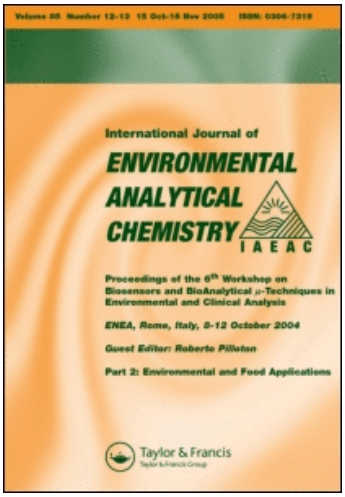

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Rapid Determination of Glyphosate Residues and Its Main Metabolite Ampa in Soil Samples by Liquid Chromatography

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RAPID DETERMINATION OF GLYPHOSATE RESIDUES AND ITS MAIN METABOLITE AMPA IN SOIL SAMPLES BY LIQUID CHROMATOGRAPHY

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Efficient procedures for the trace-level determination of glyphosate and/or the metabolite AMPA in various type of soil samples has been developed. After extraction in alkaline media an aliquout of the extract is neutralized prior to a precolumn derivatisation step with FMOC-Cl yielding highly fluorescent derivatives of the analytes. Two different LC-methods have been applied. The first one is focussed at the determination of glyphosate only and requires a liquid-liquid extraction step with ethyl acetate for the removal of the reagent excess prior to the RPLC-analyses performed on a single 25 cm C18 column under isocratic elution conditions. The second method makes use of coupled-column LC for on-line sample cleanup of diluted extracts and allows the determination of both glyphosate and AMPA.

With the developed procedures glyphosate and AMPA were succesfully recovered from different kind of soils at 400 and 40 $ng \cdot g^{-1}$ fortification levels. Sample throughput is at least 20 samples per day. Depending on the type of soil, viz. high or low clay content and organic matter, and the LC method used, limits of determination range between 100 and 10 $ng \cdot g^{-1}$ for both analytes.

KEY WORDS: Glyphosate, AMPA, soils, LC, precolumn derivatization.

INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] is a broad-spectrum, non-selective, post-emergence herbicide that has found widespread agricultural and domestic use. Several methods have been developed for the liquid chromatography determination of glyphosate and its main metabolite aminomethylphosphonic acid, AMPA, in environmental samples using either precolumn or postcolumn derivatization. Postcolumn procedures have been commonly used with o-phthalaldehyde-mercaptoethanol[1-10] or ninhydrin[11]. The rather complex experimental setup required (the use of OPA involves a hydrolisis step prior to derivatization) and the use of cation-exchange columns produces broad peaks in the final chromatogram. Moreover, a preconcentration step is needed in order to reach low detection limits. Precolumn procedures which have focused on derivatization with 9-

fluorenylmethyl-chloroformiate (FMOC-Cl) with fluorescence detection[12-14] is a good alternative to postcolumn ones.

Glyphosate has been shown to bind strongly to clay minerals[15,16] and sorption is influenced by pH and the nature of the saturating cation on the clay[17,18]. Although a number of procedures have been described for the analysis of glyphosate in soils, several workers have reported low and irreproducible recoveries[12,19].

An overview of current methodology used to analyse glyphosate and AMPA residues in soil samples is given in Table 1. It shows that several basic extracting media have been used and that most procedures requires laborious cleanup/concentration steps. In addition, low recoveries and high standard deviations are reported according to the soil type, decreasing with a high clay content[12], but also depending on pH[18]. Moreover, the recovery studies are carried out at spiking levels between 0.5 and 50 $\mu g \cdot g^{-1}$, concentrations higher than expected in soil samples. All the procedures yield limits of detection in the range of 50 $ng \cdot g^{-1}$, except the FMOC procedures, about 1 $\mu g \cdot g^{-1}$, but in any case too high for the levels expected. Hence, analytical procedures for the determination of glyphosate and AMPA in soil samples which provide (i) low limits of detection, (ii) improved recoveries and (iii) less laborious sample pretreatment are highly mandatory.

