injection:	200 µL
Detection:	Excitation wavelength - 338 nm (better sensitivity than 340 nm) Emission wavelength - 455 nm
<b>Post Column Conditions - Oxidation Solution</b>	
Flow Rate:	0.2 mL/min
Reaction Coil Size:	1.0 mL (0.02" or 0.05 cm ID X 5 meter length, TEFLON® tubing)
Reaction Time:	1.4 min
Reaction Temp:	38° C
<b>Post Column Conditions - Derivatization Solution</b>	
Flow Rate:	0.3 mL/min
Reaction Coil Size:	0.2 mL (0.02" or 0.05 cm ID X 1 meter length, TEFLON® tubing)
Reaction Time:	0.2 min
Reaction Temp:	Room Temperature

Reaction coils can be made in the laboratory, or entire post-column derivatization systems are available for purchase.

**Flow rates, reaction coil sizes, reaction times, and temperatures are critical. If you fail to follow the above instructions, low-level detection of glyphosate and its metabolite may not be possible.**

## PRP-X400

Größe	5µm	7µm	10µm	12-20µm	30-50µm
2.1 mm x 250 mm		91 79398			
4.1 mm x 150 mm		91 79717			
4.1 mm x 250 mm		91 79473			
4.6 mm x 250 mm <b>Peek</b>		91 79387			
Bulk Resin (1 gram)		91 79591		91 79592	91 79593



## HPLC Guard Columns

### Analytical Starter Kits (1 holder, 2 cartridges)

Packing Material	SS	Peek
<b>PRP-X400</b>		91 79376

### Analytical Replacement Cartridges (5/pk)

Packing Material	SS	Peek
<b>PRP-X400</b>		91 79377

### Semi-prep / preparative Starter Kits (1 holder, 1 cartridges)

Packing Material	SS
<b>PRP-X400</b>	91 79131

### Semi-prep / preparative Replacement Cartridges (2/pk)

Packing Material	SS
<b>PRP-X400</b>	91 79132