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Linker-Assisted Immunoassay and Liquid Chromatography/Mass Spectrometry for the Analysis of Glyphosate

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A novel, sensitive, linker-assisted enzyme-linked immunosorbent assay (L'ELISA) was compared to on-line solid-phase extraction (SPE) with high-performance liquid chromatography/mass spectrometry (HPLC/MS) for the analysis of glyphosate in surface water and groundwater samples. The L'ELISA used succinic anhydride to derivatize glyphosate, which mimics the epitotic attachment of glyphosate to horseradish peroxidase hapten. Thus, L'ELISA recognized the derivatized glyphosate more effectively (detection limit of 0.1 μg/L) and with increased sensitivity (10−100 times) over conventional ELISA and showed the potential for other applications. The precision and accuracy of L'ELISA then was compared with on-line SPE/HPLC/MS, which detected glyphosate and its degradate derivatized with 9-fluorenylmethyl chloroformate using negative-ion electrospray (detection limit 0.1 μg/L, relative standard deviation ±15%). Derivatization efficiency and matrix effects were minimized by adding an isotope-labeled glyphosate (2-$^{13}C^{15}N$). The accuracy of L'ELISA gave a false positive rate of 18% between 0.1 and 1.0 μg/L and a false positive rate of only 1% above 1.0 μg/L. The relative standard deviation was ±20%. The correlation of L'ELISA and HPLC/MS for 66 surface water and groundwater samples was 0.97 with a slope of 1.28, with many detections of glyphosate and its degradate in surface water but not in groundwater.

Glyphosate [*N*-(phosphonomethyl)glycine] is a broad spectrum, nonselective, postemergence herbicide that is used extensively in the United States and worldwide in various applications for weed and vegetation control. Aminomethylphosphonic acid (AMPA) is a degradation product of glyphosate and is the only major metabolite found in plants, water, and soil.[1] Glufosinate [ammonium DL-homoalanin-4-(methyl) phosphinate] is an herbicide similar to glyphosate in chemical structure and use. These herbicides kill plants by inhibiting the enzyme enolpyruvylshikimate−phosphate synthase (EPSPS). Monsanto Co. (St. Louis, MO) has inserted a bacterial gene into soybeans and other crops that produces a modified EPSPS that glyphosate does not inhibit. This procedure allows glyphosate to be used for weed control on these genetically modified crops.

During the last 7 years (1995−2001), Monsanto Co. introduced glyphosate-resistant seed for soybeans, cotton, corn, canola, and sugar beets. In 1999, 62% of United States soybean acres were planted using glyphosate-resistant seed, which is a large increase from 20% in 1995.[2] Recently (2000 and 2001), glyphosate has become the largest volume herbicide (in dollars) used worldwide.[3] Because of this widespread use and the difficulty of monitoring glyphosate at the submicrogram per liter level (glyphosate, AMPA, and glufosinate are polar and water soluble), there is yet a need for rapid, easy, and sensitive methods of analysis for glyphosate and its degradation product, AMPA. Such new methods would make it possible for routine monitoring of this important herbicide and its degradate in surface water and groundwater at environmentally relevant concentrations for both American and European Health Standards.

Several different methods of analysis are published for glyphosate in water including ELISA, gas chromatography/mass spectrometry, and liquid chromatography/mass spectrometry. The development of immunoassays for glyphosate has developed rapidly over the past 10 years from the work Hammock et al.[4] Clegg et al.[5] developed an enzyme-linked immunosorbent assay (ELISA) for glyphosate and achieved a detection limit of 7600 μg/L (IC_{50} of 154 000 μg/L) with direct analysis of the water sample. By using a preconcentration step (evaporation of a 100-mL water sample), Clegg et al.[4] were able to lower the detection limit to 76 μg/L (IC_{50} value of 1540 μg/L). The method is slow and subject to interferences during evaporation of the water sample.

Derivatization using 9-fluorenylmethyl chloroformate (FMOC) coupled with HPLC with fluorescence detection[6] or tandem mass spectrometry (MS/MS) detection[7] has been used. Fluorescence

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(1) Sprankle, P.; Meggit; W. F., Penner, D. *Weed Sci.* **1975**, *23*, 229−234.

(2) U.S. Department of Agriculture. *Agricultural Chemical Usage—1995 and 1999 Field crops summary;* National Agricultural Statistics Service: Washington, DC, 1996 and 2000.

(3) Monsanto Co. Roundup Brand is Leading Global Herbicide. In Hugh Grant/Terry Crews Financial/Investment Community Presentation: accessed November 30, 2001, at URL http://www.corporate-ir.net/ireye/ir_site.zhtml?ticker=MON&script=1200&layout=7, slide 5.

(4) Hammock, B. D.; et al. In *Immunochemical Methods for Environmental Analysis*; Van Emon, J. M., Mumma, R. O., Eds.; ACS Syposium Ser 442; American Chemical Society: Washington, DC, 1990; pp 112−137.

