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Analysis and degradation study of glyphosate and of aminomethylphosphonic acid in natural waters by means of polymeric and ion-exchange solid-phase extraction columns followed by ion chromatography–post-column derivatization with fluorescence detection

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Abstract

The quantitative trace determination of glyphosate and its major metabolite, aminophosphonic acid (AMPA) in natural waters was achieved by means of ion-exchange chromatography. Fifty ml of natural water sample was preconcentrated by a two-step procedure: first the sample was percolated through a polymeric cartridge, LiChrolut EN, then through an anion-exchange column mechanism, and finally analyzed by ion-exchange chromatography followed by post-column reaction coupled to a fluorimetric detector. Linear calibration graphs were obtained between 5 and 200 $\mu g\ l^{-1}$. Limits of detection ranged from 2 $\mu g\ l^{-1}$ of glyphosate and 4 $\mu g\ l^{-1}$ of AMPA. A study of the degradation of glyphosate in environmental waters under characteristic conditions was carried out to figure out the main degradation pathways of this compound. Half-lives of glyphosate varied from 60 h for ground water samples exposed to sunlight to 770 h for those stored under dark conditions.

Keywords: Water analysis; Environmental analysis; Stability studies; Glyphosate; Aminomethylphosphonic acid; Pesticides

1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] is a non selective, post-emergence herbicide applied to control the growth of long grasses and broad-leafed weeds. Glyphosate is in the first top pesticides used in the world. The widespread usage of this compound is due to its low mammalian toxicity, even though some researchers have recently described the presence of secondary effects in animals such as reproductive disfunctions [1–3].

Several efforts have been made in order to analyze glyphosate in environmental samples. Gas chromatographic (GC) determinations need a previous derivatization step whereas liquid chromatographic (LC) procedures involve pre- or post-column derivatization owing to the lack of chromophore of glyphosate and its metabolite, aminomethylphosphonic acid (AMPA). Pre-column procedures have focused on derivatization with 9-fluorenylmethylchloroformiate (FMOC-Cl) [4,5] and fluorescence detection. Post-column derivatization with *o*-phthalaldehyde (OPA) after separation in a cation-exchange column was first proposed by Moye et al. [6], and modifications of this method were reported [7–10]. The method proposed by the US Environ-

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mental Protection Agency (EPA) for the determination of glyphosate in water is focused on the above mentioned approach, water samples are filtered and directly injected (200-μl loop volume) into a cation-exchange column, the analytes are separated isocratically, and after elution from the analytical column at 65°C, coupled with *o*-phthalaldehyde–2-mercaptoethanol complex to give a fluorophor, which is detected by a fluorometer with excitation at 340 nm and detection of emission measured at >455 nm [8]. The method detection limits of glyphosate reported by the EPA were 6 $\mu g\ l^{-1}$ for drinking water and 9 $\mu g\ l^{-1}$ for ground water. The major disadvantage of this method is the lack of an extraction procedure, because the direct injection of samples with high amount of salts can damage the cation-exchange column. It is therefore necessary, when large amounts of natural water samples need to be determined, to set-up a preconcentration step. Glyphosate can also be determined by LC with indirect post-column detection using Al^{3+}-morin [11], with a limit of detection (LOD) between 14 and 40 ng for glyphosate and AMPA, respectively.

An important drawback in the preconcentration of such compounds from water is their high polarity, which does not allow extraction with organic solvents, making the preconcentration step quite lengthy. Due to the ionic form of glyphosate, anionic and cationic resins have been used for its preconcentration or clean-up step.

An important aspect to be considered is the binding of glyphosate to organic matter. Earlier reports [4,12] showed that humic substances adsorb glyphosate more strongly than clay minerals. Adsorption of glyphosate to humic substances can be explained by the hydrogen bonding interactions that may occur between the hydrogen acidic and the oxygen groups of both molecules.

In the present paper an analytical method for the trace determination of glyphosate and AMPA in natural waters is proposed by means of ion-exchange chromatography, post-column derivatization with OPA and fluorimetric detection. The method includes a clean-up step, the water sample was percolated through a polymeric sorbent cartridge, and afterwards 50 ml were dispensed through a strong anion-exchange column in the hydroxide form and were eluted with a citrate solution using an automated sample preparation with extraction columns system (ASPEC). The sample was separated in a cation-exchange column and derivatization was carried out in a post-column reactor which provides an OPA solution. In addition a stability study of glyphosate and AMPA in natural water was performed under different storage conditions and the influence of temperature, pH and organic matter on the degradation rate was investigated. To our knowledge, the analytical approach used here, involving two solid-phase steps, and the degradation study performed has not been described yet in the literature.

The aims of this work are as follows, (a) to develop an analytical method to determine glyphosate and AMPA, using a preconcentration procedure that allows the isolation of these compounds from natural complex matrices at ppb levels, and (b) to study the degradation behavior of glyphosate under different environmental conditions.

