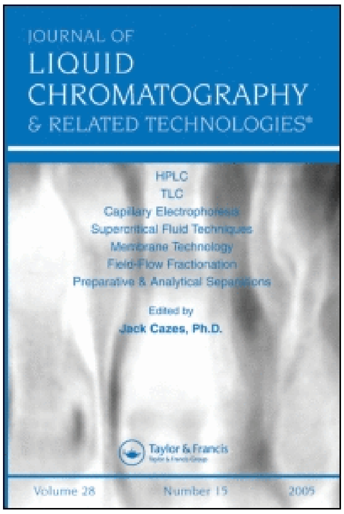

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A SCREENING METHOD FOR THE DETERMINATION OF GLYPHOSATE IN GROUND WATER

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ABSTRACT

A rugged dual column LC method was developed for the screening analysis of glyphosate in ground water. Analysis was based on a previously described pre-column fluorescence derivatization procedure. The method employed a short pre-column cartridge and a conventional reverse phase analytical column. Method precision for the screening assay was acceptable (13.1% RSD). The method was applied to a large set of pond water samples using the same analytical column without discernible loss of column efficiency during the course of analysis. The method represents an efficient and robust process for the analysis of glyphosate in ground water samples.

INTRODUCTION

Glyphosate (Roundup®) is a widely distributed broad-spectrum herbicide that has been in use for many years. The compound possesses properties (polar,

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non-chromophoric) that have rendered it problematic with regard to analysis at low levels in various environmental matrixes. Derivatization has thus been used to alter these native properties, and convert the compound into new entities that either change chromatographic properties and/or increase detectability. Although gas chromatographic methods have been reported (e.g., Mogadati *et al*.), (1) most literature reports, including an Association of Official Analytical Chemists method, (2) are based on liquid chromatographic methods. The most popular approach involves conversion of glyphosate to a fluorescent derivative (either pre- or post-column), which allows low ppb detection for most matrixes (e.g., Miles *et al*., (3) Moye *et al*., (4) Oppenhuizen *et al*. (9)). These methods are usually labor intensive and, in some cases (in our hands) not sufficiently rugged for processing large sample sets.

The need for a sensitive and rugged method that could be used for screening analysis of ground water samples was, therefore, developed based on derivatization of glyphosate with 9-fluorenylmethyl chloroformate (FMOC-Cl) followed by dual column liquid chromatography. A dual column approach has been reported (Sancho *et al*., (6) Hogendoorn *et al*. (7)) using two conventional sized analytical columns and large injection volumes to achieve high sensitivity. Our approach followed the general method of Miles *et al*., and involved the use of a small clean-up column and a backwash step to remove excess FMOC-Cl. The resultant rugged method was used to successfully screen approximately 100 ground water samples collected from several ponds in Minnesota.

EXPERIMENTAL

Sample Preparation

A 100-mL aliquot of water sample was filtered with Whatman No. 1 paper into a 250-mL round bottom flask, and 1 ml of dibasic potassium phosphate (0.1 M) was added. The sample was concentrated to approximately 1 ml by rotary evaporation. The concentrate was transferred to a 5-mL volumetric flask followed by two 2-mL rinses with reagent grade water. The reconstituted sample was filtered through a 0.45-micron glass microfiber filter (Whatman) and was stored at 4°C.

Standard Preparation

N-(Phosphonomethyl)-glycine (glyphosate - Sigma Aldrich) was dissolved in reagent grade water to prepare a stock solution at a concentration of

0.1 μg/mL. One milliliter of this solution was diluted to 100 ml in water to produce a working standard at 0.5 ppb.

Derivatization

A 0.1-mL aliquot of concentrated sample was placed in a 15-mL centrifuge tube containing 0.90 ml of 0.025 borate buffer (disodium tetraborate) and 0.90 mL of acetonitrile. To this mixture was added 0.1 ml of 9-fluorenylmethyl chloroformate (FMOC-Cl, 0.005 M in acetonitrile). The tube was shaken and allowed to react for 20 minutes at room temperature. Excess FMOC-Cl was removed by three 5 ml washes with methyl-t-butyl ether. The remaining aqueous residue was analyzed directly.

Liquid Chromatography

The LC system consisted of two pumps (Waters Associates) and two columns configured as shown in Figure 1. Automated column switching (controlled by an autosampler) was conducted using a Rheodyne 9010 6-port stainless steel valve with an Alcott 732 actuator.