(5) Clegg, B. S.; Stephenson, G. R.; Hall, J. C. *J. Agric. Food Chem.* **1999**, *47*, 5031−5037.

(6) Spark-Holland. *Application Info 53, Automated determination of AMPA/Glyphosate;* Aj Emmen, The Netherlands, 1996.

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detection has sensitivity but lacks specificity, and interferences from naturally occurring substances make this method much less reliable than a mass spectrometric method. The MS/MS method[6] used liquid chromatography/tandem mass spectrometry (LC/MS/MS) with precolumn derivatization with FMOC followed by on-line solid-phase extraction (SPE). The method is subject to considerable variation caused by irreproducibility in derivatization and fragmentation because labeled internal standards were not used and excess FMOC was not removed from the chromatogram.

Royer et al.[8] used an elaborate ion-exchange column and a mixture of trifluoroacetic anhydride (TFAA) and 2,2,3,3,4,4,4-heptafluoro-1-butanol (HFB) for derivatization prior to gas chromatography with ion trap tandem MS/MS detection. Although the detection method is reliable, variation in derivatization and the labor-intensive isolation of glyphosate from the water sample makes for a complicated method. Thus, despite these current (2001) published methods for glyphosate analysis, there yet exists a need for reliable, simple methods for low-level concentrations (~0.1 μg/L) of glyphosate.

For an immunoassay method, a sensitive antibody for glyphosate is needed that has low cross-reactivity. Two problems exist for mass spectrometry. First is the rapid isolation of the polar glyphosate and AMPA from water, and the second is the lack of an isotope-labeled standard for derivatization, which handicaps the reproducibility of a derivatization method. These problems have been overcome in the methods described herein. The linker-assisted enzyme-linked immunosorbent assay method increases sensitivity over other antibody preparation methods. The high-performance liquid chromatography/mass spectrometry (HPLC/MS) method incorporates on-line SPE, which automates the concentration of the compounds and the removal of excess FMOC. Excess FMOC shortens HPLC column life and decreases sensitivity of the MS. Use of a newly available, isotope-labeled (2-^{13}C,^{15}N)-glyphosate as an internal standard is carried through all steps of the method. The isotope-labeled glyphosate addresses variations in derivatization and fragmentation for the analysis of glyphosate. Quantitation of the samples using the standard addition method, adding a known amount of standard(s) to a matching replicate of each unknown sample, further addresses matrix variations for glyphosate, AMPA, and glufosinate.

The objectives of the research described herein were (1) to optimize the derivatization FMOC method with on-line SPE/HPLC/MS, (2) to compare the linker-assisted competitive enzyme-linked immunosorbent assay (L'ELISA) to the on-line SPE/HPLC/MS method for accuracy, precision, and cross-reactivity, and (3) to determine whether the combination of L'ELISA screening and HPLC/MS confirmation for glyphosate would be a viable and inexpensive method for large water quality surveys.

The L'ELISA method described herein is an environmental method using a derivatized immunoassay to enhance affinity for the antibody to the compound. It is also the only glyphosate ELISA with a sub-part-per-billion reporting limit, which is needed for routine, rapid, low-cost monitoring of the occurrence of glyphosate in surface water and groundwater.

(7) Vreeken, R. J.; Speksnijder, P.; Bobeldijk-Pastorova, I.; Noij, Th. H. M. *J. Chromatogr., A* **1998**, *794*, 187−199.
(8) Royer, A.; Beguin, S.; Tabet, J. C.; Hulot, S.; Reding, M. A.; Communal, P. Y. *Anal. Chem.* **2000**, *72*, 3826−3832.

EXPERIMENTAL SECTION

Reagents. Methanol and acetonitrile were HPLC-grade solvents obtained from Burdick & Jackson (Muskegon, MI). Glyphosate, AMPA, and glufosinate were obtained from Chem Service, Inc. (West Chester, PA). Isotope-labeled glyphosate (2-^{13}C^{15}N) was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA). Cysteic acid, FMOC, and ammonium acetate were from Sigma Chemical Co. (St. Louis, MO). Sodium borate and phosphoric acid were obtained from Fisher Chemical Co. (Pittsburgh, PA). Reagent water was charcoal filtered and distilled prior to use. The SPE cartridges (Waters, Milford, MA) contained 10 mg of hydrophobic lypophilic-balanced copolymer (HLB). Standard solutions were prepared in reagent water. Nitrogen gas for the MS and zero air gas for the automated on-line SPE instrument were obtained from Praxair, Inc. (Danbury, CT).

Sampling Procedure. Surface water and groundwater samples (51 and 15 samples, respectively) from 10 states in the midwestern United States were filtered at the collection site using glass-fiber filters with nominal 0.70-μm pore diameter to remove suspended particulate matter. The sites represent a cross section of localities that will be monitored for a larger survey of glyphosate in the United States in 2003. The filtrate for analysis was collected in amber glass bottles with Teflon-lined lids. Samples were chilled immediately and shipped to the laboratory via an overnight carrier. At the laboratory, samples were logged in, assigned identification numbers, and stored at 4 °C until analysis.

