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Improved method for the determination of glyphosate in water

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Abstract

A method for the determination of glyphosate (Glyph) and its metabolite aminomethylphosphonic acid (AMPA) in environmental water was developed with the emphasis on the clean-up procedure. The organic compounds in the environmental water were extracted with dichloromethane and the sample was concentrated by rotary evaporation. The concentrated sample was then passed through a strong anion-exchange (SAX) cartridge with an additional 2 ml of resin in the hydroxide form packed above the SAX packing. Glyph and AMPA were eluted with citrate buffer at pH 5.00 and determined directly by high-performance liquid chromatography with a postcolumn reactor and fluorescence detector. The detection limit and average recovery for both components were <2 μg/l and $>85\%$, respectively.

1. Introduction

Glyphosate, N-(phosphonomethyl)glycine (Glyph), is a very broad spectrum, non-selective, post-emergence herbicide. Glyphosate is the active ingredient in Roundup and Rodeo herbicides produced by Monsanto and is widely used in various applications for weed and vegetation control. Its impact on the environment is becoming more pertinent. The difficulties of obtaining a simple method for the determination of this compound at residue levels are mainly due to its properties: its relatively high solubility in water, its insolubility in organic solvents and its complexing behaviour [1].

Procedures for the determination of Glyph and its metabolite aminomethylphosphonic acid (AMPA) have been reviewed [2]. Although gas chromatographic procedures continue to be of interest, in general they suffer from tedious sample preparation because of the need to convert the analytes into volatile derivatives. Because of the requirement for a better detection limit, liquid chromatographic procedures have been developed [3].

For liquid chromatography procedures, both pre- and post-column derivatization methods have been developed. Precolumn procedures have focused on derivatization with 9-fluorenylmethyloxycarbonyl chloroformate (FMOCCl) with fluorescence detection [4]. However, other derivatization agents such as 1-fluoro-2,4-dinitrobenzene [5] and *p*-toluenesulfonyl chloride [6] have been used to form Glyph and AMPA derivatives that can be detected in the UV–Vis region. Postcolumn derivatization has been commonly used with *o*-phthalaldehyde–mercaptoethanol (OPA–MERC) [2,7]. The direct injection method recommended by the AOAC [7] was found in our initial work to damage the column, sometimes only after five injections, so frequent regeneration is required.

The main objective of this work was to develop a simpler and sensitive method for the

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determination of Glyph and its AMPA in the aquatic environment with emphasis on a simple clean-up procedure. The determination of the components is based on high-performance liquid chromatography (HPLC) with a postcolumn reactor and fluorescence detection.

2. Experimental

2.1. Reagents

All reagents were of analytical-reagent grade and used as received. The solvent methanol was of liquid chromatographic grade.

2.2. Preparation of solutions

Mobile phase: 0.005 M KH_2PO_4 buffer

A 1.36-g amount of KH_2PO_4 was dissolved in 2 l of methanol–water (4:96) and the pH was adjusted to 2.1 with H_2PO_4. The solution was filtered through a 0.22-μm membrane and degassed. The flow-rate of this mobile phase was 0.4 ml/min.

Oxidative solution

A 0.5-g amount of $Ca(OCl)_2$ was dissolved in 500 ml of deionized, distilled water using a magnetic stirrer at high speed for 30 min. A solution was prepared by dissolving 1.36 g of KH_2PO_4, 11.6 g of NaCl, 0.4 g of NaOH and 10 ml of the first solution in 1 l of deionized, distilled water, mixed thoroughly and filtered through a 0.22-μm membrane. This solution was delivered at a flow-rate of 0.4 ml/min via a postcolumn reagent system (PCRS) with mixing tees and maintained at 48°C to oxidize the analytes eluted.

o-Phthalaldehyde–mercaptoethanol solution

A 10.0776-g amount of anhydrous disodium tetraborate was dissolved in 900 ml of deionized, distilled water. Solid $Na_2B_4O_7$ was poured in a little at a time to prevent caking. A 0.800-g amount of OPA was dissolved in 10 ml of methanol in a small beaker and the OPA solution was poured into a 1-l beaker. The beaker was rinsed with $Na_2B_4O_7$ solution. The pH of the solution was adjusted to 11.5 using 60% NaOH. The solution was made up to 1 l using deionized, distilled water. The solution was then mixed completely and filtered through a 0.22-μm membrane and degassed prior to the addition of 2 ml of 2-mercaptoethanol. This solution was delivered at 0.3 ml/min to the postcolumn reactor at ambient temperature.

