

Determination of Glyphosate by Cation-Exchange Chromatography with Postcolumn Derivatization

INTRODUCTION

Glyphosate is a broad-spectrum herbicide with low mammalian toxicity. The herbicide's widespread use makes it a possible contaminant in ground water and eventually drinking water. The U.S. EPA has established Method 547 to monitor glyphosate in drinking water.¹

Alternative chromatographic methods for the analysis of glyphosate, whether gas or liquid chromatography, usually require precolumn derivatization of the glyphosate.²⁻⁶ Precolumn derivatization is tedious and can be subject to interferences. Nonchromatographic methods, such as differential pulse polarography, demonstrate poor recoveries and inadequate detection limits.⁷

This application note details a convenient chromatographic method for the analysis of glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA). Since glyphosate has been shown to rapidly decompose in chlorinated water, AMPA is the species most likely to be found in drinking water matrices.

Sample Preparation and Preservation

Drinking water samples should be collected in clean glass bottles and sealed using caps with PTFE-faced silicone septa. Samples should be filtered prior to injection and can be analyzed without any further treatment.

Summary of Chromatographic Method

The method achieves a high degree of sensitivity and selectivity by combining postcolumn derivatization with fluorescence detection. It is designed to be consistent with U.S. EPA Method 547.

After direct injection of the sample onto the column, glyphosate and AMPA are separated with a potassium phosphate buffer. After separation, the analytes pass through a postcolumn reaction system where they are reacted to form fluorescent derivatives, which are then quantified using fluorescence detection.

The analytical column specified in this application note is quality controlled by the vendor to ensure reliable glyphosate analysis. The reagents specified are also quality controlled to ensure freedom from background interferences. When analyzing a 50 μ L drinking water sample, as shown in Figure 1, method detection limits meet or exceed those cited in U.S. EPA Method 547. See the *Results* section for method performance data.

EQUIPMENT

Dionex DX 500 HPLC system consisting of:

- GP40 Gradient Pump
- Postcolumn Derivatization Module (Pickering PCX-5100, Pickering Laboratories)
- Jasco® FP-920 Fluorescence Detector (Jasco)
- Jasco Y-46 Emission Filter (Jasco)
- Eluent Organizer

PeakNet Chromatography Workstation with UI20 Universal Interface

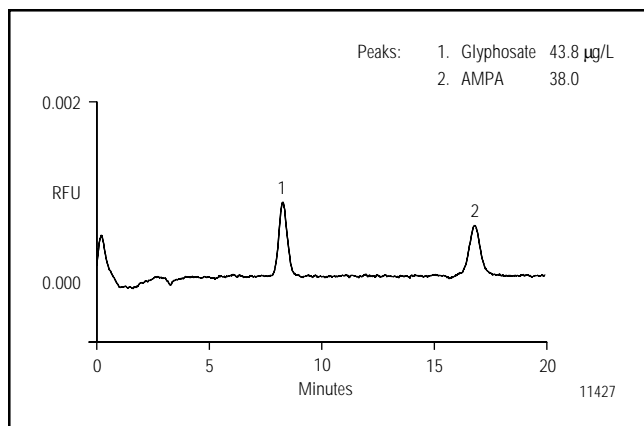


Figure 1 Drinking water fortified with glyphosate and AMPA. Injection volume = 50 μ L.

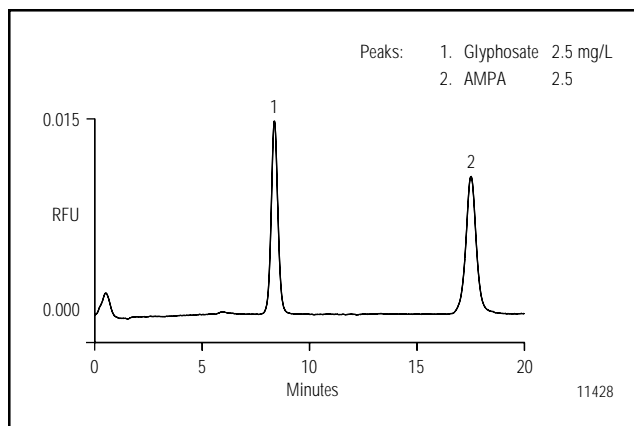


Figure 2 10- μ L injection of glyphosate and AMPA standards.