In the present paper an analytical method for glyphosate and AMPA residues in soils treated with glyphosate has been developed in order to analyse efficiently the large amount of samples generated in a monitoring programme. Basically, two methods have been developed using both a single extraction step and a subsequent precolumn derivatisation step with FMOC-Cl. The first method involves the LC-analysis of glyphosate in soils extracts pretreated with a simple low volume liquid-liquid extraction on one C18 separation column with a chromatographic run time of less than 15 min. The second method applies coupled-column LC (LC-LC) with two columns of a distincly different separation mechanism for the determination of glyphosate and AMPA in soil extracts. This somewhat more complex LC-system allows both on-line sample cleanup and high volume injection which improves sample throuthput, sensitivity and selectivity. This method is based on recent work concerning the simultaneous analysis of glufosinate, glyphosate and AMPA in environmental water samples[20,21]. As regards their analytical performance both methods (LC and LC-LC) have been compared.

EXPERIMENTAL

Chemicals

HPLC-grade methanol and acetonitrile were purchased from Scharlau (Barcelona, Spain). HPLC-grade water was obtained by purifying demineralized water in a Nanopure II system (Barnstead, USA). Ethyl acetate, pesticide residue analysis grade from Scharlau (Barcelona, Spain). Analytical-reagent grade potassium hydroxide, potassium dihydrogen phosphate, disodium tetraborate decahydrate, hydrochloric acid (37%) and 9-fluorenylmethyl chloroformiate (FMOC-Cl) were bought from Merck (Darmstat, Germany).

For the LC-LC method a 0.05 M phosphate buffer of pH 5.7 was prepared by dissolving 6.803 g KH_2PO_4 in 1 liter HPLC water and adjusting the pH with 2 M KOH.

Table 1 Residue analysis of glyphosate and AMPA in soil samples.

Sample pretreatment	*Derivatization*	*Compound*	*Spiking level ($\mu g \cdot g^{-1}$) (Recovery (%))*	*Limit of detection ($\mu g \cdot g^{-1}$)*	*Reference*
20 g soil + 2 × 150 ml KOH 0.1 M (16 h)	Post-column OPA/ME	Glyphosate	11.25 (79–86) 0.56 (80–87)	0.04	10
5 g soil + 3 × 150 ml NH3 anion and cation exchange clean-up concentration by evaporation	Post-column ninhydrin	Glyphosate	4–30 (73–79)	0.02–0.05	
		AMPA	1–7.5 (57–68)	0.01–0.02	11
30 g soil + 50 ml NaOH 0.1 M (60 min) flocculation clean-up	Pre-column FMOC	Glyphosate	200 (20–55) 50 (39–43)	5	12
2 g soil + 3 × 10 ml KOH 0.1 M	Pre-column FMOC	Glyphosate	10 (86–93) 1 (108–119)	1	14
3.5 g soil + 30 ml triethylamine 0.1 M anion exchange clean-up concentration by evaporation	Pre-column 1-fluoro-2,4-dinitrobencene	Glyphosate	1.43 (56–90)	0.05	
		AMPA	1.43 (55–90)	0.1	19

In the case the LC-method, a 0.002 M phosphate buffer of pH 6.3 was prepared by dissolving 0.27 g KH_2PO_4 in 1 liter HPLC water and adjusting the pH with 2 M KOH.

The mobile phase for the LC-method consist of acetonitrile—0.002 M phosphate buffer, pH 6.3 (7.5:92.5, v/v). For the LC-LC method a mobile phase of acetonitrile—0.05 M phosphate buffer, pH 5.7 (35:65, v/v) was used as M-1 and M-2 (see Figure 1) on both LC-columns.

Stock standard solutions (ca. 400 $\mu g \cdot ml^{-1}$) of glyphosate (Rieldel de Haën) and AMPA (Sigma) and dilutions were prepared with HPLC-grade water. A 0.025 M borate buffer solution (pH 9) and a 1000 $\mu g \cdot ml^{-1}$ FMOC-Cl solution were prepared in HPLC-grade water and acetonitrile, respectively.