2.3. Instrumentation

A Waters HPLC system was used, consisting of Waters Model 510 and 501 pumps, a reagent-delivery module (RDM), an autoinjector (WISP 712), coils, column heater and Rheodyne valve. The temperature of the column heater was maintained at 48°C with a column heater temperature control module. The postcolumn reactor consisted of a dual-pump derivatization system including two reaction coils, one maintained at 48°C and the other at ambient temperature. Glyph and AMPA derivatives were detected with a Waters Model 470 fluorescence detector with excitation at 340 nm and emission at 455 nm. The system was controlled and data were collected and analysed using a system interface module (SIM), a NEC computer and Waters Maxima 820 software.

The analytical column was a 250 mm × 4.1 mm I.D. PRP-X400 (7 μm) cation-exchange column obtained from Hamilton. The strong anion-exchange (SAX) cartridge used in the clean-up step was Supelclean LC-SAX manufactured by Supelco.

2.4. Procedure

Environmental water samples were taken from Taman Jaya Lake, Petaling Jaya, Selangor. A 250-ml sample was taken and spiked with Glyph and AMPA. The solution was filtered and extracted with 100 ml of dichloromethane to remove organic compounds. The aqueous phase which contained Glyph and AMPA was concentrated to a very small volume by rotary evaporation and the pH was adjusted to ca. 10 with 0.2 *M* NaOH. With the aid of a Supelco vacuum

system, the sample was passed through the Supelclean SAX cartridge with an additional 2 ml of Bio-Rad SAX resin in the hydroxide form packed on top of the Supelclean packing. The cartridge was washed with 20 ml of deionized distilled water to remove interferences. Glyph and AMPA were eluted using 10 ml of 0.4 *M* sodium citrate buffer at pH 5.00.

Glyph and AMPA were determined by HPLC using a cation-exchange column, which provided good separation. Glyphosate was oxidized with calcium hypochlorite in the postcolumn reactor coil at 48°C to form glycine. The glycine was treated with OPA in the presence of mercaptoethanol (MERC) in the second coil to form a fluorophore at ambient temperature, and was detected with a fluorescence detector ($\lambda_{ex} = 340$ nm, $\lambda_{em} = 455$ nm) [7]. AMPA was made to by-pass the oxidizing reagent by a switching valve. It underwent a similar reaction with OPA–MERC reagent to form another fluorophore, which was detected by the fluorescence detector under the same conditions as for Glyph. An example of the separation is shown in Fig. 1.

The efficiency of the system was checked and optimized using glycine running concurrently with Glyph. The amounts of hypochlorite required for Glyph and AMPA were also determined and optimized.

3. Results and discussion

The variables that primarily affect the fluorescence detection include the postcolumn reaction temperature and length of the coil. A series of preliminary experiments designed to establish the qualitative effect of each variable were carefully evaluated over a defined range while all other factors were held constant. The prelimin-