2. Experimental

2.1. Chemicals

Analytical-grade glyphosate and aminophosphonic acid were obtained from Aldrich (Milwaukee, WI, USA). The LC-grade water was purchased from Merck (Darmstadt, Germany) and was passed through a 0.45-μm filter before use. Potassium dihydrogenphosphate, sodium hydroxide, sodium chloride, potassium hydroxide, boric acid, hydrochloric acid and phosphoric acid were obtained from Merck. Sodium hypochlorite (5%) was obtained from Panreac (Barcelona, Spain). Anionic resins, Amberlite IRA-410, were obtained from Carlo Erba (Milan, Italy). Rhône estuary humic materials were provided by Dr. P. Scribe (Université Pierre et Marie Curie, Paris, France).

The mobile phase was 0.005 *M* KH_2PO_4, obtained by dissolving 0.68 g KH_2PO_4 in 1 l of LC-grade water and adjusting to pH 2 with phosphoric acid.

Oxidative solution was obtained as follows: dissolve 1.36 g KH_2PO_4, 11.6 g NaCl and 0.4 g NaOH in 1 l LC-grade water. Add 50 μl of 5% NaClO to the above mentioned solution.

OPA solution was obtained as follows: dissolve 30 g H_3BO_3 and 25 g KOH in 1-l LC-grade water.

Degas the solution and add 100 mg OPA previously dissolved in 10 ml of methanol. Afterwards add 2 g of thiofluor directly into the reservoir.

2.2. Chromatographic conditions

The eluent was delivered by a Model 250 binary high-pressure pump from Perkin-Elmer (Norwalk, CT, USA) coupled to a PCX 5000 post-column derivatization analysis module from Pickering Labs. (Mountain View, CA, USA). A Model LC-240 fluorescence detector from Perkin-Elmer (Buckinghamshire, UK) was used at excitation and emission wavelengths of 330 nm and 465 nm, respectively. Samples were injected via a Rheodyne 20-μl loop (Cotati, CA, USA). A cation-exchange column 150× 4 mm, K^+ form, was purchased from Pickering Labs. Column temperature was set to 55°C. The separation was performed isocratically, from 0 to 15 min, 100% 0.005 *M* KH_2PO_4, from 15 to 17 min, 100% KOH, and back to the initial conditions. Column flow-rate was set at 0.4 ml min^{-1} and both post-column reagent flows were set at 0.3 ml min^{-1}.

2.3. Sample preconcentration

Ground water samples were filtered and spiked at 20 ppb of glyphosate and aminophosphonic acid. Samples were extracted with the ASPEC XL system.

The ASPEC XL system (Gilson, Villiers-le-Bel, France) is fitted with an external Model 306 LC pump for dispensing samples through the cartridges and is connected with a Model 817 switching valve for the selection of samples. Disposable 3-ml cartridges packed with 200 mg LiChrolut EN (Merck) were used for the first step of the preconcentration procedure. The solid-phase extraction (SPE) cartridges were conditioned with 10 ml MeOH followed by 5 ml water. The sample was percolated through the cartridge with a flow-rate of 10 ml min^{-1} and collected into a reservoir. Strong anion-exchange columns were packed with 4.5 g of Amberlite IRA 410, in the hydroxide form, which is the counter-ion with lower selectivity. The resin was first conditioned with 5 ml water, and subsequently 50 ml of the water sample were percolated through the cartridge at 2 ml min^{-1}, and finally eluted with 10 ml of 0.4 *M* sodium citrate solution. The anion-exchange columns were regenerated after each analysis percolating through the sorbent 40 bed volumes of 1 *M* NaOH, and subsequently washed with 100 ml water.

2.4. Degradation studies of glyphosate

A degradation study of glyphosate was performed spiking (20 ppb) water samples from different sources at different pH and storing them under sunlight or dark conditions. Two types of water samples were studied: ground water from Barcelona (pH 7, 75 mg l^{-1} nitrate, 387 mg l^{-1} sulfate, 254 mg l^{-1} Ca, 88 mg l^{-1} Mg, conductivity 2020 μS cm^{-1}) and Ebro river water. The effect of temperature, sunlight exposition, pH, presence of humic substances and microorganisms were studied with a set of seven samples, whose main characteristics and storage conditions are shown in Table 1. In order to observe possible adsorption of glyphosate onto the particulate matter, samples were not filtered until they were analyzed. The study was carried out in Barcelona during 21 days (July 1997).

Fifty-ml water samples were periodically taken, extracted and injected into the chromatographic system.

Table 1
Main characteristics of the water samples used for the degradation study of glyphosate and their storage conditions

Sample	pH	Source	Storage conditions
1	7	Ground water	Sunlight
2	3	Ground water	Sunlight
3	7	Ground water	Dark, 4°C
4	3	Ground water	Dark, 4°C
5	7	Ground water spiked with 4 mg l^{-1} of humic substances	Sunlight
6	7	River water from Ebro Delta	Sunlight
7	3	River water from Ebro Delta	Sunlight

3. Results and discussion

3.1. Oxidation temperature optimization

A temperature optimization of the oxidation reactor was performed and compared with the published data [7,8,10]. The optimized temperatures reported in the literature range from 36 to 48°C, therefore we decided to carry out an optimization between 30 and 50°C. Table 2 shows the variation of the fluorimetric response with the temperature, leading to the conclusion that a temperature of 36°C exhibits higher response, which is close to the results of the EPA (38°C) and Pickering (36°C) methods. Higher temperatures reduce the activity of the oxidation reagent, due to the decrease of chlorine production [10].

