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Determination of Glyphosate (N-phosphonomethyl glycine) in Runoff Water

Scope: This method is for the determination of glyphosate in runoff water by using HPLC with post-column derivatization and fluorescence detection. The detection limit and reporting limit for glyphosate using this procedure are 1.755 and 2.0 µg/L respectively.

Principles: A 500 mL sample of runoff water is acidified, and concentrated on a Chelex 100 (iron form) resin column. The residues, along with iron, are eluted with 6 N HCl. The $Fe(Cl)_4^-$, is removed from the residues by passage through an AG 1 x 8 resin column, an anion exchanger. The eluent is evaporated to dryness on a rotary evaporator. The glyphosate residue is redissolved in water and analyzed using HPLC with a post column derivatization system.

Reagents, Equipment and Instrument:

Reagents: All reagents must be suitable for pesticide residue analysis. Although some specific name brands are listed, equivalent supplies can be used:

1. Glyphosate, CAS # 1071-83-6, 1 .O mg/mL in water, obtained from CDFA Standard Repository (Center for Analytical Chemistry, California Department of Food and Agriculture).
2. Chelex® 100 resin, sodium form or iron form, 100-200 mesh, BioRad Laboratories, 2000 Alfred Nobel Dr., Hercules, Ca 94547. Contact the BioRad Laboratories for the sodium form to iron form conversion procedure.
3. Anion exchanger, AG® 1 -X8 resin, Cl form, 200-400 mesh, BioRad Laboratories, 2000 Alfred Nobel Dr., Hercules, Ca 94547.
4. Deionized water, (DI water)
5. Hydrochloric acid.
6. Mobile phase: 0.005 M KH_2PO_4, pH 2.0, Pickering # K200.
7. Column Regenerant: Pickering RGO 19.
8. Hypochlorite diluent: pH 11.6, Pickering GA1 16, or dissolve 1.36 g KH_2PO_4, 11.6 g NaCl and 0.4 g NaOH in 500 mL DI water and dilute to 1000 mL with DI water.
9. Sodium hypochlorite: 5.25 % solution, Clorox™, or equivalent.
10. Hypochlorite solution: add 120 µL of 5.25% sodium hypochlerite to 1 L of hypochlorite diluent.
11. 0-phthalaldehyde diluent: Pickering GA1 04, pH 10.4, or dissolve 19.1 g of sodium borate ($Na_2B_4O_7 \bullet 10\ H_2O$) in 1 .O L of DI water and adjust pH to 10.4 with

Reagents:continued

1 N NaOH solution.

12. OPA reaction solution: dissolve 100 mg of o-phthalaldehyde in 10 mL methanol. Pour this methanol solution to 950 mL OPA diluent and mix well. Pour the solution into the reagent reservoir and add 2 g of Thiofluor directly into it. Mix well (alternate: 1 mL of 2-Mercaptoethanol can be substituted for 2 g of Thiofluor).
13. Thiofluor™, N,N-Dimethyl-2-mercaptoethylamine-Hydrochloride, Pickering Laboratories, part# 3700-2000.
14. 2-Mercaptoethanol.
15. 0-phthalaldehyde, Pierce Chemical Company.
16. Ferric chloride.

Equipment: Some specific name brands of equipment are listed, however, in most cases, equivalent equipment and supplies from various venders may be used.

1. Beakers, 150 mL
2. Flasks, 250 mL, round, flat-bottom.
3. Columns, chromatographic, with removable stopcock of PTFE and replaceable glass tip, 11 mm ID x 300 mm length, and 22 mm ID x 300 mm length, with 300 mL reservoir.
4. Steam bath with a nitrogen stream manifold.
5. Vacuum rotary evaporator, Btichi-Brinkman, RE 111.
6. Analytical column: Cation exchanger, K^+ form, 4 x 150 mm, Pickering 1954 150.
7. Tubing, stainless steel or PEEK, 0.010" ID or less after columns and 0.020" ID before columns.
8. Guard column: Pickering # 1953020.
9. Microfilter, 0.2 µm nylon Acrodisc®, Gelman.

Instrument.

1. HPLC: Perkin Elmer Series 4 with column oven.
2. Post column system: Pickering dual pumps with a reaction coil after each pump. The first reaction coil is temperature controlled.
3. Autosampler, Perkin Elmer ISS- 100.
4. Fluorescence Detector: The Toshiba model # 1000 was used to generate the validation data. Any detector capable of excitation at 340 nm and detecting an emission $\geq$ **455** nm may be used.
5. Integrator: A HP 3 3 96 series 2 integrator

Analysis:

Preparation of Chelex 100 Resin column:

1. Plug column (2.2 cm OD x 25 cm with 300 mL reservoir) with glass wool.
2. Transfer ~ 20 mL DI water into the column. Measure and transfer 11 g of Chelex 100 resin (Fe form) into the column. Rinse down any resin on the walls with DI water. Drain and discard the water.