L'ELISA Procedure. The L'ELISA for glyphosate was obtained from Osborn Group, Inc. (Olathe, KS). A 200-μL water sample was added to polystyrene test tubes (as many as 21/assay) that come prespiked with buffer and pH indicator. A 200-μL sample of reagent water was added to each of the calibration standards (0.05, 0.15, 0.45, 1.35, 4.05, 12.15, and 36.45 μg/L) and quality control (0.4, 2, and 10 μg/L) test tubes that contained known amounts of glyphosate in addition to the buffer and pH indicator. Next, 25 μL of a succinic anhydride solution was added (Figure 1). After 10 min, a solution containing glyphosate−enzyme conjugate (600 μL) (horseradish peroxidase (HRP)−glyphosate conjugate) was added, and 200 μL from each test tube (sample, standard, or quality control) was added to the polyclonal antibody-coated wells in the microtiter plate in triplicate. After a 60-min incubation in which the derivatized glyphosate in the sample competed with the glyphosate−enzyme conjugate for binding sites on the antibodies, the microtiter plate was rinsed three times with a buffered washing solution to remove excess unreacted sample and enzyme conjugate. Finally, substrate was added to the microtiter plate, and color was allowed to develop 10 min before being stopped with 1 N HCl. The microtiter plate was read at dual wavelengths (450/650 nm). Softmax Pro software (Molecular Devices Corp., Sunnyvale, CA) was used to control a Vmax microtiter plate reader (Molecular Devices Corp.) and for calculation of L'ELISA results. Glyphosate concentrations in the water samples were determined from the standard curve using a four-parameter, logistic curve-fit method.

HPLC/MS Procedure. For the HPLC/MS method, the samples were derivatized upon arrival in the laboratory (Figure 2) and then stored in a refrigerator in the dark until analyzed. For derivatization of each sample, two plastic screw-capped tubes were labeled with the laboratory identification number. The

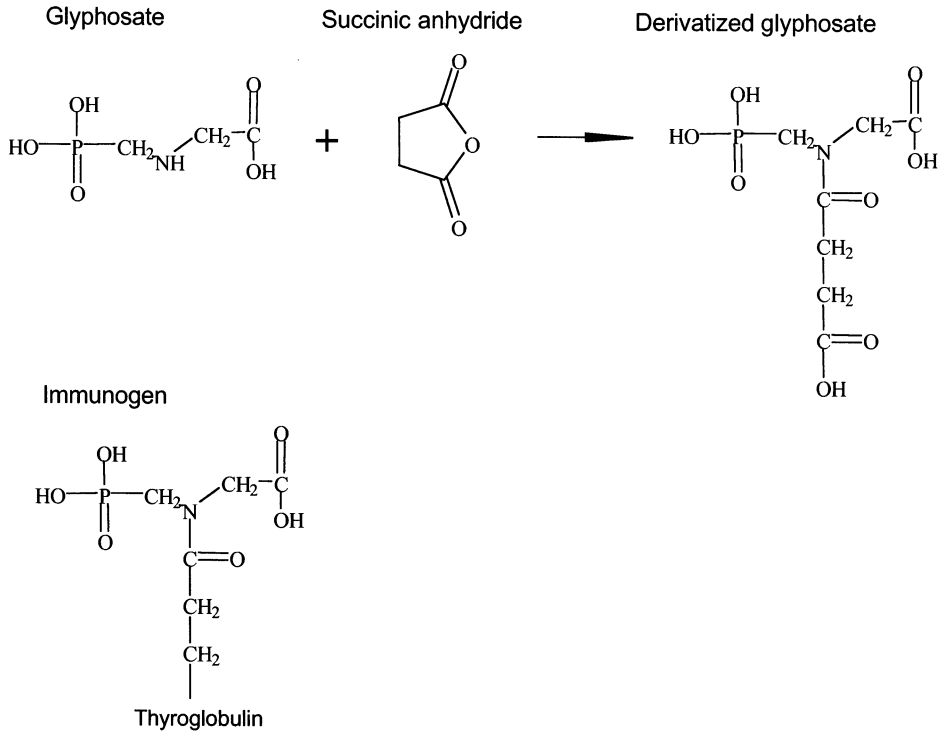

Figure 1. Chemical structures showing derivatization of glyphosate with succinic anhydride to mimic the immunogen before analysis by immunoassay.

second tube also was labeled "standard addition" (SA). A 10-mL sample was dispensed into each tube. A 100-μL aliquot of the standard addition solution was added to the SA tube. A 100-μL aliquot of reagent water was added to the first tube. A 100-μL aliquot of the isotope-labeled glyphosate, internal standard solution was added to both tubes. A 100-μL sample of the cysteic acid internal standard solution was added to both tubes followed by addition of 500 μL of 5% sodium borate in reagent water. All tubes were mixed by vortexing. A 1.5-mL aliquot of 2 mM FMOC in acetonitrile was added to all tubes, and they were mixed by inverting at least three times. All tubes were placed in a 40 °C water bath in the dark for 24 ± 1 h. The tubes were removed, and 600 μL of 2% phosphoric acid in reagent water was added to each tube. Tubes were mixed by inversion at least three times. The derivatized samples then were placed in the refrigerator (in the dark) until extracted and analyzed. Before analysis, 5.5 mL of the contents of each tube was diluted with 5.5 mL of reagent water directly into the autosampler vial.

