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Short communication

Improved coupled-column liquid chromatographic method for the determination of glyphosate and aminomethylphosphonic acid residues in environmental waters

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Abstract

An existing method for the determination of glyphosate and its main metabolite aminomethylphosphonic acid (AMPA) in water has been improved. It is based on precolumn derivatization with the fluorescent reagent 9-fluorenylmethylcloroformate (FMOC) followed by large-volume injection in a coupled-column LC system using fluorescence detection (LC–LC–FD). The derivatization step was slightly modified by changing parameters such as volume and/or concentration of sample and reagents to decrease the limits of quantification (LOQ) of glyphosate and AMPA to 0.1 μg/l. Additionally, the use of Amberlite® IRA-900 for preconcentration of glyphosate, prior to the derivatization step, was investigated; the LOQ of glyphosate was lowered to 0.02 μg/l. Drinking, surface and ground water spiked with glyphosate and AMPA at 0.1–10 μg/l concentrations were analysed by the improved LC–LC–FD method. Recoveries were 87–106% with relative standard deviations lower than 8%. Drinking and ground water spiked with glyphosate at 0.02 and 0.1 μg/l were analysed after preconcentration on the anion-exchange resin with satisfactory recoveries (94–105%) and precision (better than 8%).

Keywords: Water analysis; Glyphosate; Aminomethylphosphonic acid; Pesticides

1. Introduction

Glyphosate [(*N*-phosphonomethyl)glycine] is a broad-spectrum, non-selective, post-emergence herbicide used for weed and vegetation control. Its main degradation product is aminomethylphosphonic acid (AMPA). The determination of both compounds at sub-μg/l levels is difficult mainly because of their high polarity and solubility in water and also due to the absence of chomophores or fluorophores. There is, therefore, an interest in sensitive analytical methods for the monitoring of this herbicide in water.

In previous papers [1,2], precolumn derivatization with 9-fluorenylmethylcloroformate (FMOC) was successfully applied to determine glyphosate, AMPA and glufosinate in water in combination with large-volume injection and coupled-column liquid chromatography with fluorescence detection (LC–LC–FD). The analytes were successfully recovered from water spiked at 0.5–10 μg/l levels. A similar methodology was applied for the residue determination of glyphosate and AMPA in more complex matrices as soils and vegetal samples with satisfactory results [3,4].

These ionic compounds have been recently determined in water by liquid chromatography with mass spectrometry (LC–MS) [5,6] after derivatization with FMOC, achieving quite low detection limits and quantitative recoveries at concentrations as low as 0.8 μg/l [5] and 0.2 μg/l [6]. The use of gas chromatography coupled to MS (GC–MS) [7] or tandem MS (GC–MS–MS) [8] has also been described for the determination of glyphosate and AMPA residues in water. The analytes were satisfactory recovered from spiked mineralised and/or tap water at 0.5 μg/l [7] and 0.05 μg/l [8], but an extensive sample treatment was required to reach such low concentration levels. Two-step clean-up based on ion-exchange chromatography followed by derivatization (e.g. with trifluoroacetic anhydride) had to be used.

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Due to the amphoteric character of glyphosate and AMPA, both anionic and cationic resins have been used for preconcentration and clean-up purposes [7–10]. Thus, Mallat and Barceló [9] preconcentrated 50 ml of water by a two-step procedure and performed a post-column derivatization using *o*-phthalaldehyde (OPA) prior to fluorescence detection. Limits of detection of 2 and 4 μg/l were found in natural waters for glyphosate and AMPA, respectively. Patsias et al. [10] used on-line sample enrichment of 10 ml river water on polymeric anion-exchange cartridges, after clean-up also based on ion-exchange. The proposed method was completely automated and they achieved recoveries of 84 and 15%, for glyphosate and AMPA, respectively, with acceptable repeatability at the validation level assayed (2 μg/l) with relative standard deviations (R.S.D.) lower than 15%.