REAGENTS

5 mM Potassium phosphate, pH 2.0 (Glyphosate Eluent, Pickering P/N K200)

5 mM Potassium hydroxide (Glyphosate Column Regenerant, Pickering P/N RG019)

Hypochlorite diluent (Pickering P/N GA116)

5% Sodium hypochlorite solution

Sodium borate buffer diluent (Pickering *o*-Phthalaldehyde Diluent, P/N GA104)

OPA, Chromatographic grade (Pickering *o*-Phthalaldehyde, P/N O120)

Methanol, Optima™ grade or equivalent (Fisher Scientific)

Mercaptan reagent (Thiofluor™, Pickering P/N 3700-2000)

PREPARATION OF REAGENTS AND STANDARDS

Oxidizing Reagent (Reagent 1):

Pour one bottle of the pre-prepared hypochlorite diluent into a clean reagent reservoir that has been rinsed with methanol. Add 100 μ L of the 5% hypochlorite solution (household bleach has been found to be suitable) and swirl to mix. This amount may require

adjustment as follows to optimize detector response: after the chromatographic system is fully equilibrated, inject 10 μ L of glyphosate test mixture (Pickering P/N 1700-0080), as shown in Figure 2. If area counts for AMPA and glyphosate differ significantly, add 5% sodium hypochlorite solution to the oxidizing reagent in 20- μ L increments until the peak areas are approximately equal.

OPA Reagent (Reagent 2):

Pour the contents of the OPA diluent into a clean reagent bottle that has been rinsed with methanol. Sparge the diluent for approximately 10 minutes to remove any oxygen.

The remaining steps should be accomplished quickly since the prepared reagents are sensitive to oxygen and light: weigh approximately 100 mg of *o*-phthalaldehyde into a small beaker, dissolve in 10 mL of methanol, and add to the OPA diluent. Rinse the beaker with 1 or 2 mL of methanol and add the rinsate to the diluent. Add 2 g of Thiofluor to the reagent bottle, replace the cap, and sparge for 1 or 2 additional minutes. Swirl the bottle gently to ensure complete mixing.

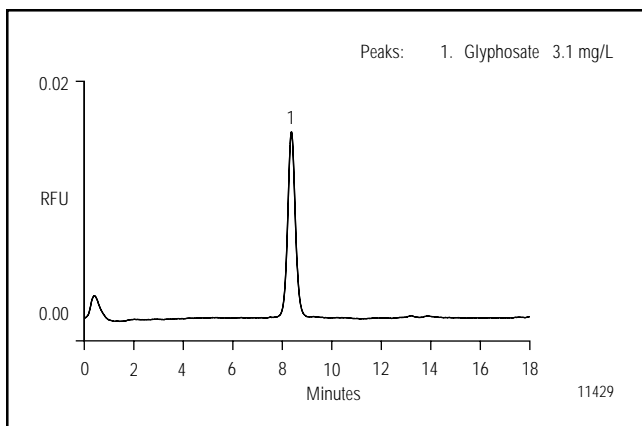


Figure 3 A 1/2500 dilution of Roundup. Injection volume = 10 μ L.

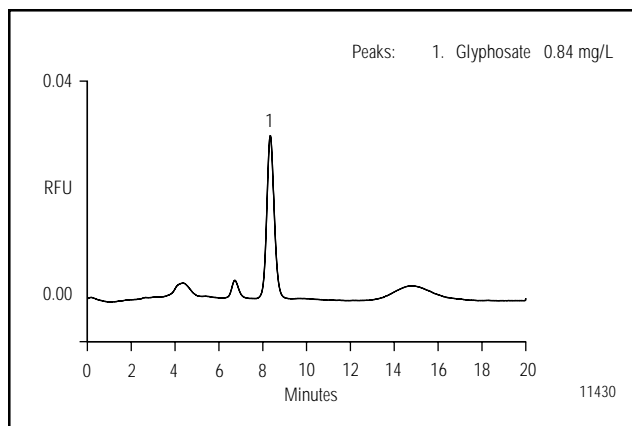


Figure 4 Glyphosate in a milkweed sample. Total concentration of glyphosate = 23 μ g/g of plant material. Injection volume = 50 μ L.

CONDITIONS

Column: Glyphosate Column, cation exchange, 4 mm x 150 mm x 8 μ m (Pickering P/N 1954150)

Guard: Glyphosate Guard Column, cation exchange, 3 mm x 20 mm x 8 μ m (Pickering P/N 1953020)

Column Temp.: 55 $^{\circ}$ C

Eluents: (A) Potassium phosphate (P/N K200)
(B) Potassium hydroxide (P/N RG019)

Gradient:	Time	A	B
	(min)	(%)	(%)
	0	100	0
	15	100	0
	15.01	0	100
	17	100	0
	25	100	0

Flow Rate: 0.4 mL/min

Postcolumn

Reagent 1: Oxidizing reagent at 36 $^{\circ}$ C

Flow Rate: 0.3 mL/min

Reagent 2: OPA reagent, ambient

Flow Rate: 0.3 mL/min

Fluorescence: Excitation: 330 nm
Emission: >460 nm (cut-off filter)

DISCUSSION AND RESULTS

Figure 3 shows the analysis of glyphosate in Roundup[®], a commercially available herbicide. The Roundup was diluted 1/2500 in deionized water and 50 μ L was injected directly onto the column. The concentration of glyphosate in this formulation was found to be about 0.8%.