The pre-column (30 × 4.6 mm) was a Brownlee Spheri-5 ODS cartridge; the analytical column (250 × 4.6 mm) was a C-18 Zorbax Rx (MacMod).

The mobile phase for the analytical pump (pump A, Figure 1) consisted of 18 parts of acetonitrile and 82 parts of buffer (0.05 M monobasic potassium phosphate at pH 6). The mobile phase for the wash pump (pump B) was methanol/water (80/20). Flow was maintained for both pumps at 2 ml/min.

An autosampler (Spark Holland Marathon) was used with injection volumes of 20 μL.

Detection was accomplished using a McPherson Fl-750B fluorescence detector with excitation at 265 nm and emission monitored at 315 nm with a 270-nm cutoff filter.

RESULTS

The methodology was developed for screening purposes, and is reported because it represents a rugged approach that allows for assay of large sample sets without the need for frequent replacement of the analytical column. The use of a small, inexpensive pre-column is a salient feature of the method. Glyphosate concentration was estimated using an external standard approach. Three concentrations of glyphosate standard were derivatized and analyzed to provide

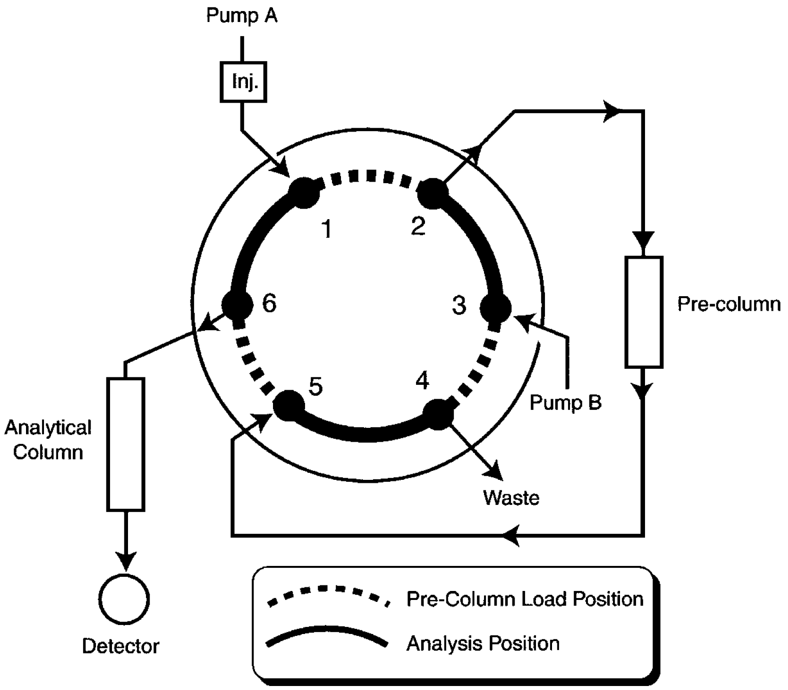

Figure 1. Flow schematic of 6-port switching valve.

a response range (approximately 0.5-10 ppb) that bracketed the 'found' concentrations for those samples in which the compound was detected.

Results for this quantitation approach are shown below. The data resulted in a regression equation (unweighted) of $y = 1{,}359x + 511.2$; $r = 0.9999$.

Glyphosate Concentration (ppb)	Response (millivolt-sec)
0.515	1238
5.15	7456
10.3	14528

Method precision was determined by processing four samples of glyphosate prepared from spiked pond water that was shown to be free of the pesticide (2 samples) and deionized water (2 samples). The samples were prepared at a glyphosate concentration of 0.5 ppb. Analysis of the four samples

Table 1. Pond Water Sample Results

Pond Water Sample	Detector Response (μV-sec)	Glyphosate Conc. (ppb)
Site A	2,108,730	1.18
Site B	9,211,380	6.40
Site C	2,478,570	1.45

using the dual column method measuring detector response (raw area counts), yielded the following data:

Sample	Area Counts (Vs)	Sample	Area Counts (μ*V*s)
DI Water 1	1,124,622	Pond Water 1	1,034,830
DI Water 2	977,728	Pond Water 2	814,185

with a precision estimate for all four samples of 13.2% RSD. From more than 90 ponds sampled, only three had quantifiable levels of glyphosate. These results are shown in Table 1.

