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 7 µm poly(styrene-divinyl-benzene) sulfonate cation exchange support (2.5 meq/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 (KH_2PO_4) pH 1.9, dissolve 0.68 g of monobasic potassium phosphate in 1 L of deionized water. Adjust the pH to 1.9 using concentrated phosphoric acid. Prior to using this preparation, filter it through a 0.45 µm nylon filter and degas.

Detection
Post-column reaction (oxidation) with calcium hypochlorite followed by derivatization with o-phthaldehyde solution provides sensitive (6 ppb 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 (6 ppb) of glyphosate and its metabolite precisely follow these instructions.

Oxidation Solution Preparation
(15 ppm calcium hypochlorite)

Stock Oxidation Solution Concentrate Preparation - To prepare the 150 ppm concentrate solution, add 0.23 g of tech grade calcium hypochlorite $Ca(OCl_2)$ to 100 mL of deionized water. With a 2 µm 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 1.36 g monobasic potassium phosphate, 11.60 g sodium chloride, and 0.40 g sodium hydroxide (or use 0.50 mL 50% w/w sodium hydroxide solution) in 950 mL deionized water. Add 10.00 mL of 150 ppm calcium hypochlorite stock concentrate solution, and dilute to 1 L. Filter through a 0.45 µm 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 19.1 g of disodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$) in 950 mL 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 1 N sodium hydroxide. Now dissolve 0.80 g phthalic dicarboxyaldehyde (Aldrich Part #P3,940-0) in 10 mL methanol. Add all 10 mL to the disodium tetraborate decahydrate solution. Then add 50 µl 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 1 L with deionized water, mixing well. Filter the mixed solution through a 0.45 µm 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.

Separation Conditions	
	Column Mobile Phase
Flow Rate:	0.5mL/min
	Isocratic. Ambient
Injection:	200 µL
Detection:	Excitation wavelength - 338 nm (better sensitivity than 340 nm) Emission wavelength - 455 nm
	Post Column Conditions - Oxidation Solution
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
	Post Column Conditions - Derivatization Solution
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

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.

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 o-phthalaldehyde solution with 1 N sodium hydroxide until the effluent pH is greater than 9.5.

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