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Rapid determination of glufosinate, glyphosate and aminomethylphosphonic acid in environmental water samples using precolumn fluorogenic labeling and coupled-column liquid chromatography

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Abstract

The approach presented in recent work [J.V. Sancho et al., J. Chromatogr. A, 678 (1994) 59] concerning the rapid determination of glufosinate in environmental water samples was successfully applied to the development of efficient procedures including the assay of glyphosate and its main metabolite, aminomethylphosphonic acid (AMPA). The methodology involves two approaches: (i) a multi-residue method allowing the simultaneous determination of the three analytes in environmental water samples to a level of 1 μg/l or (ii) single residue methods focused on the analysis of a single analyte to the sub-μg/l level. The procedures involve a precolumn derivatisation step with 9-fluorenylmethylchloroformate (FMOC-Cl) yielding highly fluorescent derivatives of the analytes which then can be determined by coupled-column LC with fluorescence detection using a reversed-phase C_{18} column (C-1) coupled to a weak ion-exchange column (C-2). The separation power of the first column (C-1) was used to achieve sensitivity, by injecting large volume samples, and automated sample clean-up was achieved by removing the less polar interferences, including the excess of hydrolysed reagent (FMOC-OH). Using these procedures, glufosinate, glyphosate and AMPA were successfully recovered from water samples at 0.50–10 μg/l fortification levels, with a sample throughput of at least 40 samples per day.

Keywords: Environmental analysis; Water analysis; Coupled columns; Glufosinate; Glyphosate; Aminomethylphosphonic acid; Pesticides

1. Introduction

Glufosinate and glyphosate are used widely as non-selective contact herbicides. Because of their high polarity and poor detectability, adequate methodology to determine these compounds at the sub-ppb level in aqueous samples is lacking [1]. As shown in Table 1, glyphosate and glufosinate are both very polar compounds with similar chemical structures which would suggest the use of a simultaneous assay containing aminomethylphosphonic acid (AMPA), the main metabolite of glyphosate. However, a literature search reveals the absence of such a procedure.

Methodology on glyphosate and AMPA is well documented using GC [2–6] and LC [7–18], techniques that require derivatization steps. The availa-

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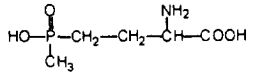
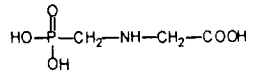
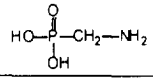
Table 1
Structural formulae and water solubilities of glufosinate, glyphosate and AMPA

Pesticide	Formula	Solubility in water (mg/l at 20°C)
Glufosinate		$>10^5$
Glyphosate		$0.1 \cdot 10^5$
AMPA		$>10^5$

bility of derivatization techniques compatible with both water samples and RPLC separation, renders LC to be the preferred technique. Two different derivatization approaches are generally used for the LC determination of glyphosate; precolumn derivatization using 9-fluorenylmethylchloroformate (FMOC-Cl) reagent [8–12] and post-column derivatization using *o*-phthalaldehyde (OPA) reagent [8,13–18].

Regarding glufosinate, manuals on pesticide residue analysis [19–23] refer only to the analytical method supplied by the manufacturer [24]. This method is very laborious, involving preconcentration on an ion-exchange column, a prederivatization step and clean-up over silica gel before GC analysis with nitrogen–phosphorus detection.

The aim of this study was to develop a method for the simultaneous determination of glufosinate, glyphosate and AMPA in water samples that is fast and, therefore, more suitable for monitoring purposes. The combination of direct large-volume injection and coupled-column LC has been demonstrated to be a suitable technique for the rapid, sensitive and selective determination of polar pesticides in environmental samples [25–27]. Recently, we have developed a single-residue method for the rapid determination of glufosinate in water samples [28]. This procedure makes use of rapid FMOC precolumn derivatization followed by large-volume sample injection and coupled-column LC.

