Analytical Residue Method for N-Phosphonomethyl Glycine and Aminomethylphosphonic Acid in Environmental Water

Method No. 86-63-1

Scope

The procedures given can determine trace quantities of N-(phosphonomethyl) glycine (glyphosate) and aminomethylphosphonic acid (AMPA) in parts per billion range in environmental water by HPLC.

Summary

Environmental water can be analyzed for glyphosate and AMPA by concentration and injection into a high pressure liquid chromatograph equipped with an *o*-phthalaldehyde (OPA) post-column reactor (PCR) and a fluorescence detector.

Sensitivity

The sensitivity of the method is 0.5 ppb after concentration of 250 mL of environmental water for both glyphosate and AMPA. If the environmental water is injected straight into the HPLC-PCR system, a concentration of 25 ppb can be determined directly by external standard calibration.

Apparatus and Equipment

High vacuum pump, Sargent-Welch Model 1400B or equivalent
Rotary Evaporator
Cold finger condenser (45 cm long) filled with dry ice
3 mL disposable syringes with Luer-Lok® tips (Becton-Dickinson
Arcodisc® disposable filter assembly, 0.45 µg pore size
6 inch cotton tipped applicators
500 mL round bottom flask
Vials for autosampler

Reagents

A. Chemicals and Materials

Deionized water

Hydrochloric acid, reagent grade

Potassium dihydrogen phosphate (HPLC grade)

Methanol (HPLC grade)

Phosphoric acid, concentrated (HPLC grade)

Disodium ethylenediamine tetraacetate dihydrate, certified A.C.S.

B. Fortification Solutions

1. Glyphosate and AMPA

Weigh and dissolve 0.1000 g of N-(phosphonomethyl) glycine (Glyphosate) and 0.1000 g of aminomethylphosphonic acid (AMPA) in 1000 mL of deionized water. This concentrate contains 100 micrograms of glyphosate and AMPA per milliliter.

Standard solutions for fortifications are made by diluting the standard concentrate with deionized water as follows:

From 100 Microgram Concentrate

Milliliters Concentrate	Standard Dilution	Concentration Micrograms/mL
50.0	100.0	50.0
30.0	100.0	30.0
20.0	100.0	20.0
10.0	100.0	10.0
5.00	100.0	5.0
3.00	100.0	3.0
2.00	100.0	2.0
1.00	100.0	1.0
0.50	100.0	.50
0.25	100.0	.25

2. Source of Standard Material Used in Fortifications and HPLC Standard Solutions.

Glyphosate reference N-(phosphonomethyl) glycine > 99

AMPA reference (aminomethylphosphonic acid), 99%

C. HPLC Standards

HPLC standard solutions are prepared in a similar manner as above except that 0.001 M disodium EDTA solution is used in place of deionized water to make dilutions. The 100 and 10.0 microgram per milliliter stock solutions are made as for the fortication solutions with 0.001 M disodium EDTA solution. The solutions for HPLC standards are made by diluting the 10.0 microgram per milliliter stock with 0.001 M disodium EDTA solution as follows:

From 10.0 Microgram Dilution

Milliliters Concentrate	Standard Dilution	Concentration Micrograms/mL
10.0	100.0	1.00
5.00	100.0	0.50
1.00	100.0	0.10
0.50	100.0	.050
0.25	100.0	.025

Procedure

Sample Preparation

Thaw frozen water sample, shake thoroughly, and transfer approximately half of the 250 mL sample (filter through glass wool or filter paper if there is a lot of suspended particles in the sample) into a 500 mL round bottom flask. For recovery samples, analyte fortifications are made at this stage. Add 5 mL of concentrated hydrochloric acid to the sample in the flask and 5 mL to the sample remaining in the bottle. Concentrate the sample on a rotary-film evaporator by slowly increasing the temperature of the water bath from 20° C to 60° C. Before the sample is evaporated to dryness, add the remainder of the sample and rinse the bottle twice with approximately 5 mL of deionized water, adding the rinses to the flask also. Concentrate the sample to dryness and remove the final traces of moisture with a stream of dry nitrogen if necessary. Combine the residue with 2.9 mL of HPLC buffer (0.005 M KH_2PO_4 in 4% methanol/deionized water adjusted to pH 2.1 with concentrated phosphoric acid) using a long cotton swab

to remove any solid residues from the bottom and a pipette to rinse the sides of the flask. Leaving the swab in the flask, add 0.1 mL of 0.03 M disodium EDTA solution and mix thoroughly by rinsing the sides. Transfer the solution into a disposable syringe and filter through an attached 0.45 μm size pore membrane filter. The sample is now ready for quantification of glyphosate and AMPA by using the HPLC – OPA post-column reactor system.