The aim of the present paper is to improve the sensitivity of our previous LC–LC method [2], to enable quantification of glyphosate and AMPA at 0.1 μg/l concentrations in water. For this purpose, the prederivatization reaction with FMOC was slightly modified and enrichment on an anion-exchange resin was included to further decrease the limit of quantification (LOQ) for glyphosate.

2. Experimental

2.1. Chemicals

Glyphosate and AMPA (content >99%) were obtained from Riedel-de-Haën (Seelze, Germany) and Sigma (St. Louis, MO, USA), respectively. Acetonitrile and methanol, both of HPLC-grade, were purchased from Scharlab Science (Barcelona, Spain). Analytical reagent-grade potassium dihydrogen phosphate, disodium tetraborate decahydrate, potassium hydroxide and sodium chloride were obtained from Scharlab Science. FMOC-Cl was purchased from Fluka (Switzerland). HPLC-grade water was obtained by purifying demineralised water in a Nanopure II system (Barnstead, Newton, MA, USA). Amberlite® IRA-900 was obtained from Fluka (Switzerland).

Stock standard solutions of glyphosate (500 μg/ml) and AMPA (517 μg/ml) were prepared in HPLC water and stored in freezer. Diluted standards were prepared in HPLC-grade water and stored in fridge. Standard mixture solutions at levels lower than 500 μg/l were prepared weekly.

Solutions of 0.025 and 0.05 M borate buffer (pH 9) in HPLC-grade water and solutions containing 1000 and 2000 μg/ml of FMOC-Cl in acetonitrile were used for the derivatization step prior to the LC–LC analysis.

2.2. Equipment

The modular LC system consisted of an automatic sample processor (ASPEC XL) from Gilson (Villers-le-Bel, France) equipped with two Rheodyne six-port valves time controlled by the sampler, one equipped with a 2.0 ml loop used to perform large-volume injections and the second valve to perform column switching; a model 9013 ternary gradient LC pump (Varian, Walnut Creek, CA); a model 2150 isocratic LC pump (LKB, Bromma, Sweden); a model 1046A fluorescence detector (Hewlett-Packard) set at 263 nm (excitation) and 317 nm (emission); a 30 mm × 4.6 mm i.d. first-separation column (C-1) packed with 10 μm Kromasil 100 (Scharlab Science) and a 250 mm × 4.6 mm i.d. second-separation column (C-2) packed with 5 μm Hypersil APS (Scharlab Science).

Chromatograms were recorded with a Hewlett-Packard Chemstation for LC (Rev. A.05.03[273]). A MicropH 2001 pH meter and Pipetmans (1000 and 5000 μl) were obtained from Crison Instruments (Barcelona, Spain) and Mettler Toledo (Barcelona, Spain), respectively. A Minipuls 3 peristaltic pump (Gilson, France) was used for sample enrichment.

2.3. Procedure

2.3.1. Precolumn derivatization

The derivatization procedure was the following: 1.5 ml of water was introduced into a glass tube with 0.5 ml of 0.05 M borate buffer (pH 9) and followed by 0.5 ml of FMOC reagent (2000 μg/ml). The tube was shaken and left for 30 min at room temperature. An amount equal to 7.5 ml of 0.025 M borate buffer was added to dilute this solution prior to the LC–LC–FD analysis.

2.3.2. LC–LC analysis

The mobile phase used in both columns consisted of a mixture acetonitrile–0.05 M phosphate buffer (pH 5.5, adjusted with 2 M KOH) (35:65, v/v) set at a flow rate of 1 ml/min.

A 2 ml volume aliquot of the diluted solution obtained after derivatization was injected into C-1. After clean-up with 2.4 ml of mobile phase, C-1 was switched on-line to C-2 for 0.9 min to transfer the fraction containing both glyphosate and AMPA derivatives from C-1 to C-2. After the transfer, C-1 was rinsed with the mobile phase to remove the excess of FMOC reagent as well as retained compounds, while glyphosate and AMPA were separated on C-2. The analytes were quantified by external calibration with standard solutions prepared in HPLC-grade water.