Sample Concentration with Chelex 100 Resin.

1. Mix sample well and then pour 500 mL into a beaker and record the weight.
2. Acidify the water sample with 6 N HCl to a pH of 2.0-2.3.
3. Add the acidified sample onto the column and elute at a rate of ~8 mL per minute. (If column becomes plugged and will not drain the top surface of sediment can be stirred gently so as not to disturb the column.)
4. After the sample has eluted, rinse the column walls with 50 mL DI water. **Next** turn the stopcock wide open and rinse with 100 mL 0.1 N HCl.
5. Add 3 mL 6 N HCl carefully, so as not to disturb the column and elute at a rate of ~ 10 drops per minute. Discard the eluent. Add 4 more mL and discard.
6. Elute the glyphosate with 6 mL of 6 N HCl at a rate of ~ 10 drops per minute. Collect the eluent into a 150 mL beaker. Repeat the elution procedure two more times collecting all eluent.
7. Add an additional 5 mL 6 N HCl onto the column and collect the eluent into the previously collected fraction. Add 5 mL concentrated I-ICI to the eluent to ensure the eluted iron complex is in the negatively charged form.

Preparation of Anion exchange column.

1. Plug a column (1.1 cm ID x 30 cm) with glass wool and add ~ 5 mL of DI water.
2. Transfer 7 g of AG 1-X8 anion exchange resin into the column.
3. With the stopcock wide open rinse the column with about 20-50 mL DI water.
4. Rinse the column twice with ~ 30 mL of 6 N HCl.
5. With the stopcock wide open, rinse the column with ~ 10 mL of 6 N HCl shortly before applying the sample.

Sample clean-up with an anion exchange column: AG 1x8 Resin

1. Transfer the sample onto the anion exchange column and elute with stopcock wide open. Collect the eluent into a 250 mL flat bottom flask.
2. Rinse the sample container with ~ 6 mL 6 N HCl and apply to the column.
3. Rinse the sample container with an additional 6 mL 6 N HCl and apply to the column.
4. Collect the rinse eluents into the corresponding 250 mL flask.

Concentration of the sample:

1. Evaporate the sample just to dryness on a rotary vacuum evaporator in a 65 °C water bath with 28-29 inches of vacuum. **To avoid sudden bumping, immerse the flask approximately 2-3 cm into the water for the first 3-5 minutes of evaporation.**
2. Place the flask on a 90 °C steam bath under a gentle stream of N_2 for 2-3 minutes to dry completely, then remove from the steam bath.
3. After the flask has cooled to room temperature, rinse down the sides of the flask with 2-mL DI water. Filter extract through a 0.2 µm filter into a 2-mL auto sampler vial for analysis.

Instrument Conditions:

Instrument: Perkin Elmer Series 4 HPLC with column oven and a Pickering post column system

Detector: Fluorescence: Excition, 340 nm & Emission, 465 nm

Column: Pickering Potassium Cation Exchange 4 mm x 1.50 mm x 8 µm

Instrument Conditions:continued

Guard Column: Glyphosate guard column k^+ form 3 x 20 mm
Column Temperature: 55 °C
Mobile Phase:
Eluent A: 0.005 M KH_2PO_4, pH 2.0
Eluent B: Column regenerent, or RGO 19

Time (min.)	Eluent A %	Eluent B %
1.0	100	0.0
15	100	0.0
2	0.0	100
6	100	0.0

Flow Rate: 0.4 mL/min.
Injection volume: 10 µL

Post Column System: Pickering
Derivatization Reagents: Hypochlorite solution & OPA solution
Flow Rate: 0.3 mL/min
Reaction Temperature: 3 1 °C
Retention time: Glyphosate, 8.6 ± 0.2 minutes

Calculation:

$$\mu g/L \text{ glyphosate} = \frac{\text{peak area of sample x final volume (mL) x 1000 (g/L)}}{\text{response factor x sample weight(g)}}$$

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$$\text{Where: response factor} = \frac{\sum (\text{peak area, / std concentration, ug/mL})}{n}$$

n = number of standards

Method Performance:

Quality Control:

1. A 4 point calibration curve of 0.5, 1 .O, 2.0, and 4.0 ng/µL glyphosate was obtained at the beginning and the end of each set of samples.
2. Each sample shall be injected two times to insure reliability of the analysis. If the signal of a sample is greater than that of the highest standard in the calibration curve, dilute the sample. Reinject the diluted sample together with standards twice more. A sample set is usually comprised of 8 samples, a blank and a spike.