Samples that need to be diluted to remain within the standard concentration range must be diluted with a 0.001 M EDTA solution.

Note: Standards used for quantification of concentrated samples containing EDTA must also contain EDTA. If environmental water is injected straight into HPLC-PCR system, filter through a 0.45 μm filter and compare to standards dissolved in deionized water.

HPLC OPA Post-Column Reactor System

Glyphosate and aminomethylphosphonic acid (AMPA) may be separated and detected utilizing a high pressure liquid chromatograph and a post column reaction specific for primary amines. Glyphosate is oxidized with calcium hypochlorite and the product (glycine) and AMPA are coupled with O-phthalaldehyde in the presence of mercaptoethanol (OPA-MERC) to give fluorophors detected by a fluorometer with excitation at 340 nm and emission measured at 455 nm. The components needed for the construction of this detection system are outlined below. A general schematic is presented in Figure 1 and several general comments on the assembly are included.

Equipment and Supplies

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Solvent Buffer Reservoir
HPLC pumps (two required)
Temperature controller for HPLC column and oxidation reaction coil (Kratos URA 200 or equivalent)
$Ca(OCl)_2$ solution reservoir
OPA solution reservoir
Post Column Derivatization System (Kratos URS 051 dual pump Reagent Delivery Module or equivalent)
1 mL reaction coil (2)
stainless steel tubing { 1/16 inch × O.D.0.020 inch I.D.
1/16 inch × O.D.0.010 inch I.D.
Fluorescence Spectrometer (Perkin-Elmer LS-4 or equivalent)
Solvent filter (Millipore GSWP 04700 or equivalent)
Electronic integrator or computer

Reagents

Deionized water
Potassium dihydrogen phosphate (HPLC grade)
Methanol (HPLC grade)
Phosphoric acid, concentrated (HPLC grade)
Fluoraldehyde®, o-phthalaldehyde reagent solution (Pierce Chemical Company)
Disodium ethylenediamine tetraacetate dihydrate, certified A.C.S. grade

Alternate Solution for Fluoraldehyde®

1. Boric acid
2. 2-Mercaptoethanol
3. Potassium hydroxide, 45% solution
4. Brig®35, 30% solution
5. Deionized water, with resistance of 16 megohm/cm
6. Fluoropa o-phthalaldehyde, Dionex) Pierce Chemical Company
7. Methanol

Calcium hypochlorite, certified, 70.89% available chlorine, Fisher Scientific Company

Sodium Chloride, analytical reagent

Sodium hydroxide, certified A.C.S. reagent

Solution Preparation

1. HPLC Buffer

   Prepare 0.005 M potassium dihydrogen phosphate (KH_2PO_4) by dissolving 2.72 g in four liters of 4% methanol/deionized water. This solution is adjusted to pH 2.1 with concentrated phosphoric acid. Normal HPLC degassing procedures are followed, and the solution is filtered through a 0.22 μm filter.

2. Oxidative Solution

   In a 500 mL volumetric flask, dissolve 0.5 g $Ca(OCl)_2$ in 500 mL of deionized water using a magnetic stirrer at high speed for 45 minutes. In a 1 liter volumetric flask, dissolve 1.36 g KH_2PO_4, 11.6 g $NaCl$, and 0.4 g $NaOH$ in 500 mL solution) and dilute to 1 L with deionized water, mixing well. Filter the solution through a 0.22 μm filter.