Equipment

The HPLC set-up used for both the LC and the LC-LC method is illustrated schematically in Figure 1. The modular system consisted of a Model 1050 autosampler (AS, Hewlett-Packard, Waldbron, Germany) using the default automatic injection valve equipped with a 100 µl loop for the LC-method and the additional installed manual injection valve equipped with a 2.0 ml loop for the LC-LC method, a Model 1050 gradient pump (P-1, Hewlett-Packard), a Model C6W six-port switching valve (HV) driven by a WE-II actuator from Valco (VIGI, Schenkon, Switzerland) and time controlled by the sampler, a Model 2150 pump (P-2) from LKB (Bromma, Sweden), a Model 1046A fluorescence detector (Hewlett-Packard) set at 263 nm (excitation) and 317 nm (emission).

The LC-method employs a 250 × 4.6 mm I.D. column packed with 5 µm Spherisorb ODS2 (Hewlett Packard) kept at 30°C in the column heater of the Model 1050 pump and directly connected to the fluorescence detector. The LC-LC-method uses a 30 × 4.6 mm I.D. column packed with 3 µm Spherisorb ODS2 (Scharlau, Barcelona, Spain) as C-1 and a 250 × 4.6 mm I.D. column packed with 5 µm Adsorbosphere NH2 as C-2 kept at 30°C.

Recording of chromatograms and quantitative measurament of peak areas were performed with a Hewlett Packard HPLC ChemStation (G1034A). A MicropH 2001 pH meter and Pipetmans (100, 200, 1000 and 5000 µl) were obtained from Crison Instruments (Barcelona, Spain) and Gilson, respectively.

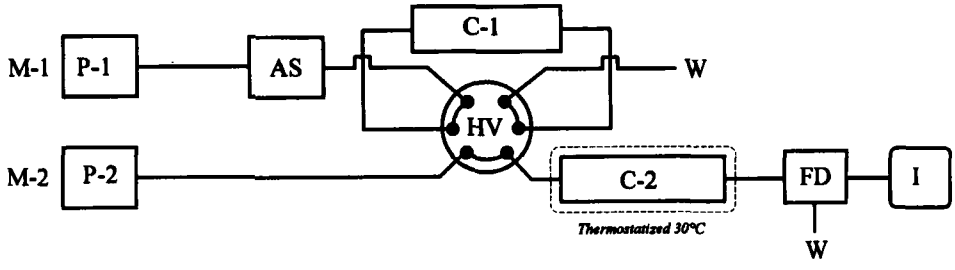

Figure 1 HPLC set-up for column-switching. AS = sample injector with 2-ml loop (L); HV = six-port high-pressure valve; P-1 = gradient LC pump; P-2 = isocratic LC pump; C-1 = first separation column; C-2 = second separation column; M-1 and M-2 = mobile phases on C-1 and C-2, respectively; FD = fluorescence detector; I = integrator system; W = waste.

Soil fortification

Soil samples were taken at different depths at two spanish locations, a clay soil from Tenerife island (Canary Islands) and a sandy soil from Maresme area (Barcelona). Soils characteristics are shown in Table 2.

Air-dried soil samples were homogenized and 5.0 g subsamples were transferred to centrifuge tubes (50 ml). Samples were fortified using automatic pipet to deliver appropiate volumes of mixed standard, yielding fortification levels of 40–400 $ng \cdot g^{-1}$. Samples were equilibrated under dark conditions for 2 h prior to extraction.

Soil extraction

Samples were extracted by shaking with 0.6 M KOH (10 ml) on a mechanical shaker at 120 oscillations/min for 30 min, and then centrifuged (2500 rpm for 20 min). 3.5-ml clear supernatant was transferred to a 5-ml vial and dropwise neutralized with HCl to a pH between 5–8, and then the precolumn derivatization step was carried out on a 1-ml aliquot.

Precolumn derivatization

The derivatization procedure is based on the previous work of Sancho *et al.*[20,21]: 1-ml aliquot of the neutralized supernatant was pipetted into a 9-ml glass tube together with 0.5 ml of borate buffer and 1 ml of FMOC reagent. The tube was swirled and left for 30 min at room temperature.