The autosampler, Triathlon, type 900 (Spark-Holland), and an automated on-line SPE instrument, the Prospekt, type 795/796--900 (Spark-Holland) were programmed for the HPLC/MS method. Each sample and its matching standard addition sample were loaded into the sample tray of the autosampler. The automated on-line SPE instrument was loaded with the HLB SPE cartridges. The automated on-line SPE instrument performed one complete cycle of a cartridge before proceeding to the next cartridge (sample). The cartridge was activated with methanol, 2 mL/min for 2 min, and conditioned with reagent water, 2 mL/min for 2 min. Then 10 mL of sample was loaded onto the cartridge from the autosampler at a rate of 2 mL/min. The cartridge was washed with reagent water at the same flow rate for 15 s.

The loaded SPE cartridge was placed in the flow path of the Hewlett-Packard (Wilmington, DE) series 1100 LC/MS prior to the HPLC column. The column used was a Prodigy C-18, 250 × 3.0 mm 5 μm (Phenomenex, Torrance, CA). Operating conditions for the HPLC/MS were as follows: flow rate 0.5 mL/min; HPLC oven at 35 °C; MS detector in electrospray, negative-ion mode; drying gas flow at 9 L/min at 250 °C; nebulizer gas pressure at 25 lb/in.^{2}; capillary voltage at 3500 V; and fragmentor voltage set at 70 V. The compounds were eluted using the mobile phase consisting of a gradient, beginning with 95% mobile phase A (5 mM ammonium acetate in reagent water) and 5% mobile phase B (acetonitrile) to 83% mobile-phase A and 17% mobile phase B over the first 8.5 min and then changed to 60% mobile phase A and 40% mobile phase B over the next 10 min.

A 3.5-min column rinse at 10% mobile phase A and 90% mobile phase B completed the analysis. The cartridge remained in the flow path for the first 9 min. The data were acquired using the HP Chemstation software (Wilmington, DE). The ions m/z 170, 392 (labeled glyphosate-FMOC), 168, 390 (glyphosate-FMOC), 390 (cysteic acid-FMOC), 110, 136, 332 (AMPA-FMOC), and 180, 206, 402 (glufosinate-FMOC) were acquired using the selected ion mode from 10 to 18 min during the analysis (Figure 3). The diode array detector and total ion chromatogram signals were acquired from 0 to 22 min during analysis. Determination of the concentration in each sample was accomplished using the labeled glyphosate as the internal standard and the standard addition replicate calculated by the following equation:

$$C = [(A_c/A_i)/(A_{csa}/A_{isa} - A_c/A_i)]SA_cDF$$

where C is the concentration of the selected compound in the sample, A_c is the area of peak of the ion for the selected compound, A_i is the area of peak for the internal standard, A_{csa} is the area of the peak for the selected compound in the standard addition sample, A_{isa} is the the area of the peak for the internal