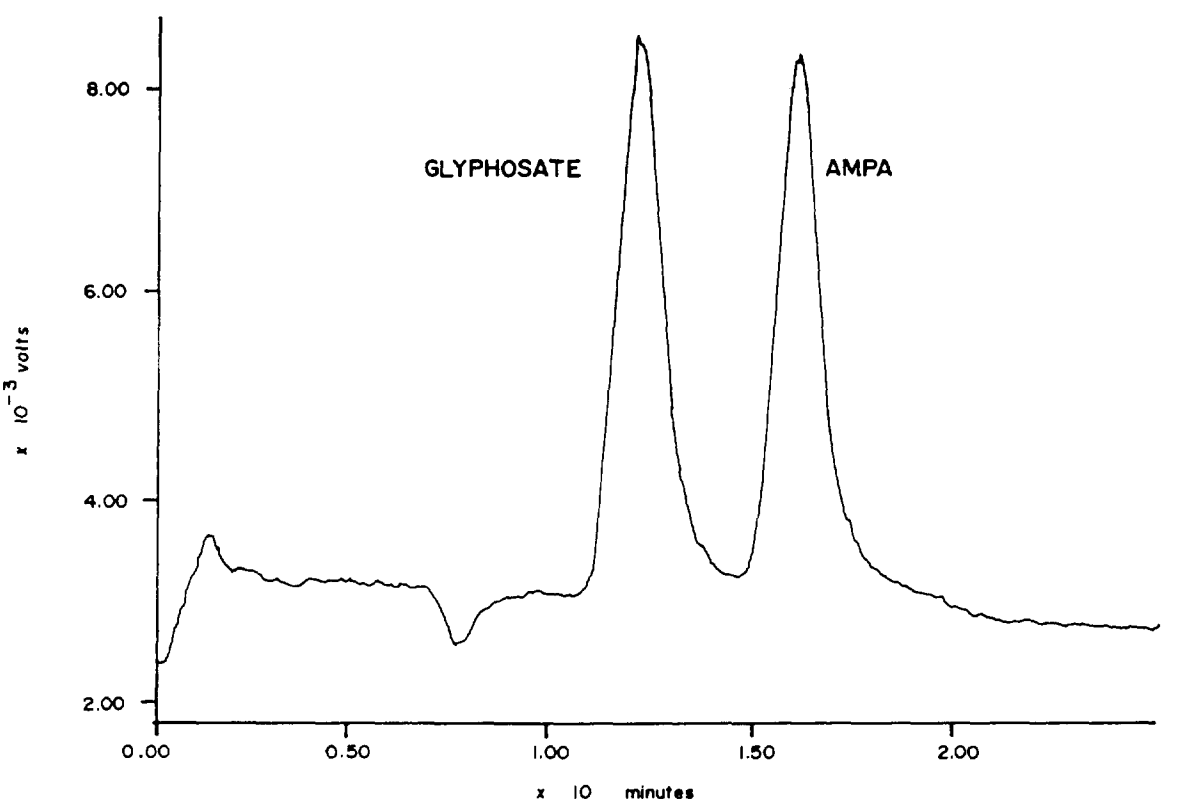

Fig. 1. Typical chromatogram for Glyph and AMPA, both at 1.0 mg/l concentration.

ary experiments demonstrated that a column flow-rate of 0.4 ml/min and the postcolumn flow-rate of 0.3 ml/min each for calcium hypochlorite and OPA–MERC solutions were optimum for Glyph and AMPA.

The purpose in optimizing each variable was to obtain an accurate, reproducible change in fluorescence that would correspond to the best detection limits for Glyph and AMPA. When the mixing coil beyond the mixing tee was increased in length, while other variables were held constant, the reaction of the analyte with calcium hypochlorite was more complete and the fluorescence became larger, as can be seen in Table 1. Table 1 also indicates that a coil length of 15 feet is appropriate for this purpose and it thus used in this work.

Using the same conditions but changing the temperature indicated that the optimum reaction coil temperature for glyphosate was 48°C (Fig. 2). At higher temperatures, the peak area began to decrease because the effectiveness of calcium hypochlorite to produce chlorine and disproportionate in water to form hypochlorous acid decreased [8]. The optimum temperature seems to be critical, as indicated in Fig. 2. However, this effectively poses little difficulty because the temperature control module used can easily and precisely control the required temperature.

The optimum concentration of hypochlorite solution was also investigated. It was found that the fluorescence intensity of AMPA was substantially reduced when passed through the hypochlorite reaction coil (Fig. 3). This phenomenon is unlike the earlier reported findings that AMPA is relatively unreactive towards hypochlorite and undergoes a similar reaction with OPA–MERC reagent [7]. AMPA, a primary amine, reacted directly with OPA–MERC. However, AMPA also reacted with the hypochlorite reagent to form chloramine and small amounts of ammonia. This substantially decreased the ultimate fluorescence response of the AMPA, as shown in Fig. 3. As AMPA does not require hypochlorite, the present postcolumn set-up was modified to incorporate a switching valve so the hypochlorite solution does not enter the reaction coil whenever AMPA is supposed to be in the coil. This is done automatically and is controlled by the Maxima 800 software.

The linear response of the detector was checked using five standard solutions (0.025, 0.050, 0.100, 0.500 and 1.00 μg/l). The concentration of the five standard solutions was plotted on the ordinate against the peak-area response of the respective analyte on the abscissa. The correlation coefficient was 0.999 for both Glyph and AMPA.

Resin in the hydroxide form was chosen and used for a clean-up step in the cartridge because it has the lowest selectivity in anion-exchange chromatography and therefore would be readily exchanged by another anion. However, the commercially available resin in the hydroxide form has a large particle size (20–50 mesh), which produced a fast elution flow-rate, so a resin of smaller mesh size is required. This was achieved by converting the resin in the chloride form into the hydroxide form using 20 volumes of 1 *M* NaOH. The surface area-to-volume ratio of the

Table 1
Percentage conversion of glyphosate to glycine

Concentration of glyphosate (μg/l)	Conversion of glyphosate to glycine (%)	
	10 ft. × 0.02 in. I.D. coil	15 ft. × 0.02 in. I.D. coil
0	0	0
500	85.5	95.2
1000	84.3	95.6
5000	85.6	95.1
10000	85.7	95.7