LC analysis

Unreacted FMOC was removed from the medium by shaking the mixture twice with 2.5 ml of ethyl acetate and removing a portion of the water phase with a syringe for chromatographic analysis on the C18 column. The mobile phase was set at a flow-rate of 1 $ml \cdot min^{-1}$ and a volume of 100 µl of the aqueous phase was injected in the LC system.

Table 2 Chemical and physical soil properties

	Soil descriptor				
Parameter	*TF1*	*TF2*	*M1*	*M2*	*M3*
Depth (cm)	10	65	20	100	200
pH	6.40	7.26	8.25	7.51	7.89
Organic matter (%)	2.4	1.2	1.1	< 0.1	< 0.1
Moisture (%)	23.6	23.3	2.9	5.6	7.1
Sand (%)	22.0	37.1	81.6	85.2	87.3
Silt (%)	33.3	21.3	11.4	8.1	6.0
Clay (%)	44.7	41.6	7.0	6.7	6.7

TF: sample from Tenerife Island (Canary Islands).
M: sample from Maresme area (Barcelona).

Quantification of glyphosate was done by external calibration with standard solutions in water which were processed with the precolumn derivatization procedure.

LC-LC analysis

For the simultaneously determination of glyphosate and AMPA, after FMOC reaction, 17.5 ml of borate buffer were added and the tube was swirled again for thorough mixing. A volume of 2.00 ml of the solution obtained was injected on to C-1. After clean-up with 2.12 ml of M-1 (injection volume included), C-1 was switched on-line with C-2 for 0.41 min to transfer the fraction containing both glyphosate and AMPA derivatives to C-2. Quantification of analytes was done also by external calibration with standard solutions in water which were processed with the precolumn derivatization procedure.

For a more sensitive and selective analysis of AMPA, only 7.5 ml of borate buffer were added, applying a clean-up volumen of 2.30 ml of M-1 and transferring AMPA fraction with 0.19 ml of M-2.

RESULTS AND DISCUSSION

Based on previous studies, the extraction of soils for the determination of glyphosate using potassium hydroxide 0.6 M was chosen as the best approach[10,12,14,22]. One can expect similar recoveries for the metabolite AMPA as both show similar adsorption coefficients[23].

The derivatization reaction yield for glyphosate and AMPA with FMOC is optimum at pH 9 and decreases at higher pH values. As the borate solution cannot buffer properly the sample extract (extraction with KOH 0.6 M), a neutralizing step is necessary before the derivatization. Any attempt of fixing the volume of HCl necessary to neutralize the KOH excess failed due to the different nature of the soils. This step must be done manually adding drops of HCl 6 and 0.6 M until the pH range was between 5 and 9, when the borate buffer can act properly. During this stage, precipitates can appear and a filtering step should be carried out, but resulting in an analyte loss. Thus, precolumn derivatizating procedure must be done on the neutralized unfiltered extract, leaving the mixture settling for 30 min and taking the clear supernatant for analysis.

As regards the speed of analysis the possibility to perform analyses of soil sample extracts on a single column LC-system was firstly investigated. For this purpose a C18-silica bonded column[24] and an amino-silica bonded column acting as a weak anion exchanger[13] were studied. Because of their amphoteric property the analytes will always behave as ionic compounds in a reversed phase system and hence C18 retention is limited. Adequate conditions as regards retention and separation were found in selecting a 250 × 4.6 mm I.D. 5 μm C18 column with a mobile phase consisting of acetonitrile-0.002 M fosfate buffer, pH 6.3 (7.5:92.5, v/v). Under these conditions the capacity factors (k-values) of glyphosate-FMOC and AMPA-FMOC are 0.48 and 2.60, respectively. Concerning the band broadening of glyphosate-FMOC sample injection volume was limited to about 100 μl.

An amino column acting as anion exchanger can provide easily sufficient retention of the analytes. In this case optimal separation conditions were taken over from our previous study. On a 250 × 4.6 mm I.D. 5 μm NH2 column and a mobile phase of acetonitrile-0.05 M fosphate buffer, pH 5.7 (35:65, v/v) the capacity factor of AMPA-FMOC and glyphosate-FMOC are 1.75 and 5.35, respectively. Despite the improved

retention in comparison to the reversed phase separation the maximum sample injection volume on the amino column was similar to that of the C18 column. From experimental work it appeared that removal of the excess of hydrolysed reagent (FMOC-OH) was necessary because it affected the column life very fast. This removal was achieved by extracting twice with 2.5 ml ethyl acetate.