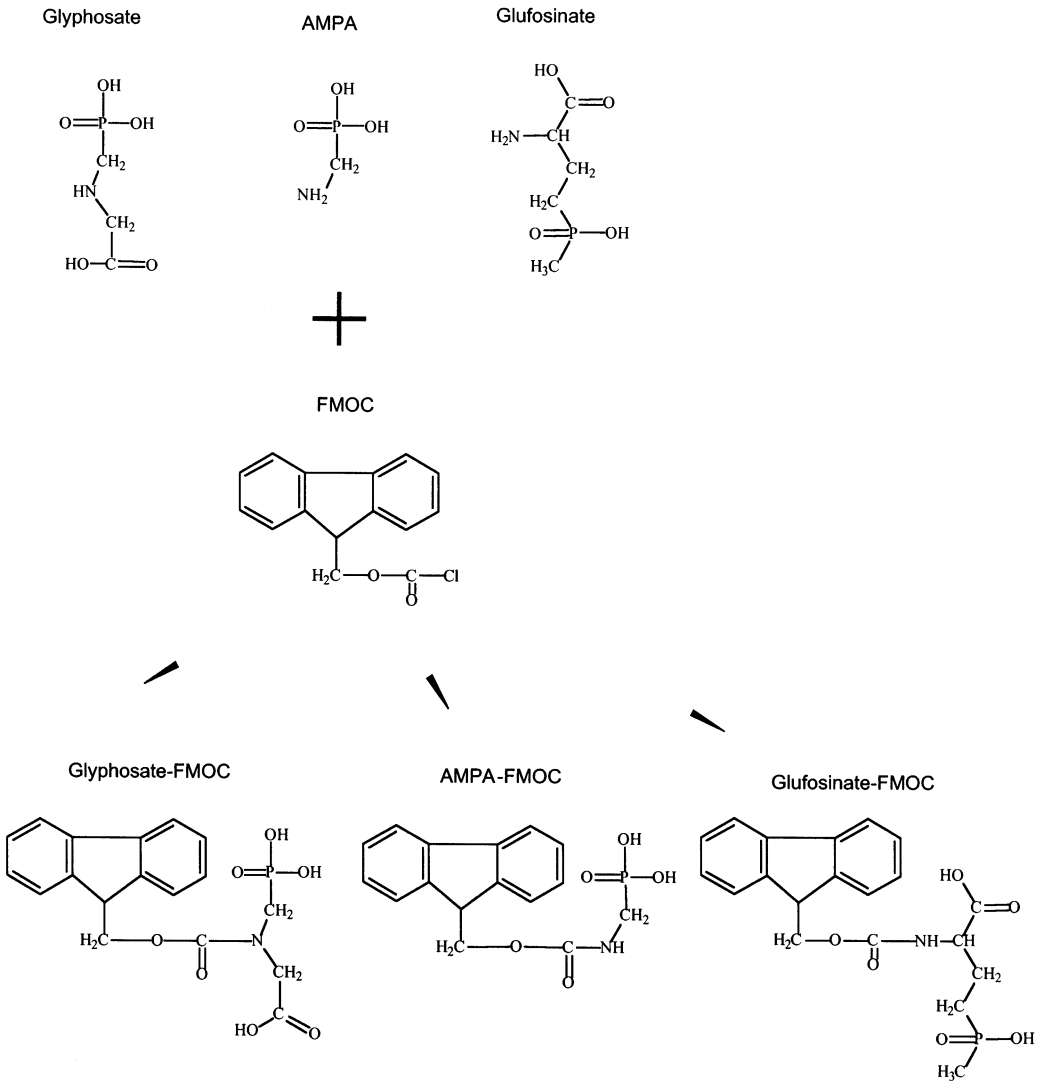

Figure 2. Chemical structures showing derivatization of glyphosate, AMPA, and glufosinate with FMOC and derivatized compounds.

standard in the standard addition sample, SA_c is the concentration of the standard addition, and DF is the dilution factor.

RESULTS AND DISCUSSION

L'ELISA. Polyclonal glyphosate antibodies were produced from rabbits using glyphosate directly coupled to thyroglobulin (TG). The conjugate was prepared by activating the carboxylic groups of TG with 1-ethyl-3- (3-diaminopropyl)carbodiimide hydrochloride (EDC) followed by their coupling to the secondary amino group of glyphosate (patent pending[9]). HRP−glyphosate was prepared by activating the carboxylic groups of the enzyme horseradish peroxidase with 1-ethyl-3-(3-diaminopropyl)carbodiimide followed by their coupling to the secondary amino group of glyphosate. The compounds in a water sample are first derivatized with succinic anhydride (Figure 1) to mimic the epitopic structure, thereby enhancing the affinity. This is an improvement over previous attempts at glyphosate ELISAs and may be applicable to other negatively charged small molecules such as 2,4-dichlorophenoxyacetic acid, which currently (2001) lacks a sensitive ELISA method.

(9) Bhullar, B. S. Linker-Assisted Immunoassay for Glyphosate. PCT Patent Application WO 99-US20494, Priority US 98−99612, 1999.

The portion of the standard curve from 0.4 to 10 μg/L was linear when concentration was plotted versus absorbance on a log scale. After derivatization, cross-reactivity was measured to determine specificity. Cross-reactivity is measured as the concentration required for 50% inhibition (IC_{50}) for compounds with similar structure, including AMPA and glufosinate. It was found that the response of glyphosate was greatest with an IC_{50} of 3 nM (0.5 μg/L). Glyphosine, a compound similar to glyphosate, had an IC_{50} of 20 μM with 0.04% cross-reactivity, AMPA had an IC_{50} of 400 μM with 0.002% cross-reactivity. Glufosinate, glycine, and lysine all had 0% cross-reactivity at greater than 10 mM concentrations. Calcium chloride, when added to the assay buffer up to 5 mM, had no adverse effect on the assay. Because each sample is buffered when added to the test tube provided in the L'ELISA kit, pH did not affect this assay.

Three quality control samples with concentrations of 0.40, 2.0, and 10.0 μg/L were analyzed within each of 34 assay runs. They represent interassay comparisons. The mean concentrations were 0.36, 2.09, and 8.08 μg/L, respectively, and the respective standard deviations were 0.085, 0.22, and 1.09 μg/L. Precision (CV) average ± 20% and recovery was 91, 105, and 81%, respectively. These assays were done by three different analysts and included