2.3.3. Preconcentration procedure

Preconcentration of sample is optional and it is only mandatory to determine glyphosate at concentrations below 0.1 μg/l.

The resin Amberlite® IRA-900 was dried at 65 °C, prior to use, until constant weight. 0.5 g of the sorbent were packed into a glass column (length 140 mm, inner diameter 5 mm). After swelling the resin with HPLC-grade water, 50 ml of solution containing glyphosate were passed through the column at a 1 ml/min flow rate using the peristaltic pump. The resin was then washed with 10 ml of HPLC-grade water

and, after that, 10 ml of 1 M NaCl were passed to elute glyphosate. The content of glyphosate was determined by the LC–LC–FD method described earlier.

3. Results and discussion

3.1. Direct LC–LC–FD analysis

In our previous paper [2], adequate LC–LC conditions were established enabling clean-up and large-volume injection on the first short C_{18} column, and efficient separation of the analyte on the second amino column, using as mobile phase acetonitrile–0.05 M phosphate (pH 5.5) in water (35:65, v/v). We proved that sensitivity could be considerably enhanced, so a derivatized sample volume of 2 ml was introduced into the LC–LC system. However, the derivatization mixture (with 40% of acetonitrile coming from the FMOC solution) had to be eight-fold diluted to obtain good peak shape on C-1. Under these conditions, the simultaneous transfer of glyphosate and AMPA from C-1 to C-2 was feasible. In the present work, the derivatization step was slightly modified by increasing in the volume of water sample derivatized and reducing the final dilution.

The linearity of the response was checked at low levels of concentration. Six standard solutions from 0.1 to 10 μg/l were derivatized and injected in duplicate in the LC–LC–FD system achieving calibration curves for glyphosate (response = 20.065 × concentration (μg/l) − 0.0503) and AMPA (response = 31.213 × concentration (μg/l) − 0.143) with coefficients of regression higher than 0.999 in both cases.

The improved derivatization reaction followed by the direct LC–LC–FD procedure was applied to drinking, surface and ground water spiked at of concentrations of 0.1–10 μg/l in order to evaluate the accuracy and the precision. The results achieved were satisfactory with regard to both recoveries and relative standard deviations (%); they are shown in Table 1. Thus, a LOQ of 0.1 μg/l can be proposed for both analytes, as this was the lowest concentration level assayed (validated) with satisfactory recovery and precision.

Fig. 1 shows chromatograms for different water samples spiked at the lowest level assayed (0.1 μg/l). One can conclude that the proposed modification in the derivatization reaction allows the quantification of glyphosate and AMPA at 0.1 μg/l level without any preconcentration. The global procedure is quite selective, mainly for glyphosate, since two columns (C-1 and C-2) with different retention characteristics are used. No clean-up was required prior to derivatization with FMOC since the first C_{18} column allowed an automated clean-up of the derivatized sample. The chromatograms show that AMPA elutes in a crowded area with many potential interferences. Although the results achieved showed that quantification of AMPA was satisfactory at the low levels assayed, its determination could cause problems in more contaminated samples. It is, then, recommended to apply a selective transfer only for AMPA in order to reduce the amount of interferences transferred from C-1 to C-2. This approach was successfully applied for soil analysis [3].

Table 1
Recoveries and R.S.D. values (%) of test analytes in water spiked at 0.1–10 μg/l, using LC–LC–FD[a]

Type of water	Glyphosate (%)	AMPA (%)
Level 0.1 μg/l		
Ground water	94 (7)	104 (5)
Drinking water	97 (3)	101 (3)
Surface water	87 (1)	106 (3)
Level 1 μg/l		
Ground water	101 (1)	101 (1)
Drinking water	98 (1)	97 (1)
Surface water (3)[b]	100 (3)	100 (2)
Level 10 μg/l		
Surface water (3)[b]	97 (3)	102 (5)

The values given in parentheses are R.S.D. values.
[a] $n = 3$ or 4 in all cases.
[b] Three different water samples.