Preliminary experiments with soil sample extracts indicated that the amino could not provide sufficient separation between interferences and analytes. Therefore, the C18 column was chosen as the separation column for further experiments. The C18 column provides sufficient separation between the analytes and the later eluting excess of FMOC-OH. However, a liquid-liquid extraction step with ethyl acetate was still preferred to avoid a gradient elution step after the elution of the analytes and, thus, decreasing considerably the time of the chromatographic run.

The procedure performance was studied on different kind of soils fortified with glyphosate and AMPA at several levels. It appeared that this rather simple method was useful for the determination of glyphosate at levels such low as 400 $ng \cdot g^{-1}$ in different soil types (TF2 and M2, analyzed by quintuplicate yielded recoveries of 101 and 96.2% with RSDs of 7.8 and 12%, respectively). The results show that for sandy soil sample types (M2) are also satisfactory at levels of 40 and 20 $ng \cdot g^{-1}$ (recoveries of 92.7 and 105% with RSDs about 9%), yielding a limit of detection about 5 $ng \cdot g^{-1}$.

Unfortunately, the method did not allow the determination of AMPA due to the presence many interferences limiting the correct identification and quantitation of this metabolite at required low levels. An attempt to retain the interferences (mainly humic acid substances) by filtering a neutralised soil extract (to a pH of about 5) through a 500 mg C18 SPE cartridge did not improved substantially the selectivity.

In order to enhance (i) sample throughput, (ii) selectivity and (iii) sensitivity the coupled-column LC technique developed for the determination of glufosinate in environmental water samples[21] was investigated on its applicability for the determination of glyphosate and AMPA in soil extracts. The coupled-column method is based on a large volume injection of the sample after FMOC-reaction on a C18 separation column followed by a small analyte containing transfer volume to a second amino separation column. During separation on the second column the excess of FMOC is removed providing a fully automated processing of sample extracts. In this approach the large volume of sample injected acts as a mobile phase and in order to obtain sufficient peak compression of the analyte during injection the eluotropic strength (content of acetonitrile) of the sample solution plays a crucial role. For glufosinate sufficient peak compression was obtained by diluting the sample solution after reaction three times with a borate buffer.

Experiments indicated that applying two ml sample the acetonitrile content should be lowered more in order to avoid excessive band broadening of the analytes. In addition, the dilution factor appeared to be different for glyphosate and AMPA. Satisfactory results were obtained with dilution factors of 4 and 8 for AMPA and glyphosate, respectively. Selecting the 8 times dilution step after precolumn derivatisation soil samples were processed with LC-LC using accurate adjusted clean-up and transfer times for glyphosate and AMPA (clean-up 2.12 min, transfer 0.41 min). Figure 2 shows the chromatogram obtained following this procedure for a soil sample (M3) fortified with both analytes at 400 $ng \cdot g^{-1}$ level.

The LC-LC method was applied to the two different kind of soils fortified with glyphosate and AMPA at 40 and 400 $ng \cdot g^{-1}$. The results on recoveries and relative standard deviations are listed in Table 3. Concerning the determination of glyphosate the results of both methods (LC and LC-LC) are in good agreement.

