Technical Information

Glyphosate Analysis using the PRP-X400

Polymeric cation exchange packing for separation of glyphosate and its metabolite in drinking water

- Faster analysis compared to other glyphosate columns
- Detection levels of less than 10 ppb when using the post column, OPA derivatization technique
- Applicable to other separations, such as inositol and sugar alcohols

Product Features

- One particle size: 7 µm
- Three column internal diameters: 2.1 to 4.6 mm
- Two column materials: 316 stainless steel and PEEK
- Analytical and semiprep/preparative guard columns

PRP-X400 Advantages

Faster Separation

The PRP-X400 column provides a fast separation for glyphosate and its metabolite.

Use at Room Temperature

The PRP-X400 column does not have to be heated to 65° C, so you don't need to purchase a column heater for this method.

No Methanol Required

PRP-X400 columns do not require the use of methanol in the mobile phase.

Lower Cost

PRP-X400 columns cost much less than other glyphosate columns.

Use the PRP-X400 Column for EPA Method #547

Separation Mechanism

The PRP-X400 is a 7 µm poly(styrene-divinylbenzene) sulfonate cation exchange support (2.5 meq/gm) column. It separates glyphosate and aminomethylphosphonic acid according to charge in less than 10 minutes. This separation requires post-column oxidation and derivatization (see Figure 1).

Mobile Phase Preparation

To prepare 0.005 M monobasic potassium phosphate (KH_2PO_4) pH 1.9, dissolve 0.68 gm of monobasic potassium phosphate in 1 Liter 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-phthalaldehyde solution provides sensitive (6 ppb or lower) and selective (primary and secondary) amine detection. To achieve low-level detection (6 ppb) of glyphosate and its metabolite, follow the instructions listed under Separation Conditions.

Separation Conditions

Column Mobile Phase

Flow Rate	0.5 mL/min (Isocratic)
Temperature	Ambient
Injection	200 µL
Detection	Excitation λ: 338 nm (better sensitivity than 340 nm) Emission λ: 455 nm

Post Column Conditions - Oxidation Solution

Flow Rate	0.2 mL/min
Reaction Coil Size	1.0 mL (0.02 in or 0.05 cm ID x 5 m length tubing*
Reaction Time	1.4 min
Reaction Temperature	38° C

Post Column Conditions - Derivatization Solution

Flow Rate	0.3 mL/min
Reaction Coil Size	0.20 mL (0.02 in. or 0.05 cm ID X 1 meter length tubing*)
Reaction Time	0.2 minutes
Reaction Temperature	Ambient

Separation Procedure

Oxidation Solution Preparation (15 ppm calcium hypochlorite)

Stock Concentrate Solution Preparation - To prepare the 1500 ppm concentrate solution, add 0.23 gm of tech grade calcium hypochlorite 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. The shelf life is several freeze/thaw cycles.

Working Oxidation Solution Preparation

Dissolve 1.36 gm monobasic potassium phosphate, 11.60 gm sodium chloride, and 0.40 gm sodium hydroxide (or use 0.50 mL 50% w/w sodium hydroxide solution) in 950 mL deionized water. Add 10 mL of 1500 ppm calcium hypochlorite stock concentrate solution, and dilute to 1 Liter. 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 gm of disodium tetraborate decahydrate in 950 mL of 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 and 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 gm 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 2-mercaptoethanol, as the fumes are noxious.) Dilute the concentrate to 1 Liter with deionized water, mixing well. Filter the mixed solution through a 0.45 µm nylon filter, and degas before using. Store the solution in an inert atmosphere (helium or nitrogen). Refrigerate the unused solution. Shelf life is one or two weeks.

Reaction coils can be made in the laboratory (see Separation Conditions) or entire post-column derivatization systems are available for purchase.

Troubleshooting Guide

Problem	Solution
A loss in sensitivity for glyphosate relative to aminomethylphosphonic acid.	Prepare a new calcium hypochlorite stock solution. Oxidation reaction is required for detection of glyphosate, not aminomethylphosphonic acid.
A loss in sensitivity for both glyphosate and aminomethylphosphonic acid.	Prepare new o-phthalaldehyde derivatization solution.
There is poor resolution between glyphosate and aminomethylphosphonic acid.	Ensure the pH of the mobile phase is 1.9. You may need to regenerate the column.
Neither compound is detected.	Check to determine if the pH of the effluent coming out of the detector is higher than pH 9.5. If not, increase the pH of the o-phthalaldehyde solution with 1 N sodium hydroxide until the effluent pH is greater than 9.5.

Source: https://www.hamiltoncompany.com/technical-information/hplc_glyphosate-analysis?p=1