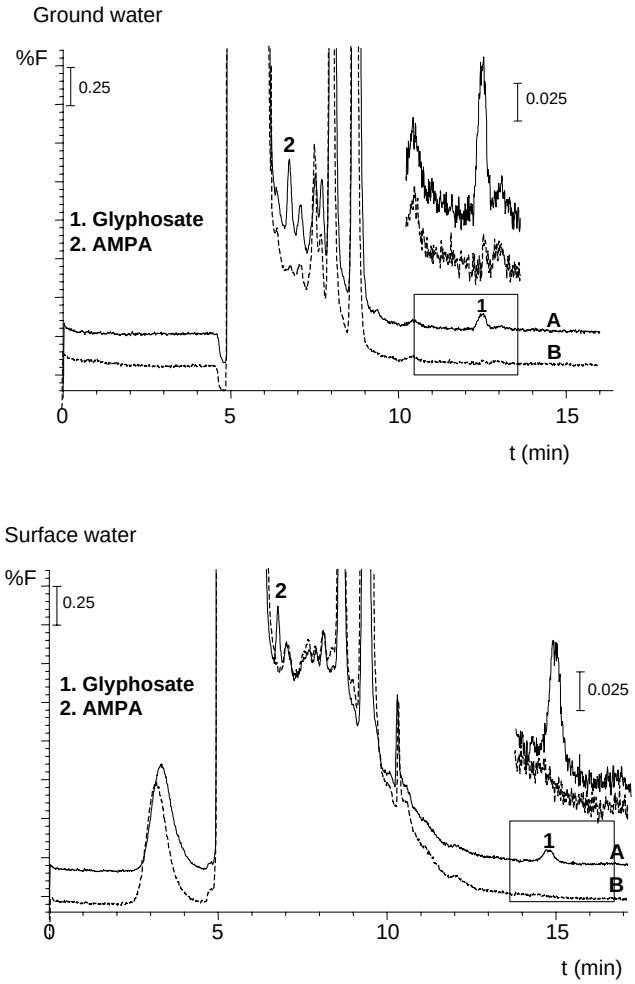

Fig. 1. LC–LC–FD chromatograms of ground and surface water spiked at 0.1 μg/l (A) and their blanks (B).

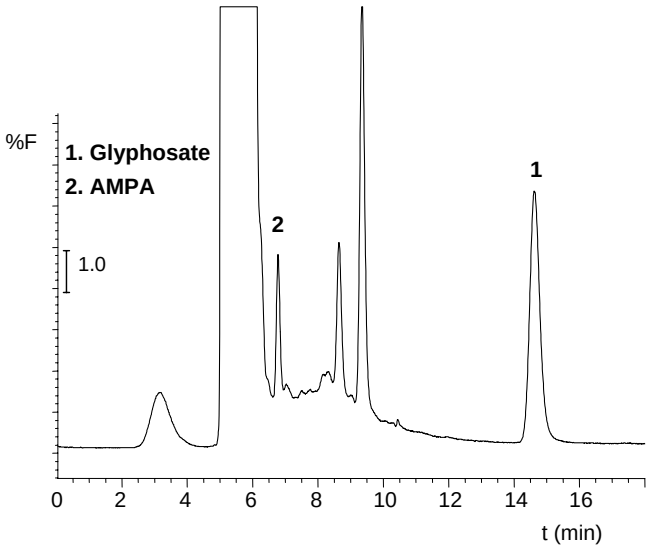

Fig. 2. LC–LC–FD chromatogram of a surface water containing glyphosate (7.5 μg/l) and AMPA (1 μg/l).

The improved procedure was applied to surface water collected from a wet area (Castellón Province, Spain), where glyphosate is widely used for citric crops. Average concentrations ($n = 3$) of 7.5 and 1 μg/l were obtained for glyphosate and AMPA, respectively, with R.S.D. of 1 and 3%. Fig. 2 corresponds to one of the replicates analysed.

3.2. Preconcentration

Given that both glyphosate and AMPA can be present an anionic form in a broad pH range, we used of an anion-exchange resin, Amberlite® IRA-900, to preconcentrate the analytes in order to further decrease the LOQ.