This paper presents the development of a coupled-column method for the rapid determination of glufosinate, glyphosate and AMPA, after FMOC derivatization, in environmental water samples using a short C_{18} separation column to perform large-volume injection and to obtain efficient separation of the analytes and reagent excess, coupled to a second amino separation column for the anion-exchange separation of the derivatives.

2. Experimental

2.1. Chemicals

Glufosinate and glyphosate (content >99%) were obtained from Riedel-de Haen (Seelze, Germany). AMPA was purchased from Sigma (St. Louis, MO, USA). Acetonitrile and ethyl acetate, both of HPLC grade, were purchased from Scharlau Science (Barcelona, Spain). Analytical-reagent grade potassium dihydrogenphosphate, disodium tetraborate decahydrate, orthophosphoric acid (50% pure), hydrochloric acid (37%), potassium hydroxide and FMOC-Cl were bought from Merck. HPLC-grade water was obtained by purifying demineralized water in a Nanopure II system (Barnstead, Newton, MA, USA).

Stock standard solutions (ca. 400 μg/ml) of glufosinate, glyphosate and AMPA, as well as mixed diluted standards, were prepared with HPLC-grade water. 0.125 *M* and 0.02 *M* borate buffer solutions (pH 9) in HPLC-grade water and solutions containing 100 and 1000 μg/ml of FMOC-Cl in acetonitrile were used to perform derivatization and dilution prior to the LC analysis.

Acetonitrile–0.05 *M* phosphate (pH 5.5) in water (35:65, v/v) was used both as the first (M-1) and the second (M-2) mobile phase. The pH of the aqueous buffer solution was adjusted with 2 *M* KOH and 1 *M* HCl.

2.2. Equipment

The modular LC–LC–fluorescence detection (FD) system (for scheme see Ref. [28]) consisted of a Model 1050 sampler (Hewlett-Packard, Waldbronn, Germany), a manual injector equipped with a 2.0-ml loop, that was used to perform large-volume injections (LVIs), a Model 1050 gradient LC pump, Hewlett-Packard), a model C6W six-port switching valve driven by a WE-II actuator from Valco (VIGI,

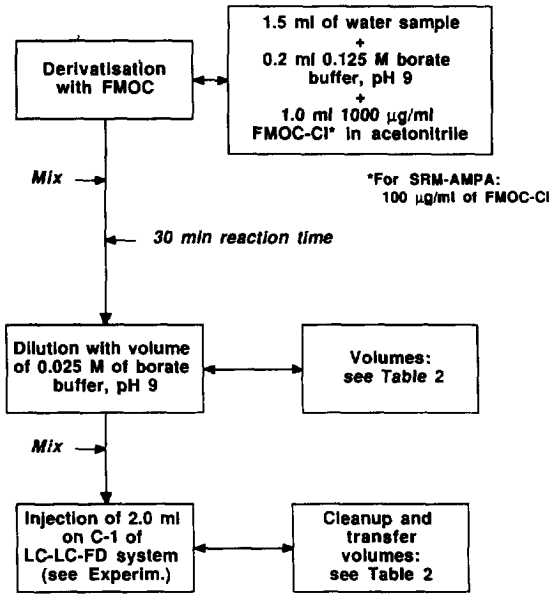


Fig. 1. Scheme of analysis (see also Section 2 and Table 2).

Schenkon, Switzerland) and time-controlled by the sampler, a Model 2150 isocratic LC pump from LKB (Bromma, Sweden), a Model 1046A fluorescence detector (Hewlett-Packard) set at 263 nm (excitation) and 317 nm (emission), a 30×4.6 mm I.D. first separation column (C-1) packed with 5 μm Spherisorb ODS-2 from Scharlau Science and a second 250×4.6 mm I.D. separation column (C-2) packed with 5 μm Adsorbosphere NH_2 from Alltech (Carnforth, UK) or 5 μm Spherisorb NH_2 from Scharlau Science. C-2 was kept at 30°C in the column heater of the Model 1050 pump. The flow-rate of the mobile phases was set at 1 ml/min.