Roundup was then applied to a milkweed plant. After approximately 6 hours, a 7.2-g sample of the milkweed was homogenized with 200 mL of the potassium phosphate eluent in a blender. The sample was centrifuged at 5000 rpm for 15 minutes, and the supernatant filtered through a 0.45- μ m filter (Gelman Acro[™] LC13, P/N 4453). A sample size of 50 μ L was injected into the system and the results are shown in Figure 4.

Postcolumn Chemistry⁸

The postcolumn system features two reagent pumps, two reactors (one heated), and a column oven. A built-in pressure switch shuts down the reagent pumps if it senses that the analytical pump pressure has dropped below 3.4 MPa (500 psi). This feature prevents back-flow of postcolumn reagents onto the analytical column. A schematic diagram of the system hardware is shown in Figure 5.

After it is eluted from the column, the glyphosate is oxidized by hypochlorite at 36 $^{\circ}$ C to form glycine. The glycine is then derivatized with *o*-phthalaldehyde and *N,N*-dimethyl-2-mercaptoethylamine hydrochloride

(Thiofluor) to form a highly fluorescent isoindole compound. AMPA reacts directly with the OPA reagent to form a similar isoindole, as shown in Figure 6.

The Thiofluor reagent is a solid that may be substituted for the liquid 2-mercaptoethanol that is traditionally used for this application. The advantage of Thiofluor is that it is much more stable in solution and is relatively odorless.

Method Detection Limits

The method detection limit for a 50- μ L injection of glyphosate in reagent water is 1.8 μ g/L, which is less than the 6.0 μ g/L MDL for a 200- μ L injection volume cited in U.S. EPA Method 547. Dionex recommends

an injection of ≤ 50 μ L to preserve peak shapes for all possible matrices; however, 200- μ L injection volumes can be used if the pH of the sample is adjusted to ≤ 2 .

Linearity

Glyphosate and AMPA standards of 0.05, 0.5, 2, 4, 6, 8, and 10 mg/L were injected in duplicate for this study. The method was found to be linear for glyphosate over the range tested.

AMPA is not linear over this range ($r^2 = 0.9983$), but a quadratic fit of the data gave an r^2 value of 0.99999. For more accurate work, a quadratic fit should be employed.