DISCUSSION

Dual column chromatography has been employed for many years as a means of obviating the need for either greater sample purification or lengthy chromatographic run times. With pre-column derivatization, excess reagent can present a problem because it is normally present in grossly excessive amounts. In the present case, after reaction of glyphosate with FMOC-Cl, the excess reagent is largely removed by extraction with an organic solvent (in our work, methyl-t-butyl ether – MTBE). Without exhaustive and time-consuming extraction schemes, some reagent can remain associated with the sample solution. This can be a significant problem if, as is reported in some of the cited literature, an aminopropyl LC column packing is used, which can potentially react with FMOC-Cl. An additional problem that we encountered stemmed from the presence of trace quantities of extracting solvent that contaminated the LC column and produced a variety of difficulties (excessive back pressure, unstable retention times).

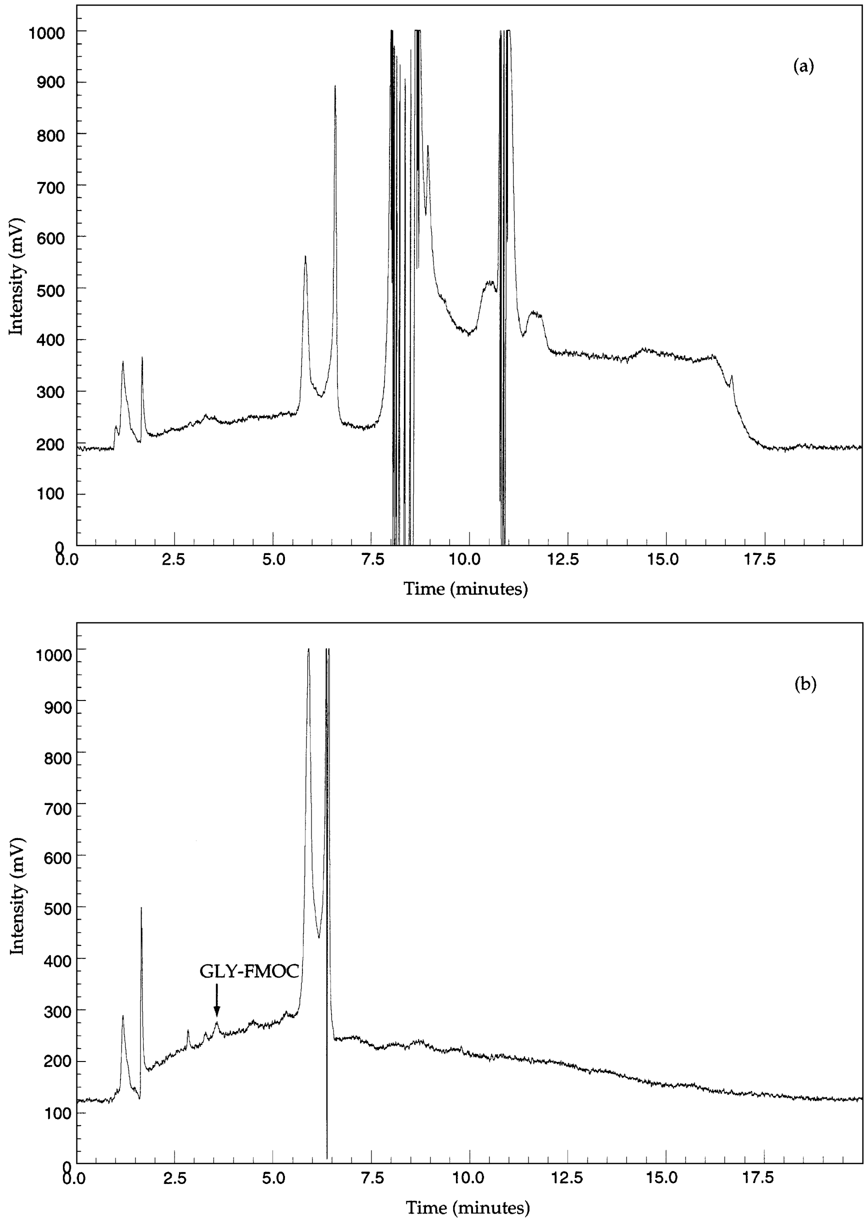

Figure 2. Chromatograms of derivatized pond water. (a) Pond water – single column analysis; (b) Spiked pond water (0.5 ppb) – dual column analysis; (c) Water blank – dual column analysis; (d) Representative pond water field sample – dual column analysis.