Recording of chromatograms and quantitative measurements of peak areas were performed with a Hewlett-Packard HPLC ChemStation (software version G1034A). A MicropH 2001 pH meter and Pipetmans (200, 1000 and 5000 μl) were obtained from Crison Instruments (Barcelona, Spain) and Gilson, respectively.

2.3. Procedures

The scheme for the analysis by both the multi-residue method (MRM) and the single-residue method (SRM) is given in Fig. 1. Data on the applied dilution volumes and the various clean-up and transfer volumes used in coupled-column LC are given in Table 2.

3. Results and discussion

In our previous work [28] on the determination of glufosinate in aqueous samples, adequate LC–LC conditions were established enabling clean-up and large volume injection on the first short C_{18} colume and efficient separation of the analyte on the second amino separation column. Based on the information in Ref. [11], a mobile phase of acetonitrile–0.05 *M* phosphate (pH 5.5) in water (35:65, v/v) was selected on both columns, providing satisfactory results. The study revealed that sensitivity could be enhanced considerably by LVI after some dilution of the sample after derivatisation with a volume of borate buffer. In this study we investigated the potential of this approach for the simultaneous determination of glufosinate, glyphosate and AMPA.

Table 2
Information on experimental conditions (cf. Fig. 1)

Method	MRM: Glyphosate, AMPA glufosinate	SRM		
		Glyphosate	AMPA	Glufosinate
Dilution volume (ml)	17.5	17.5	7.5	7.5
Clean-up volume (ml)	2.21	2.21	2.55	2.32
Transfer volume (ml)	0.53	0.28	0.18	0.18

3.1. Second column conditions

The first step in method development was the establishment of suitable conditions for the simultaneous separation of glufosinate, glyphosate and AMPA on the 250×4.6 mm I.D. amino column used as the second separation column (C-2). Selecting acetonitrile and phosphate buffer as the constituents of the mobile phase, the influence of the percentage of modifier, the pH and the concentration of phosphate buffer were investigated. Starting with the mobile phase composition used for the determination of glufosinate (see above), the parameters mentioned above were varied. A liquid–liquid extraction with ethyl acetate after the FMOC derivatization of standards [11] was applied prior to the LC analysis, using an injection volume of 20 μl of sample onto the amino separation column.

When varying the percentage of modifier (tested range, 25–45% acetonitrile), it appeared that 35% acetonitrile provided full separation and sufficient retention ($1<k'<10$) of the three analytes.

The influence of the pH on retention is illustrated in Fig. 2A. It shows that for glyphosate and glufosinate, maximum retention is obtained at pH 4, while for AMPA the influence of pH is negligible. A pH value of 5.5 was chosen as a good compromise between resolution, analysis time and amino column stability (silica-based column).

The influence of the ionic strength of the phosphate buffer (pH 5.5) is displayed in Fig. 2B. This experiment reveals that a decrease in the ionic strength increases the retention considerably, being more noticeable for glyphosate and glufosinate, producing excessive band broadening. Hence, as a good compromise between separation and peak shape, acetonitrile–0.05 *M* phosphate (pH 5.5) in water (35:65, v/v) was selected as a mobile phase for the efficient separation of all analytes.