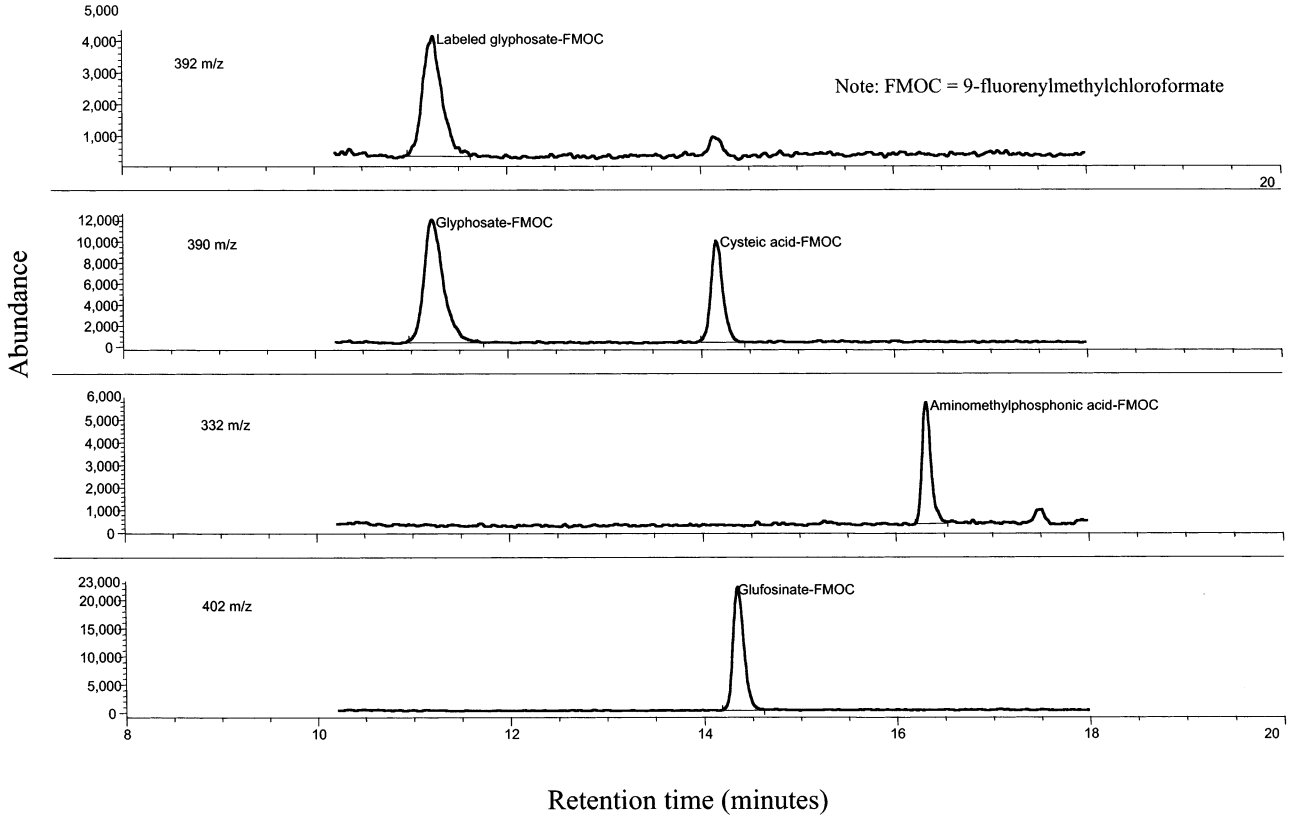

Figure 3. Selected ion chromatograms of a FMOC-derivatized 2.0 μg/L spiked, surface water sample from Clinton Lake (Kansas) for molecular FMOC ions *m*/*z* 332, 390, 392, and 402 analyzed using an HPLC/MS method.

assays from several different lots of reagents during a 15-month period.

The L'ELISA method (patent pending[9]) increases the affinity of the antibody (Ab) for the epitope by careful selection of the linkage between the hapten and the carrier in the immunogen and the derivatization of the compound to mimic the epitopic structure of the antigen (Ag). Immunoassays are based on the fundamental concept that Ab prepared in animals can recognize and bind with specificity the Ag that stimulated their production. The binding forces involved in specific interactions between Ab and Ag are of a noncovalent, purely physiochemical nature. Thus, for an effective Ab−Ag interaction, a large number of noncovalent interactions need to be present, and there should be a close fit between the Ab and the Ag for these noncovalent forces to operate (<1 Å or 1×10^{-7} mm).[10] The affinity (K_{aff}) of the Ab for the epitope of the Ag is the sum total of noncovalent interactions between a single binding site on an Ab and a single epitope. Figure 4 shows that a nearly 1000 times lower concentration of derivatized glyphosate than nonderivatized glyphosate is needed to produce the same response. Addition information regarding glyphosate immunoassays can be found in *Research Disclosures*.[11,12] Thus, the L'ELISA method proved to be sensitive on standards prepared in water.

HPLC/MS. Figure 2 shows the derivatization of glyphosate, AMPA, and glufosinate with FMOC, which increases hydrophobicity and allowed for concentration of the compounds on the HLB SPE cartridges. SPE also concentrated the unused excess FMOC, which overloads the chromatogram causing problems with the separation of the compounds and greatly reducing the life of the HPLC column. They also foul the MS detector and source. This problem was overcome by separation of excess FMOC from the derivatized glyphosate, AMPA, and glufosinate by automated on-line SPE, which separated the derivatized compounds from the excess FMOC before the analytical column (Figure 3). The derivatized compounds are eluted ahead of the excess FMOC, which stays behind on the disposable SPE cartridge.