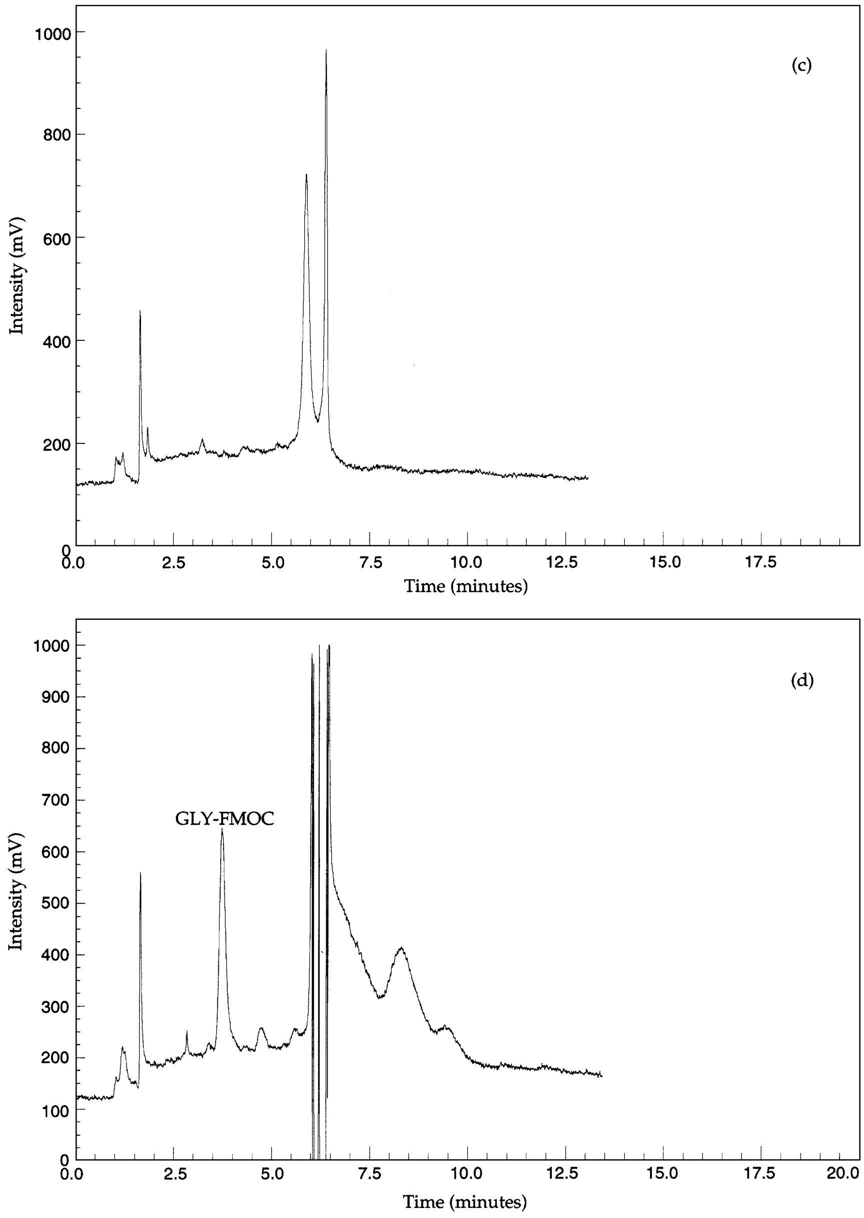

Figure 2. Continued.

Our method was based on the use of a C-18 column, which provided separation of derivatized glyphosate, an early eluter under our conditions, and unreacted FMOC-Cl, which was highly retained. This circumstance allowed us to develop a dual column method using a short pre-column to retain the late-eluting reagent, while allowing rapid introduction of the analyte onto the analytical C-18 column. As shown in Figure 1, operation of the valve provided flow of wash solvent onto the pre-column in preparation for the next injection. All LC operations were optimized to give a total cycle time (time from one injection to the next) of 12 minutes.

The benefit of the dual column approach is illustrated in Figure 2. Using a single column, the modified procedure of Miles *et al.* (2) produced chromatograms as presented in Figure 2A. The large peak at approximately 8 minutes represents unreacted FMOC-Cl, which, with other late-eluting species, increases analysis time and, ultimately, shortens the life of the analytical column. Figure 2B depicts results of analysis of a spiked sample using the dual column configuration. The run time is substantially reduced, and, of greater importance, the lifetime of the analytical column was extended significantly. A water blank chromatogram is shown in Figure 2C, and 2D shows a chromatogram of a representative field sample of pond water.

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