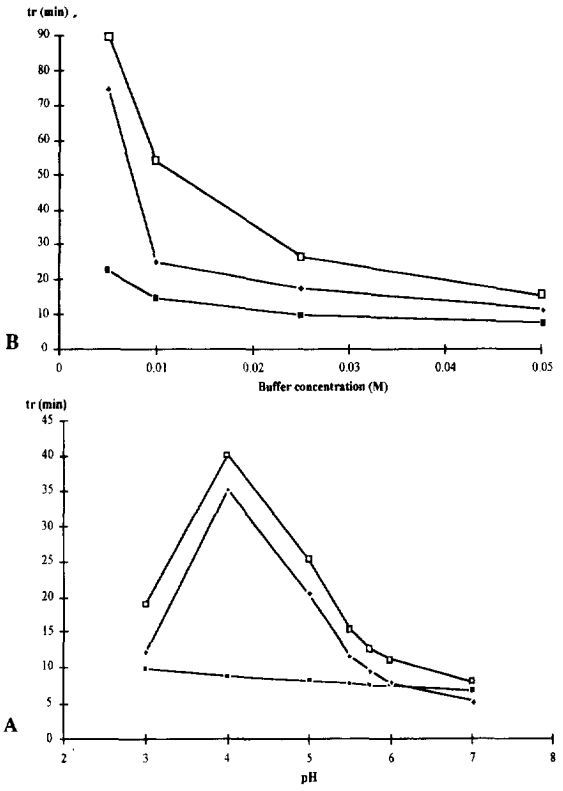


Fig. 2. Optimisation of the separation conditions for analytes on the 250×4.6 mm amino separation column (C-2). (**A**) Effect of the pH of the buffer on retention of analytes in a mobile phase consisting 0.05 *M* phoshate buffer–acetonitrile (35:65; v/v). (**B**) Effect of the concentration of the phosphate buffer. ■=AMPA; □=glyphosate; ◇=glufosinate. For further information, see text.

3.2. Sample pretreatment

For the analysis of glufosinate [28], a standard procedure [11] was applied to perform precolumn derivatization with FMOC-Cl using 0.5 ml of aqueous sample, 1 ml of FMOC solution (1000 μg/ml in acetonitrile) and 1 ml of 0.025 *M* borate buffer, with a reaction time of 20 min at room temperature. It was established that at least a 40% acetonitrile content in the reaction mixture was necessary to avoid precipitation of the reagent and, consequently, an additional filtration step prior to the LC analysis. In order to improve sensitivity, the volume of the water sample was increased and the volume of the borate buffer was decreased in such a way that in the final mixture the acetonitrile and borate concentrations were similar to the ones used in the selected procedure. Satisfactory conditions were found with 1.5 ml of water sample and 0.2 ml of 0.125 *M* borate buffer, improving the volume of water sample processed in comparison to the standard procedure by about three-fold [11].

3.3. First column conditions

As discussed earlier [25–27], the attainable sensitivity and selectivity of a coupled-column procedure depend on the amount of sample that can be injected on the C-1 column without excessive band broadening of the analyte (sensitivity), the clean-up volume that can be applied on C-1 after injection (selectivity) and the volume of mobile phase required to transfer the analyte-containing fraction from C-1 to C-2 (selectivity). Considering the first two aspects, sample injection volume and clean-up volume are distinctly determined by the analytes retention on C-1, which in turn can be controlled, to a certain degree, by the selected separation power of the first C_{18} column and the eluotropic strength of the mobile phase. Elution on C-1 must be considered as a step gradient in which the sample volume acts as the first mobile phase. For the direct processing of water samples, the attainable analytes C_{18} retention on C-1 during injection is maximal and, consequently, sample injection volume. However, in this application, samples after FMOC derivatization contain a percentage of acetonitrile. Hence, the eluotropic strength of the sample solution will play a major role during injection, and should be kept as low as possible to minimize band broadening. For example, under the optimised LC–LC conditions for glufosinate, the combination of a 30×4.6 mm I.D. column packed with 5-μm C_{18} and a mobile phase of acetonitrile–0.05 *M* phosphate (pH 5.5) in water (35:65, v/v), used on C-1, the maximum injection volume of the sample containing 40% acetonitrile was about 100 μl. It was established that the overall volume of sample that could be loaded could be increased significantly by an aqueous dilution of the sample prior to injection. For glufosinate, a good result for both sensitivity and selectivity with respect to sample dilution was obtained by a three-fold dilution of the sample with borate buffer after derivatisation. Under the selected conditions mentioned above, the diluted solution (15% acetonitrile) permitted the use of injection volumes of up to 2.0 ml, without significant elution (band broadening) of the analyte during injection. After elution of the injection volume through the C_{18} column, the mobile phase (35% acetonitrile) elutes FMOC–glufosinate almost unretained, providing an efficient separation between analyte and the less polar excess of FMOC reagent. When investigating this favourable approach for AMPA and glyphosate, it appeared that somewhat more dilution was necessary in order to avoid excessive band broadening of the FMOC derivatives during injection. Employing a 1.1-ml sample injection on C-1, the dramatic effect of the modifier content (% acetonitrile) on the band broadening of glyphosate–FMOC is nicely illustrated in Fig. 3. Experiments indicated that in a similar way to the determination of glufosinate, a 2.0-ml sample injection volume could be applied for the assay of AMPA and glyphosate, when dilution factors of 4 and 8 with borate buffer were used, respectively, corresponding to a final acetonitrile content of 10 and 5% in the sample solution.