To accommodate for the variation of derivatization rates observed in different matrixes, an isotope-labeled glyphosate (2-$^{13}C^{15}N$) was incorporated into the method as an internal standard. To further enhance accuracy, quantitation was accomplished by standard addition, the process of adding a known amount of standard to a replicate of each sample. Cysteic acid, another chemically similar compound, also was incorporated as a secondary internal standard and as a retention time check (Figure 3).

The molecular ion for each compound was monitored (Figure 3) as well as the fragment ion. The derivatized ions of glyphosate, AMPA, labeled glyphosate, and glufosinate all fragmented to give their $M - H^-$ ions. The cysteic acid did not fragment with the fragmentor voltage set at 70. However, the sulfur isotope of cysteic acid was monitored at *m*/*z* 392 for confirmation (Figure 3). A third fragment ion was monitored for AMPA (136 amu) and for glufosinate (206 amu) for further confirmation.

Breakthrough of the derivatized compounds and the excess FMOC on the HLB SPE cartridge was checked by utilizing the absorbance of FMOC at 254 nm and the diode array detector

(10) Kuby, K. *Immunology;* W. H. Freeman & Co.: New York, 1992.
(11) *Research Disclosure*, 239, No. 33606, April 1992.
(12) *Research Disclosure*, 127, No. 35924, March 1994.

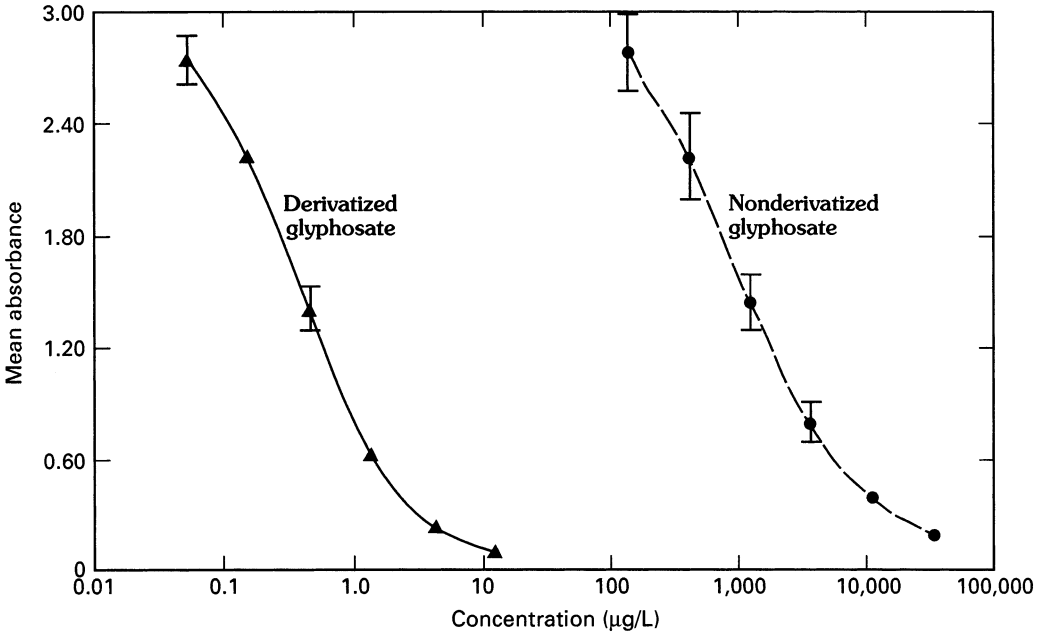

Figure 4. Antibody affinity for derivatized glyphosate ((▲) vs nonderivatized glyphosate (●). For nonderivatized glyphosate to obtain the same response as derivatized glyphosate, the concentration would need to be increased 1000 times.

(DAD) of the HPLC. The Prospekt was connected directly to the DAD flow cell with the flow rate set at 0.5 mL/min. The DAD was zeroed with distilled water, and then a flow of undiluted derivatized surface water was passed through the DAD until a stable reading was achieved in ~4 min. The HLB SPE cartridge then was placed into the flow path preceding the DAD. The absorbance reading returned to zero due to the removal of absorbance of the FMOC. Once the cartridge had reached its holding capacity, the absorbance reading began to increase. The net time during which the FMOC was being completely absorbed was 23 min, corresponding to 11.5 mL of undiluted, derivatized sample. The sample volume used for the method was 5 mL, diluted 1:1 with reagent water. The breakthrough value for the cartridge was ~230% of the quantity that was normally loaded onto the SPE cartridge to ensure complete recovery of the derivatized compounds.