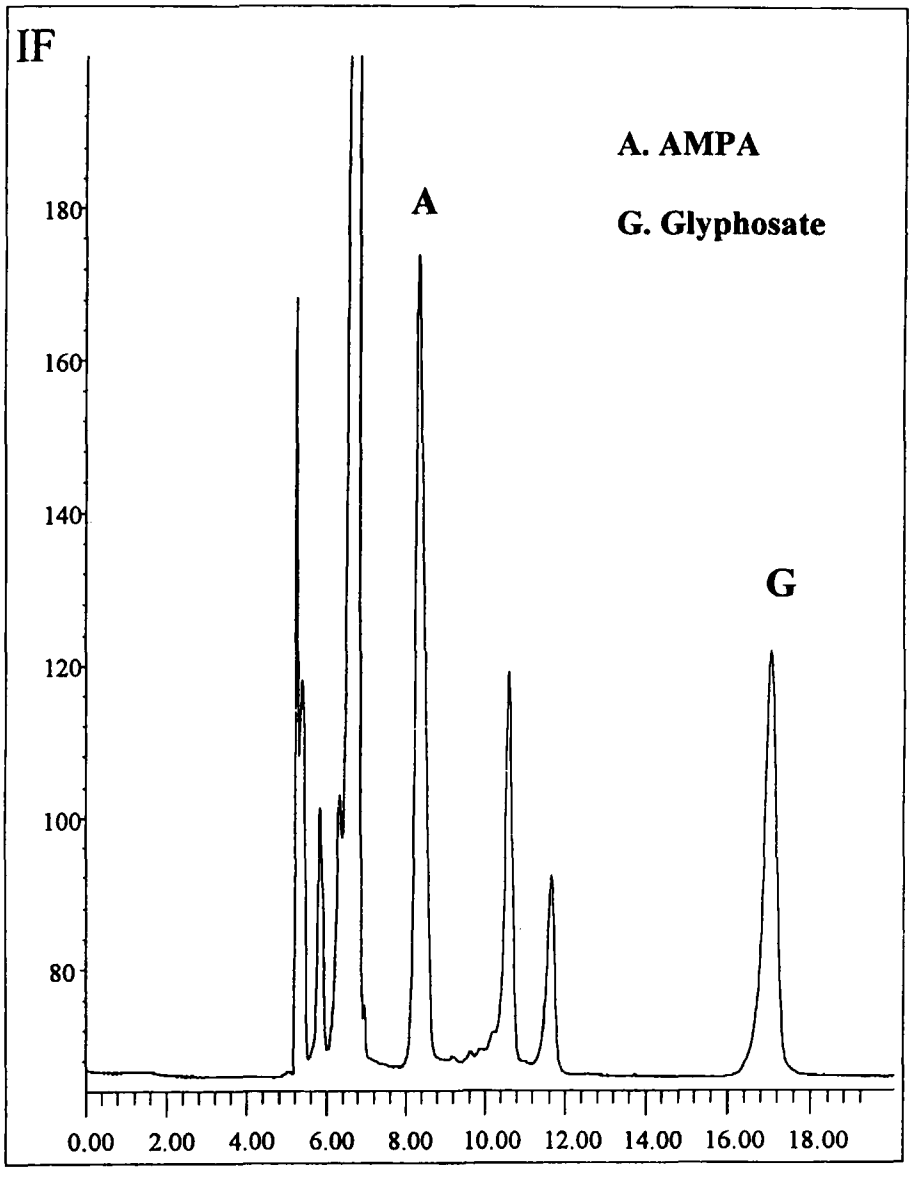

Figure 2 LC-LC chromatogram from an eight times diluted extract obtained from a soil (M3) fortified at 400 ng·g^{-1} with glyphosate and AMPA (cleanup time 2.12 min, transfer time 0.41 min).

For a soil sample characterized by a high organic matter and clay content, as TF2, fortified at 400 ng·g^{-1} the LC-LC procedure produces a satisfactory clean-up allowing the analysis of both glyphosate and AMPA, of which the later compound could not be analyzed by the LC method. As regards this type of soil samples the limits of determination of LC-LC method are at least 40 ng·g^{-1} and 100 ng·g^{-1} for glyphosate and AMPA, respectively.