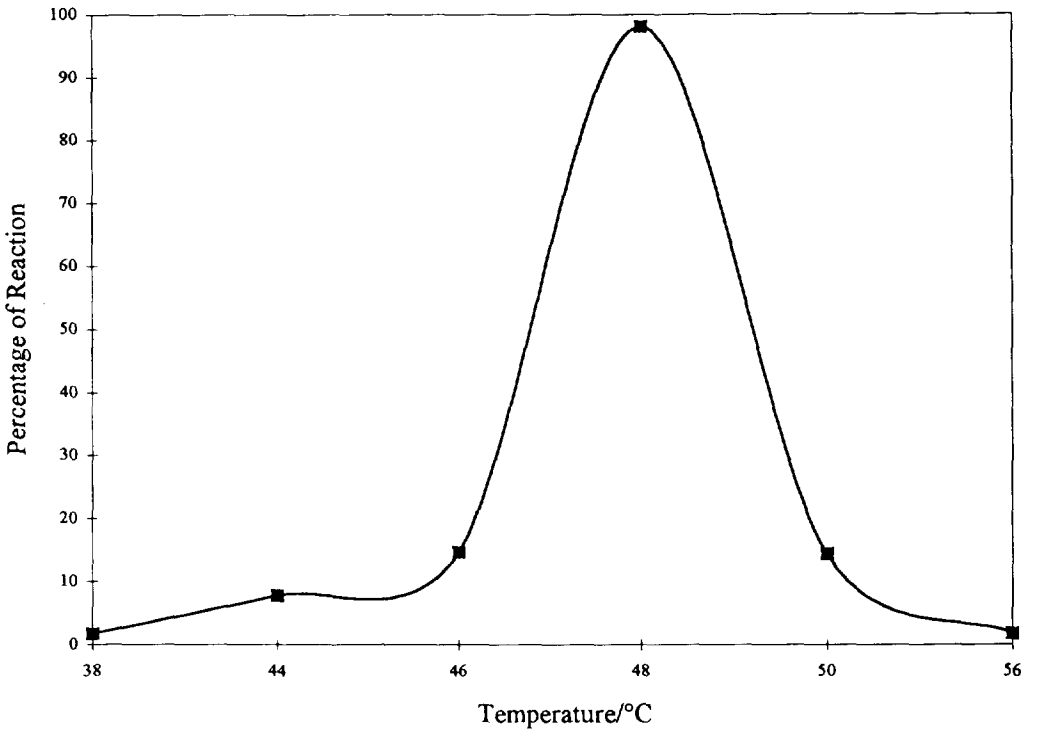

Fig. 2. Effect of temperature on oxidation of Glyph.

small particle resin would be greater than that of the large particle resin. As this resin is packed on top of the Supelclean packing, the flow-rate was controlled by the finer material in the Supelclean packing.

The acid dissociation constants of Glyph were determined to be $pK_1 = 2.32 \pm 0.03$, $pK_2 = 5.86 \pm 0.03$, $pK_3 = 10.86 \pm 0.03$ [9]. During the clean-up procedure, it is very important that the pH of the sample be maintained near the pK_3 value and that the pH of the sample be adjusted to near the pK_3 value before elution in order to obtain adequate recoveries from the SAX cartridge.

It was found that 0.1 *M* KH_2PO_4 buffer solution (pH 1.9) could not elute the analytes from the cartridge [10]. Sodium citrate buffer at 0.4 *M* (pH 5.00) was used as the elution solvent because it shows high relative selectivity in anion-exchange chromatography.

When the clean-up procedure was applied to a standard sample, virtually all the Glyph and AMPA were retained by the cartridge and 10 ml of 0.4 *M* sodium citrate buffer (pH 5.00) could give high recoveries (>95%) for both components.

When the same clean-up procedure was applied to the Taman Jaya Lake water samples, neither Glyph nor AMPA could be detected, which indicates their absence. The recoveries were then assessed by adding known amounts of Glyph and AMPA (in the range 2–40 μg/l) to these water samples. The results in Table 2 were calculated by comparison of the detector response with that for a standard solution containing both analytes at the same concentration as in the eluate. The mean recoveries of Glyph and AMPA were 91.6% and 88.6% with relative standard deviations of 6.2% and 7.4%, respectively. The method detection limit found for both components based on a signal-to-noise ratio of 3:1 is better than 2 μg/l.