Recovery Data

The analytical method was validated using 4 sets of spike samples. Each set contained 3 levels of spikes and a matrix blank. The matrix background water was supplied by Dept. of Pesticide Regulation. All samples were processed through the entire analytical method.

Analyte	Spike Level (µg/L)	Results (µg/L)	Recovery (%)
Glyphosate	4.0	2.35	58.8
		2.96	74.0
		2.37	59.3
		2.72	68.0
	20	14.9	74.9
		14.4	72.0
		14.8	74.0
		16.2	81.0
	100	81.7	81.7
		70.1	70.1
		78.4	78.4
		74.1	74.1

Method Detection Limit (MDL).

Method Detection Limit (MDL) refers to the lowest concentration of analytes that a method can detect reliably in either a sample or blank. To determine the MDL, 7 samples each containing 500 mL of background surface water were spiked with 4 ug glyphosate. The standand deviation derived from the 7 spikes was used to calculate the MDL using the following equation:

$$MDL = S\ t_{4}$$

where:

t is the student's "t" value for the 99% confidence level with n-l degrees of freedom (n- 1,1 -a = 0.99). n represents the number of replicates
S denotes the standard deviation obtained from replicate analyses.

Method Detection Limit (MDL): continued

Spike Recoveries for MDL Determination

Spike	Recovery µg/L
1	5.29
2	4.97
3	5.86
4	5.37
5	4.59
6	5.83
7	6.20

The standard deviation ascertained for glyphosate is 0.558 µg/L
The MDL is 1.755 µg/L for glyphosate.

Reporting Limit (RL):

RL refers to the level above which quantitative results may be obtained. The MDL was used as a guide for determining the RL. The reporting limit for this method is 2.0 µg/L which is the value obtained for the MDL rounded to the nearest whole number.

Discussion:

AG 1-X8 resin was successfully regenerated in our study. This was acomplished by adding approximately 30 mL of DI water to the column to wash off the iron. If the column starts to change back to its orginial color regeneration is possible. Let the water drain ~ half way down and then add ~ 10 mL of 6 N HCl. The column should turn a light yellow color. Let this solution drain completely and then wash the column with ~ 30 mL of DI water. The column should be back to the orginal color. Continue with step *4* in *Preparation of Anion Exchange Column* and the column is ready to reuse. The chemist must be alert to any adverse effects after several times of reuse.

The HPLC column should be stored in regenerant solution when not in use to prolong the life of the column. The column may need to be treated with Restore occassionaly when peak shape starts to broaden. Treat the column with Restore for 60 minutes, then rinse with the mobile phase for 30 minutes and try the column again. If this does not work it may be necessary to replace the column.

Irreversible damage to the column may be caused by solvent passing through the analytical column or running the column at high flow rates.

References:

1. Lee, Paul, *Determination of Glyphosate (N-phosphonomethyl glycine) and AMPA (Aminomethyl phosphonic acid) in Well Water by HPLC, with Post-column Derivatization and Fluorescence Detection,* 10-30-95, Enviromental Monitoring Methods, California Department of Food and Agriculture.

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2. Cowell, J., et al., " *Validation of an Analytical Residue Methodfor Analysis of Glyphosate and Metabolite: An Interlaboratory Study",* J. Agric. Food Chem. 1986, 34, 955-960.

3. Jerry R. Steinmetz *"Analytical Methodfor Glyphosate and AMPA in Raw Agricultural Commodities, and Their Processed Fractions, Document #Res-008-90*", Environmental Science Department, Monsanto Company, 700 Chesterfield Parkway North, St. Louis, Missouri 63 198. Fax Number: (3 14) 537-6134.

4. US Environmental Protection Agency, " *Determination of Glyphosate in Drinking Water by Direct-Aqueous-Injection HPLC, Post-Column Derivatization, and Fluorescence Detection",* EPA-500 Series Supplement I, July 1990.

5. Communication with *Donna Harding of BioRad Laboratories* during September 1995, Customer Technical Support, BioRad Laboratories.

6. Communication with *Tony Le and Mark Tracy of Pickering Laboratories* during September 1995, 195 1 Colony Street, Suite S, Mountain View, California 94043.

7. Mark E Oppenhuizen and John E. *Cowell "Liquid Chromatographic Determination of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Environmental Water: Collaborative Study"* J. Assoc. Off. Anal. Chem. 74, January/ February 1991 Issue.

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