3.4. Multi-residue procedure

As mentioned above, for the simultaneous determination of glufosinate, glyphosate and AMPA, the sample solution after derivatization must be diluted eight times before the LC–LC analysis. The applied modifications for sample pretreatment (see above) and dilution will decrease the sensitivity to some extent, compared with the single residue method for glufosinate [28]. Furthermore, the C_{18} retention of AMPA is about 0.30 min longer than glyphosate and glufosinate, which means that, in comparison with glufosinate, a larger volume will be necessary to transfer the three analytes from C-1 and C-2. The performance of the final multi-residue procedure (see Section 2 is depicted in Fig. 4, which shows the chromatogram of a surface water sample spiked at 4 μg/l. This procedure, with a high sample throughput of at least 40 samples per day, allows the simultaneous determination of glufosinate, glyphosate and AMPA in water samples to a level of at least 1 μg/l (signal-to-noise ratio=3).

3.5. Single-residue methods

In order to improve sensitivity, the possibility of lowering the limits of detection to the sub-μg/l level by simply concentrating a certain volume of water sample by evaporation, as has been demonstrated for glufosinate [28], was investigated. Unfortunately, this concentration step resulted in poor recoveries for

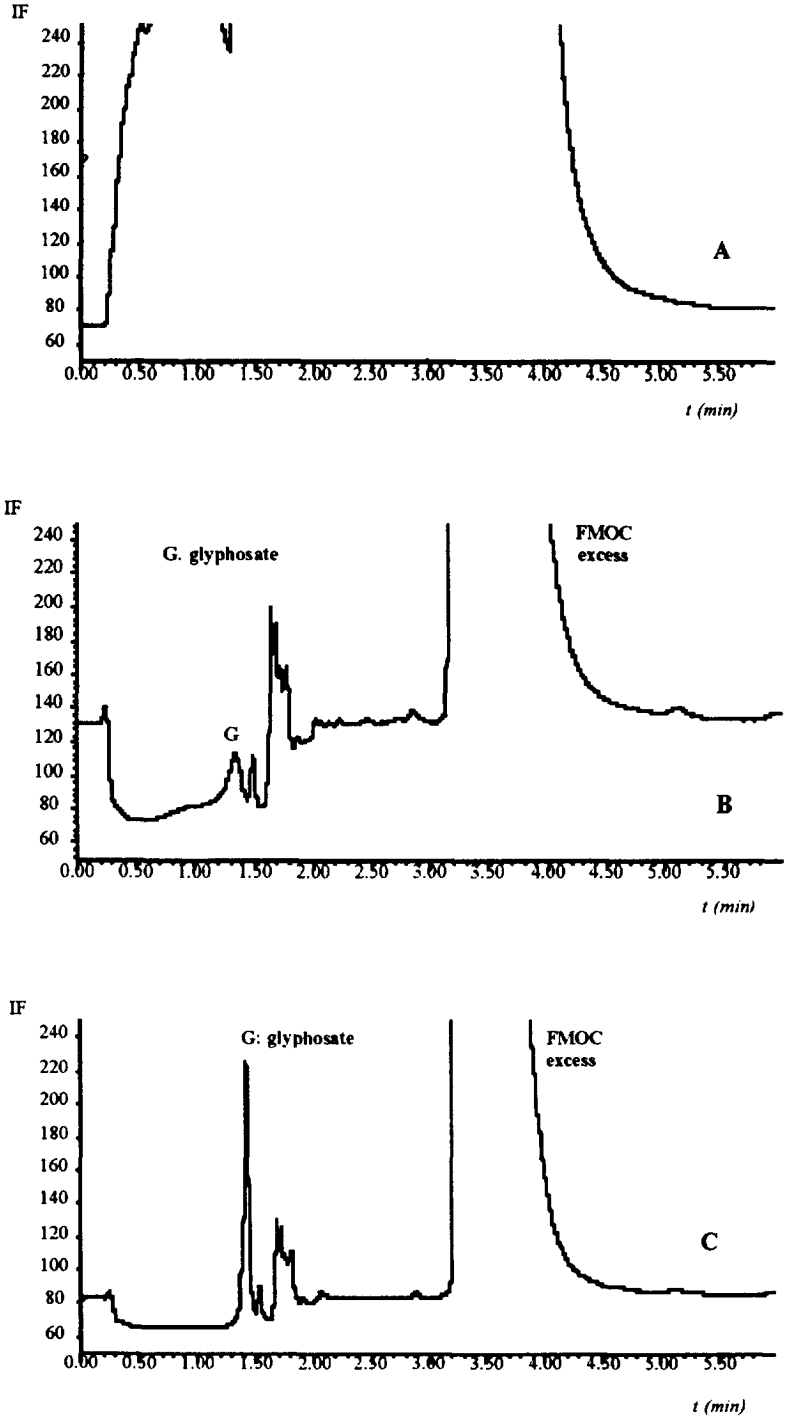