In a preliminary study, we had evaluated the use of two anion-exchange resins (the microporous Amberlite® IRA-416 and the macroporous Amberlite® IRA-900) and a solid-phase extraction cartridge, ISOLUTE-NH_2 in the protonate form for the extraction and preconcentration of glyphosate and AMPA from natural water samples. The influence of flow rate, sample pH, eluent, sample volume and analyte concentration on the extraction procedure were investigated. Glyphosate was quantitatively adsorbed in the range 0.1–500 μg/l in all the sorbents tested whilst only Amberlite® IRA-900 allowed a total retention of AMPA.

Table 2
Recoveries and R.S.D. values (%) of glyphosate in enrichment experiments

Type of water	Recovery (%)	
	0.02 μg/l	0.1 μg/l
Ground water	97 (1)	97 (4)[a]
Drinking water	105 (7)	94 (4)[b]

The values given in parentheses are R.S.D. values.
[a] $n = 3$.
[b] $n = 4$.

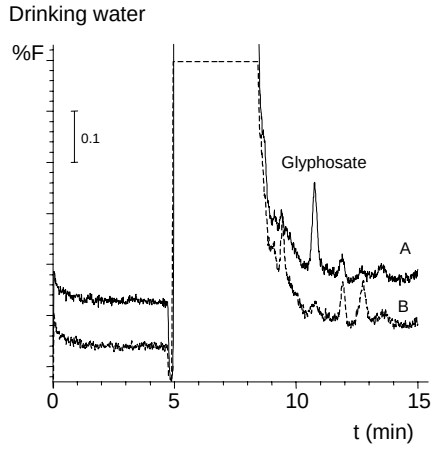

Fig. 3. LC–LC–FD chromatogram of a drinking water spiked at 0.02 μg/l after preconcentration on Amberlite® IRA-900 (A) and its blank (B).

However, when using this resin to process solutions containing the analytes at lower concentrations, quantitative adsorption was not achieved for AMPA. Thus, in subsequent experiments only glyphosate was analysed. The elution of glyphosate was attempted with 0.4 M sodium citrate, 0.4 M phosphate buffer, 0.1 M hydrochloric acid, and 0.1 and 1 M sodium chloride. Complete recovery was obtained using 1 M NaCl. Similar recovery rates were obtained with 0.1 M NaCl and 0.1 M HCl indicating that elution processes occur as a consequence of the exchange of the Cl^- present in the solution for the glyphosate sorbed in the resin.

Fifty millilitres of drinking and ground water samples spiked at 0.02 and 0.1 ng/ml were passed through the resin. After washing with HPLC-grade water, glyphosate was eluted with 10 ml of 1 M NaCl. The results obtained are shown in Table 2, where it can be seen that glyphosate can be quantified satisfactorily at concentration levels as low as 0.02 μg/l.

Fig. 3 shows the chromatograms corresponding to a drinking water sample spiked at 0.02 μg/l together with its blank (non-spiked water sample passed through the resin).

4. Conclusions

An improved LC–LC–FD method was used for the rapid and sensitive determination of glyphosate and AMPA in water after derivatization with FMOC. A first short C_{18} column (3 cm) was used to perform large-volume injection (2 ml) and effect the efficient separation between the derivatized analytes and the excess of FMOC. It was coupled to a second amino analytical column (25 cm) for the anion-exchange separation of the derivatives. Some minor changes in the derivatization reaction together with the efficient resolution and automated clean-up offered by the LC–LC system allowed us to reach LOQ of 0.1 μg/l (without preconcentration) or 0.02 μg/l (after preconcentration of 50 ml water sample on an anionic resin).

The proposed method can be used to monitor of glyphosate and AMPA at sub-μg/l levels with little sample handling; no expensive or sophisticated equipment is required. Although AMPA was satisfactorily recovered at the concentration levels assayed, the abundance of peaks in the chromatogram could make its confirmation in real world samples problematic. Selective transfer of this analyte from C-1 to C-2 by adequate adjusting of the LC–LC parameters is then required.

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