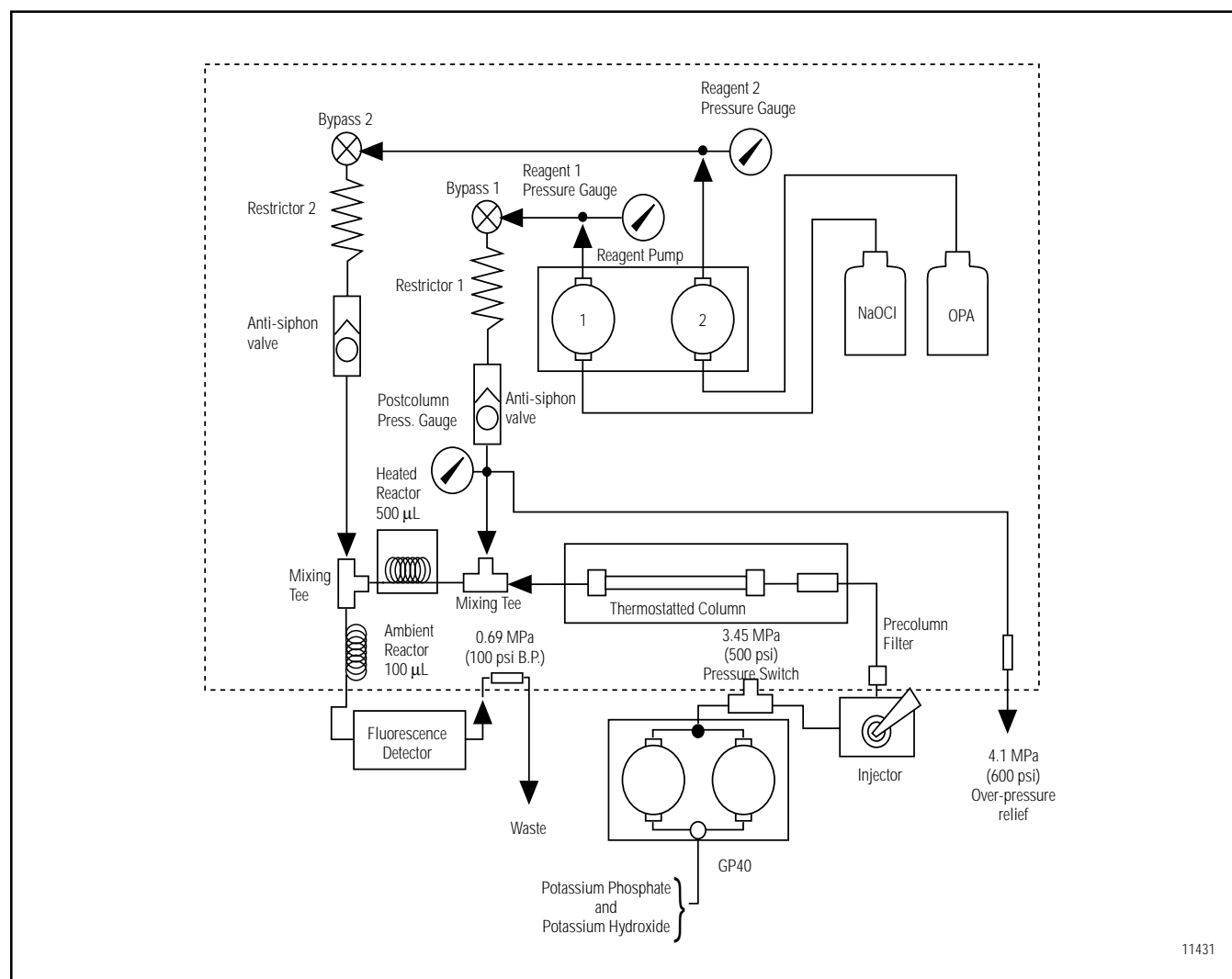


Figure 5 Schematic of glyphosate analysis system. The chromatography column and postcolumn reactor are represented by the portion of the diagram inside the dotted line.

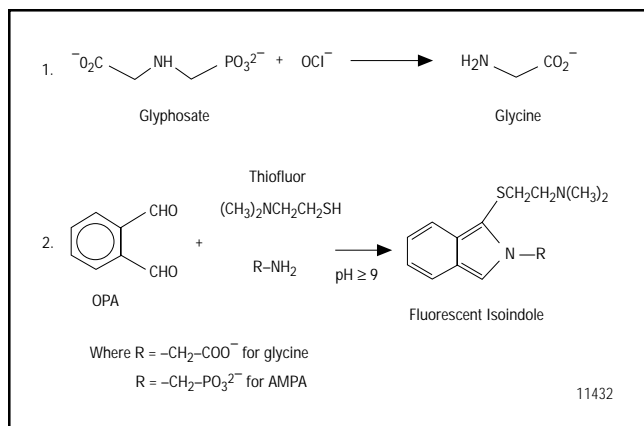


Figure 6 Postcolumn reaction chemistry of glyphosate and AMPA.

PRECAUTIONS

Contamination

This method is sensitive to amines from fingerprints and other sources of contamination. We recommend using gloves while preparing reagents and rinsing reagent bottles and eluent lines with methanol before use.

The U.S. EPA method calls for the use of calcium hypochlorite in the oxidizing reagent. The substitution of sodium hypochlorite, however, is equally effective and reduces the chance of plugging the postcolumn reactor.

Reagent Compatibility

The cation-exchange column is *not* solvent compatible and care should be taken to ensure that methanol from the postcolumn system does not back up into the analytical column.

The column regenerant (Eluent B) is strongly alkaline and all system components should be compatible with high pH. Many standard injection valve rotor seals are made of VespeI® or other incompatible polymers. For this application, the seal should be made of Tefzel®.

Reagent Storage

Oxidizing Reagent, when kept under helium, can be used for about three days. After this period, fresh reagent should be prepared.

OPA reagent is oxygen sensitive. If stored under helium, it is stable for up to one week.

Aqueous samples and standards should be kept refrigerated until they are ready to use.


REFERENCES

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8. *PCX 5100 Postcolumn Derivatization Instrument User's Manual*, Pickering Laboratories, Mountain View, CA, 1993.

LIST OF SUPPLIERS

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 Jasco, 8649 Commerce Drive, Easton, Maryland, 21601-9903, USA, 1-800-333-5272.
 Pickering Laboratories, 1951 Colony Street, Suite S, Mountain View, California, 94043, USA, 1-800-654-3330.

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