Figure 3 shows chromatograms for a sandy type soil sample (M2) fortified at 40 $ng \cdot g^{-1}$ obtained by LC and LC-LC methods. The estimated determination limits for glyphosate

Table 3 Recoveries and relative standard deviations for different soils fortified with glyphosate and AMPA at several levels analyzed by LC-LC (n = 5)

	400 $ng \cdot g^{-1}$		*40 $ng \cdot g^{-1}$*	
	Glyphosate	*AMPA*	*Glyphosate*	*AMPA*
	R (RSD)	*R (RSD)*	*R (RSD)*	*R (RSD)*
TF1	101 (5.3)	73 (9.8)	93 (14)	i
TF2	99 (0.5)	95 (8.0)	97 (12)	i
M1	93 (14)	79 (12)	101 (13)	i
M2	88 (7.9)	101 (11)	96 (14)	97 (16)
M3	99 (7.5)	88 (16)	109 (7)	103 (10)

i: interferences

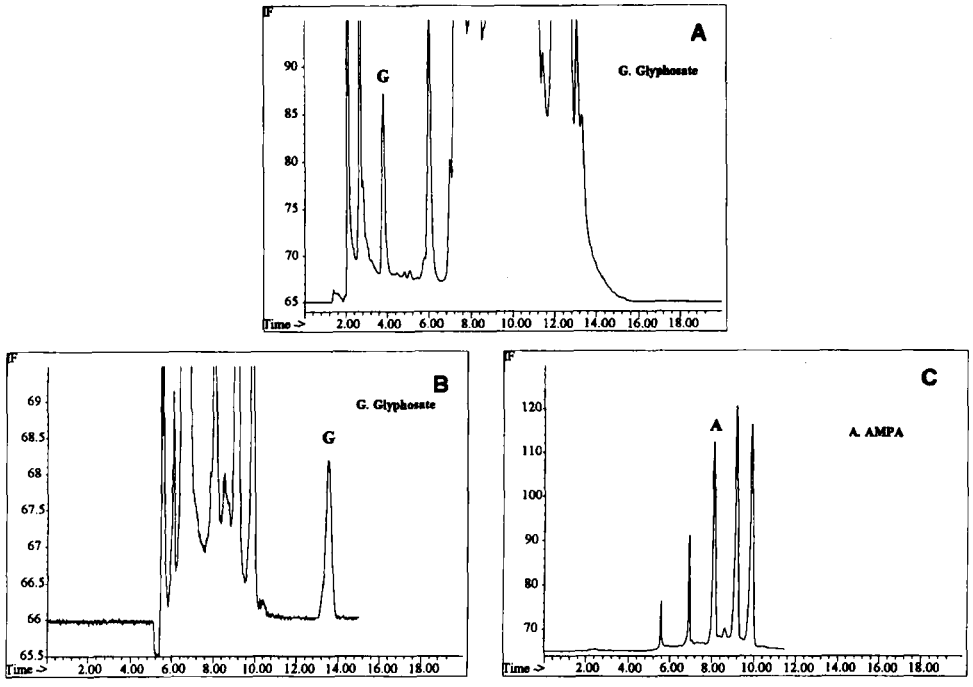

Figure 3 LC and LC-LC chromatograms from a soil extract obtained from a soil (M2) fortified at 40 $ng \cdot g^{-1}$ with glyphosate and AMPA.

(A) LC chromatogram

(B) LC-LC chromatogram from an eight times diluted extract (cleanup time 2.12 min, transfer time 0.41 min)

(C) LC-LC chromatogram from a four times diluted extract (cleanup time 2.30 min, transfer time 0.19 min)

and AMPA in these type of soils were found to be at least 10 $ng \cdot g^{-1}$. Figure 3 also indicates that the LC-method can be applied for the determination of glyphosate at this low level.

The LC-LC procedure was applied to analyse the soil samples of the monitoring. The concentrations found in soil samples taken at different depths reached maximum glyphosate values of 185 $ng \cdot g^{-1}$ (soils from Barcelona) and 250 $ng \cdot g^{-1}$ (soils from Canary Islands). These maxima were found in superficial soils samples. For AMPA, maximum concentrations of 250 $ng \cdot g^{-1}$ were found in superficial soil from Barcelona. Moreover, glyphosate was detected at depths up to 150 cm (Barcelona) or 30 cm (Canary Islands). The concentration profiles in depth for these compounds were obtained in this project.