Fig. 3. Effect of sample dilution after FMOC derivatization on the band broadening of analytes on the first C_{18} column (C-1). Chromatograms obtained following the injection of 1.1 ml of processed glyphosate standard (0.4 μg/ml) on C-1 connected to a fluorescescence detector. (A) Without dilution (40% acetonitrile); (B) four-fold dilution (10% acetonitrile); (C) eight-fold dilution (5% acetonitrile). For further conditions, see Section 2.

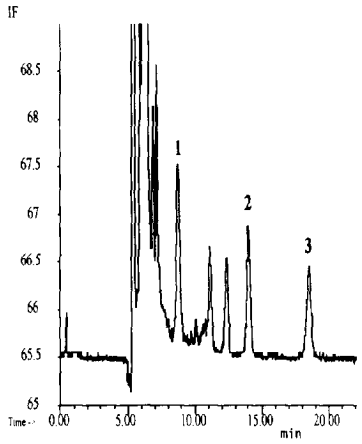


Fig. 4. LC–LC–FD of the MRM for a surface water sample spiked with the analytes at a level of 4 μg/ml. Peaks: 1=AMPA; 2=glufosinate; 3=glyphosate.

glyphosate and AMPA (20–30%), which may be caused by adsorption to glass surfaces [11], similar to the well known adsorption onto soils by glyphosate [29,30]. The addition of 0.1–0.05 *M* phosphate buffer to the water sample before concentration [11], to reduce the adsorption of the analytes, did not improve the recovery sufficiently (48–54%) at low levels (tested range, 0.5–5 μg/l).

Therefore, we investigated the possibility of lowering the limit of detection by the application of methods accurately focused at the single-residue determination of each analyte. AMPA and glufosinate require only a four-fold dilution (see Table 2), hence, improved sensitivity for both analytes could be expected. Applying the four-fold dilution step and accurate adjusted clean-up and transfer volumes, as listed in Table 2, AMPA and glufosinate could easily be determined in water samples to the sub-μg/l level. Results of this approach are illustrated in the first and third chromatogram of Fig. 5, showing the LC–LC–FD analyses of a surface water sample spiked with AMPA and glufosinate at a level of 0.4 μg/l.