Method detection limit (MDL) was defined as the minimum concentration of a substance that could be identified, measured, and reported with a 99% confidence that the compound concentration was greater than zero. MDLs were determined according to procedures outlined by the U.S. Environmental Protection Agency.[13] Eight replicate samples of buffered reagent water spiked with 0.20 μg/L of each of the compounds were analyzed to determine MDLs. Each sample was analyzed on different days during August 2001 so that day-to-day variation is included in the results. The estimated mean MDL was 0.084 μg/L for glyphosate, 0.078 μg/L for AMPA, and 0.057 μg/L for glufosinate.

Mean recoveries in reagent water, surface water, and groundwater samples were determined by comparing the mean analyzed concentrations from the eight replicate samples spiked at 0.2 and 2.0 μg/L. Mean recoveries for glyphosate ranged from 88.0 to 116%. Mean recoveries were highest overall in reagent water samples at the 2.0-μg/L spiked concentration and lowest overall in the surface water sample from Clinton Lake at the 0.2 μg/L spiked concentrations. Relative standard deviation of the recoveries was ± 15%.

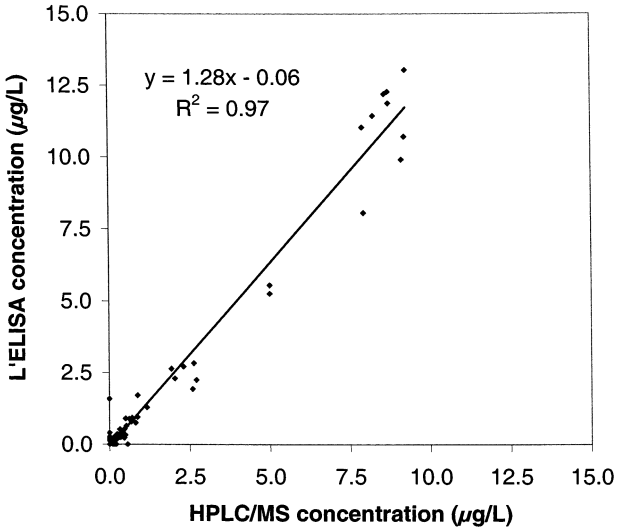

Figure 5. Comparison of L'ELISA and HPLC/MS analytical results for glyphosate in various water matrixes.

L'ELISA and HPLC/MS Comparison. The correlation between glyphosate samples analyzed by L'ELISA versus HPLC/MS was 0.97 (Figure 5), and the slope of the regression line was 1.28. The intercept was 0.06. These results included analysis of 66 samples collected throughout the United States representing various matrixes that included various surface water and groundwater samples, as well as reagent water samples made up in the laboratory. In all, more than 10 different matrixes were included in the comparison. The L'ELISA exhibited 18% false positives when compared to the HPLC/MS at concentrations less than 1.0 μg/L.

(13) U.S. Environmental Protection Agency. *Guidelines Establishing Test Procedures for the Analysis of Pollutants-—Appendix B, part 136, Definition and Procedures for the Determination of the Method Detection Limit.* U.S. Code of Federal Regulations, Title 40; 1992; pp 565—567.

Two false negatives (6%) were found for the L'ELISA at concentrations greater than 0.1 μg/L; these concentrations were 0.1 and 0.2 μg/L and were near the listed MDL for both methods. Cross-reactivity for the L'ELISA ranged from 0.002% for AMPA (IC_{50} = 80 mg/L) to 0% for glufosinate (IC_{50} > 2000 μg/L). Analysis of the HPLC/MS standards, which also contained AMPA and glufosinate, by L'ELISA did not show any cross-reactivity for these compounds. However, the 18% false-positive rate between 0.1 and 1.0 μg/L suggested that there were some natural substances that were derivatized by succinic anhydride and that cross-reacted with the glyphosate L'ELISA. The most likely possibilities include the naturally occurring phosphate, sulfate, and carbonate anions in the water samples with IC_{50}'s of 1−3 mM. The fact that the majority of the cross-reactivity occurred below 1.0 μg/L suggested that the cross-reacting substances occurred frequently but did not have a high cross-reactivity.

The L'ELISA method described showed a good correlation, r = 0. 97, with the HPLC/MS method for the analysis of glyphosate in a series of surface water and groundwater samples. The affinity enhancement afforded by derivatization before reacting with the Ab gives the L'ELISA method substantially greater sensitivity. Positive aspects of the L'ELISA are that the analysis can detect low concentrations of glyphosate, from 0.1 to 10 μg/L. It uses a limited amount of sample, 200 μL, and the analyses can be performed rapidly. No sample preparation is necessary. The L'ELISA method requires HPLC/MS confirmation for concentrations less than 1.0 μg/L, and for good quality assurance in monitoring, ~25% of the samples should be checked for concentrations above 1.0 μg/L. Precolumn derivatization using FMOC followed by HPLC/MS may be used in conjunction with the L'ELISA as a confirmation technique. The HPLC/MS method is easily automated and can provide a low-cost technique for the confirmation. These methods are currently being used in a large water quality survey of glyphosate in the United States by the U.S. Geological Survey.

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