CONCLUSION

The developed LC-LC method offers a simple, sensitive and selective determination of glyphosate and AMPA using precolumn fluorogenic labelling with FMOC at residue levels in soils of very different kinds. Concerning soil organic matter and clay content the procedure can reach determination limits of at least 10 $ng \cdot g^{-1}$ for both analytes. For soil samples with a high organic matter and clay contents determination limits are in the range of 40 $ng \cdot g^{-1}$ for glyphosate and 100 $ng \cdot g^{-1}$ for AMPA.

In comparison with the LC-LC method, the simple LC-method only allows the determination of glyphosate after FMOC precolumn derivatisation and a simple liquid extraction in both kind of soil samples down to a level of 10 $ng \cdot g^{-1}$ in sandy samples and 100 $ng \cdot g^{-1}$ in clay and organogenic samples.

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References

1. H. A. Moye, C. J. Miles and S. J. Scherer, *J. Agric. Food Chem.*, **31**, 69–72 (1983).
2. T. E. Archer and J. D. Stokes, *J. Agric, Food Chem.*, **32**, 586–588 (1984).
3. HMSQ, Her Majesty's Stationery Office, Methods for the Examination of Waters and Associated Materials, *The determination of glyphosate in rive water*, pp. 37–42 (1985).
4. J. E. Cowell, J. L. Kunstman, P. J. Nord, J. R. Steinmetz and G. R. Wilson, *J. Agric. Food Chem.*, **34**, 955–960 (1986).
5. L. G. M. Th. Tuinstra and P. G. M. Kienhuis, *Chromatographia*, **24**, 696–700 (1987).
6. T. W. Winfield, W. J. Bashe and T. V. Baker, U. S. Environmental Protection Agengy, Method 547, 59–75 (1990).
7. Y. Y. Wigfield and M. Lanouette, *Analytica Chimica Acta*, **233**, 311–314 (1990).
8. Y. Y. Wigfield and M. Lanouette, *J. Assoc. Off. Anal. Chem.*, **74**, 1317–1322 (1991).
9. M. E. Oppenhuizen and J. E. Cowell, *J. Assoc. Off. Anal. Chem.*, **74**, 317–323 (1991).
10. K. P. Spann and P. A. Hargreaves, *Pestic. Sci.*, **40**, 41–48 (1994).
11. D. G. Thomspon, J. E. Cowell, R. J. Daniels, B. Staznik and L. M. McDonald, *J. Assoc. Off. Anal. Chem.*, **72**, 355–360 (1989).
12. R. L. Glass, *J. Agric, Food Chem.*, **31**, 280–282 (1983).
13. C. J. Miles, L. R. Wallace and H. A. Moye, *J. Assoc. Off. Anal. Chem.*, **69**, 458–461 (1986).

14. C. J. Miles and H. A. Moye, *J. Agric. Food Chem.*, **36**, 486–491 (1988).
15. P. Sprankle, W. F. Meggit and D. Penner, *Weed Sci.*, **23**, 224–228 (1975).
16. R. J. Hance, *Plant Soil*, **45**, 291–293 (1976).
17. J. S. McConnell and L. R. Hossner, *J. Agric. Food Chem.*, **33**, 1075–1078 (1985).
18. R. L. Glass, *J. Agric. Food Chem.*, **35**, 497–500 (1987).
19. L. N. Lundgren, *J. Agric. Food Chem.*, **34**, 535–538 (1986).
20. J. V. Sancho, *Determination of polar herbicides residues by high performance liquid chromatography. Application of coupled-column techniques,* Doctoral Thesis (1994).
21. J. V. Sancho, F. J. López, F. Hernández, E. A. Hogendoorn and P. van Zoonen, *J. Chromatogr.*, **678**, 59–67 (1994).
22. A. J. Aubin and A. E. Smith, *J. Agric. Food Chem.*, **40**, 1163–1165 (1992).
23. J. Beltran, F. Hernandez and R. Gerritse, *Estudios de adsorción de herbicidas en diferentes suelos de Western Australia,* XXIII Reunión Internacional del Grupo de Cromatografia y Técnicas Afines, Peñiscola, Spain (1994).
24. R. Schuster and A. Gratzfeld-Husgen, Application Note No. 12–5091–3621E, Hewlett Packard, Avondale (1992).