The required eight-fold dilution step hampers the sensitivity obtained for glyphosate. However, when using a precisely adjusted transfer volume of 280 μl for the FMOC–glyphosate-containing fraction, a limit of detection of about 0.2 μl/l can be reached. The performance of this procedure is depicted in the second chromatogram of Fig. 5, obtained for the analysis of a surface water sample spiked with glyphosate at 0.4 μg/l.

3.6. Results

The response of glufosinate, glyphosate and AMPA derivatives was linear for standard solutions in water, with concentrations between 1 and 400 μg/l ($r>0.999$, $n=4$). The multi-residue procedure was validated by analysing surface water samples spiked with glufosinate, glyphosate and AMPA. Recoveries made at two levels are given in Table 3.

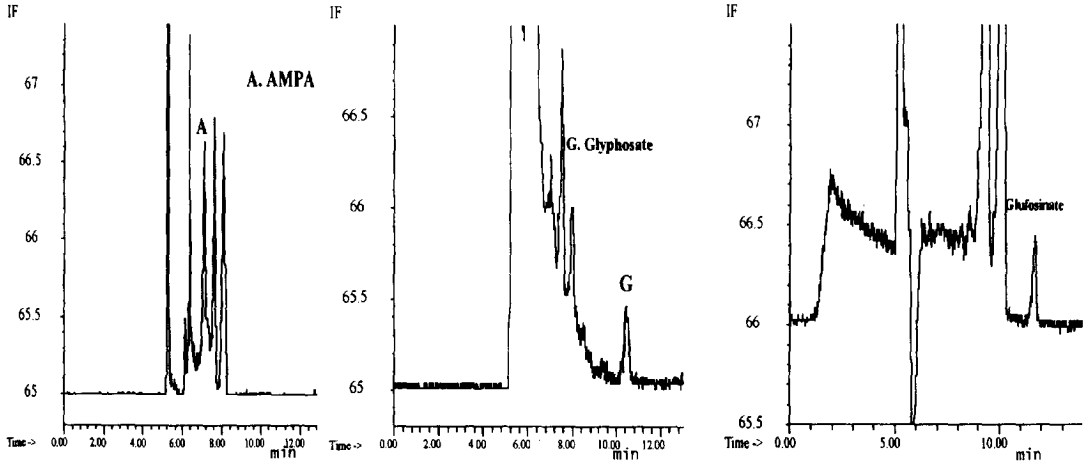


Fig. 5. LC–LC–FD of the SRMs for surface water samples spiked with an analyte at the 0.4 μg/ml level.

Table 3
Recoveries and relative standard deviations (R.S.D.s) for surface water samples spiked with AMPA, glufosinate and glyphosate ($n=5$)

Pesticide	10 μg/l		4 μg/l	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
AMPA	94.9	3.2	95.5	4.1
Glufosinate	95.6	2.8	93.1	4.3
Glyphosate	86.0	4.9	88.0	5.3

The single-residue methods were tested by the analysis of surface water samples spiked with each analyte at a level of 0.4 μg/l ($n=5$). The corresponding recoveries and relative standard deviations (R.S.D.s) for AMPA, glyphosate and glufosinate were 95% (R.S.D., 11%), 107% (R.S.D., 5%) and 92% (R.S.D., 10%), respectively.

4. Conclusions

The combination of precolumn fluorogenic labeling with FMOC and coupled-column LC with LVI provides an efficient method for the simultaneous determination of glufosinate, glyphosate and AMPA in environmental water samples, to a level of 1 μg/l. With the same approach but directed to the determination of a single analyte, methods were found that enabled the determination of analytes at the sub-μg/l level. The high sample throughput of the developed procedures makes them highly suitable for screening purposes.

Acknowledgements

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