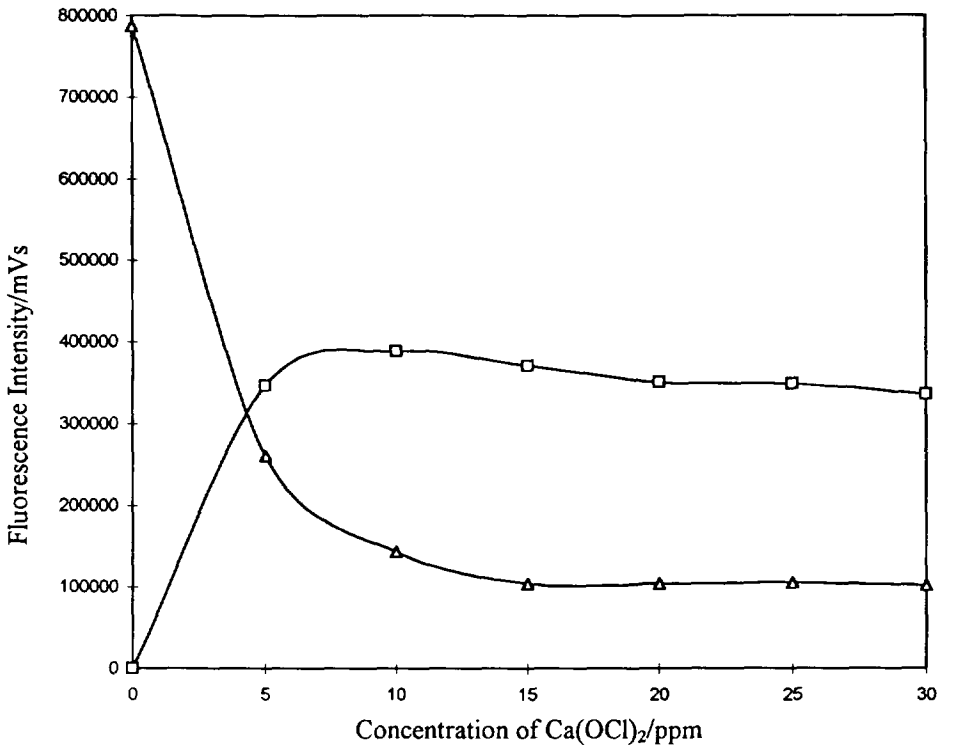

Fig. 3. Effect of $Ca(OCl)_2$ on (□) Glyph and (△) AMPA.

Table 2
Recoveries of Glyph and AMPA from environmental water samples

Analyte	Concentration added ppb (μg/l)	No. of additions	Range of recoveries (%)	Mean (%)	S.D. (%)	R.S.D. (%)
Glyph	2–40	30	81.3–97.8	91.6	5.7	6.2
AMPA	2–40	30	79.6–97.4	88.6	6.6	7.4

4. Conclusion

The sample preparation procedure developed is efficient with a method detection limit of less than 2 μg/l and mean recoveries of more than 85%. The efficiency of the oxidative cleavage in the first reaction coil could be checked and optimized by the simultaneous injection of glycine and glyphosate. Calcium hypochlorite solution was found to decrease the fluorescence intensity of AMPA substantially, which justified the use of a switching valve. This procedure offers a simple, sensitive and reproducible determination of Glyph and AMPA at residue levels in water. The elution order can be reversed depending on whether the separation is carried out on an anion- or cation-exchange column.

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References

[1] M. Yusof, *Ph.D.* Thesis, University of Glasgow, Glasgow, 1988.
[2] P.C. Bardalaye, W.D. Wheeler and H.A. Moye, in E. Grossbard and D. Atkinson (Editors), *The Herbicide Glyphosate*, 1985, p. 263.
[3] M.J. Lovdahl and D.J. Pietrzyk, *J. Chromatogr.*, 602 (1992) 197.
[4] C.J. Miles, L.R. Wallace and H.A. Moye, *J. Assoc. Off. Anal. Chem.*, 3 (1986) 458.
[5] L.N. Lundgen, *J. Agric. Food Chem.*, 34 (1986) 535.
[6] S. Kawai and M. Tomita, *J. Chromatogr.*, 540 (1991) 411.
[7] M.E. Oppenhuizen and J.E. Cowell, *J. Assoc. Off. Anal. Chem.*, 74 (1991) 317.
[8] C.J. Miles and G. Leong, *LC–GC Mag.*, 10 (1992) 452.
[9] D. Wauchope, *J. Agric. Food Chem.*, 24 (1976) 717.
[10] Y.Y. Wigfield and M. Lanouette, *Anal. Chim. Acta.*, 233 (1990) 311.