3. EDTA Solutions

   a. 0.03 M EDTA Solution

      Dissolve 11.17 g of disodium ethylenediamine tetraacetate dihydrate in 1 L deionized water using a magnetic stirrer and filter through a 0.22 μm filter.

   b. 0.001 M EDTA Solution

      Dissolve 0.37 g of disodium ethylenediamine tetraacetate dihydrate in 1 L deionized water using a magnetic stirrer and filter through a 0.22 μm filter.

4. Alternate OPA Solution

   a. Dissolve 25 g boric acid in 950 mL deionized water using a magnetic stirrer.

   b. While monitoring the pH with a pH meter, titrate with the potassium hydroxide solution (approximately 30 mL will be required) to a final pH of 10.40 ± 0.2.

   c. Filter through a 0.22 μm filter.

   d. Add 3 mL 30% Brig 35 solution.

   e. Add 2.0 mL 2-mercaptoethanol.

f. Dissolve 800 mg Fluoropa in 10 mL methanol at room temperature using gentle swirling.

g. Add the methanol-Fluoropa solution.

Storage Stability

Fluoropa and 2-mercaptoethanol are subject to atmospheric oxidation and these oxidation products can contribute to increased background fluorescence. Thus, unless the reagent solution is protected from atmospheric oxygen, it should be prepared fresh daily. The solution can be stored in closed glass bottles under atmospheric conditions at 4° C for up to two weeks without appreciable increases in background fluorescence or it can be stored under nitrogen for indefinite periods.

Note: The Fluoraldehyde® (Pierce OPA reagent solution) is a specially formulated OPA solution which has outstanding shelf life with no increase in background fluorescence with time.

HPLC Conditions

Pre-Column: RP-18 Spheri-10, 3.6 cm x 4.6 mm I.D., guard column, Brownlee Labs Inc., Santa Clara, California.

Column: Aminex A-9, 30 cm x 4.6 mm I.D., Bio-Rad Laboratories, Richmond, California.

Column Temperature: 50° C

Buffer Flow: 0.5 mL/min

Oxidation solution flow rate: 0.5 mL/min

OPA solution flow rate: 0.5 mL/min

Pressure: approximately 1500 psi Buffer

Fluorometer settings: { Excitation 340 nm / Emission 455 nm

Injection volume: 200 µL

See Figure 1 for an outline of the system.

General Comments

The system is designed to permit the continuous analysis of samples. A pre-column or guard column is installed to protect the analytical column. This pre-column can be changed periodically or when necessary.

The analytical column should be packed using the HPLC buffer as eluant. The column end fittings should accomodate 1/16 x 0.001 inch I.D. tubing and must be capable of withstanding a backpressure of 4000 psi.

When starting, turn all pumps and the detector on for thirty minutes prior to use. If an air bubble becomes trapped in the detector cell, it can be removed by disconnecting the waste line from the detector and alternately drawing and forcing liquid through the cell with a syringe containing water or methanol, until the bubble is removed. When shutting the system down, it is advisable to flush the system (pumps and fluorometer) with deionized water.

The relative peak response of glyphosate to AMPA can be adjusted by optimizing the flow rates of hyphochlorite and OPA. Increasing the hyphochlorite flow rate will decrease the AMPA response relative to the glyphosate response.

A buffer of pH 2.1 was used for analyzing glyphosate and aminomethylphosphonic acid in water samples. Sometimes a close peak interfering with the integration can be further separated by changing the pH of the buffer. Should it become necessary to change the pH of the solvent buffer to effect a better separation, the hypochlorite and OPA flows should also be adjusted to optimize the greatest response for glyphosate and AMPA. The pH and the amount of excess hypochlorite and OPA solutions present do effect the reactions that produce the fluorogenic responses.

The $Ca(OCl)_2$ reaction coil can be run at room temperature but temperature should be constant throughout the run. Variation of temperature by several degrees can effect the response of the glyphosate peak. To prevent problems from temperature variation, the $Ca(OCl)_2$ reaction coil should be placed in a temperature controlled module such as the Kratos URA 100 or 200 reaction chamber/column temperature control module. A temperature of 40–C gives the optimum response for both glyphosate and AMPA.

Using the HPLC conditions given above, the analysis time between injections is approximately 30 minutes, depending on the quality of the column.