3.2. Preconcentration procedure

In a first step, the use of C_{18} bonded silica sorbent led to recoveries ranging from 25% for glyphosate to 12% for AMPA, thus indicating the low capacity of this type of sorbent to retain organic compounds of medium and high polarity. Nevertheless, the use of a polymeric sorbent improved the percentage of extraction. Two types of crosslinked polymeric sorbents, Isolute ENV+ and LiChrolut EN, were tested and the results showed that the use of a LiChrolut EN polymeric cartridge allowed the extraction of glyphosate and AMPA with percentages of 90 to 83% and relative standard deviations (R.S.D.s) of 10 to 12% ($n=8$), respectively.

In this study a polymer-based ion exchanger was used in the second step of the extraction procedure, which has the advantage of being utilized over a larger pH range [1–13] than the silica-based ion-exchange sorbents. The kinetics of ion-exchange are somewhat lower than the kinetics of other type of interactions and so 2 ml min^{-1} flow-rate was used. Values above this limit could not allow the appropriate ion transfer mechanism, leading to poor recoveries of the compounds. 0.4 *M* sodium citrate buffer solution gave satisfactory results for both compounds owing to the high selectivity of this counter-ion with an elution volume of 10 ml.

The selection of a two-step extraction is recommended for ionic or ionizable solutes, when the use of an ion-exchange sorbent cannot overcome the presence of organic and inorganic interferences. The removal of organic compounds, which can have additional interactions with the matrix of the ion-exchange sorbent, such as hydrophobic interactions, is performed by means of a non-polar sorbent. Several approaches for the extraction of highly polar compounds based on mixed mechanisms are described in the literature [13]. In the present work percolation through a polymeric sorbent, LiChrolut EN, followed by anion-exchange extraction was proposed to isolate glyphosate and aminophosphonic acid.

3.3. pH optimization

Acid dissociation constants of glyphosate were previously reported [14], the values found in the literature are pK_1 0.8, pK_2 2.2, pK_3 5.4 and pK_4 10.2. It is noticeable that the zwitterionic structrure of glyphosate from pH 1 to 10, enhances the ability of this compound to be extracted by an ion-exchange mechanism. The preconcentration of glyphosate was studied over the pH range 6 to 10, and the subsequent variation of the fluorimetric response can be observed from Table 3. It should be taken into account that there is no dependence of the fluorimet-

Table 2
Relative fluorescence values corresponding to glyphosate and aminophophonic acid at different oxidation reaction temperatures (°C)

Temperature (°C)	Relative fluorescence	
	Glyphosate	AMPA
30	13.3	73.4
34	11.2	13.2
36	38.9	99.0
40	28.1	57.3
45	23.8	51.6

Table 3
Relative fluorimetric response values of glyphosate at different extraction pH values

pH	Relative fluorescence response (%)	R.S.D. (%)
6	97.1	6.9
7	80.2	4.6
8	62.9	0.8
9	94.6	3.9
10	36.9	0.2

Table 4
Calibration data obtained with off-line preconcentration of 50 ml of water and LC–post-column derivatization–fluorimetric detection of spiked ground water samples (n=5), between the linear range from 5 to 200 ppb

Compound	Calibration equation	r^2
Glyphosate	y=12 288x+46 446	0.995
AMPA	y=9514x+76 974	0.996

ric response with pH between pH 6 and 9, whilst at pH 10 the peak area begin to decrease due to the proximity of the pK_4 value.

3.4. Quantification

Calibration was performed spiking ground water samples at pH 7 over the concentration range of 5 μg l^{-1} to 500 μg l^{-1}. The relationship between the fluorimetric response and the concentration was found to be linear for both compounds over the range of 5 μg l^{-1} to 200 μg l^{-1}. The linear regression equations and correlation coefficients are shown in Table 4.

The LODs at a signal-to-noise ratio of 3 are 2 μg l^{-1} and 4 μg l^{-1} for glyphosate and AMPA, respectively, after the preconcentration procedure and analysis by LC. The difference observed between the LODs of the two compounds might be due to the lowest extraction efficiency of AMPA in comparison to glyphosate. The obtained values are quite reasonable since the US EPA has fixed the Health Advisory Level (HAL) for each individual pesticide, and in the case of glyphosate the HAL is 700 μg l^{-1}.

Industrial effluents and environmental waters were analyzed and quantified by the proposed method. For instance, Fig. 1 shows a natural sample from an European lake, where glyphosate and AMPA were present at low-ppb levels. This indicates the capa-

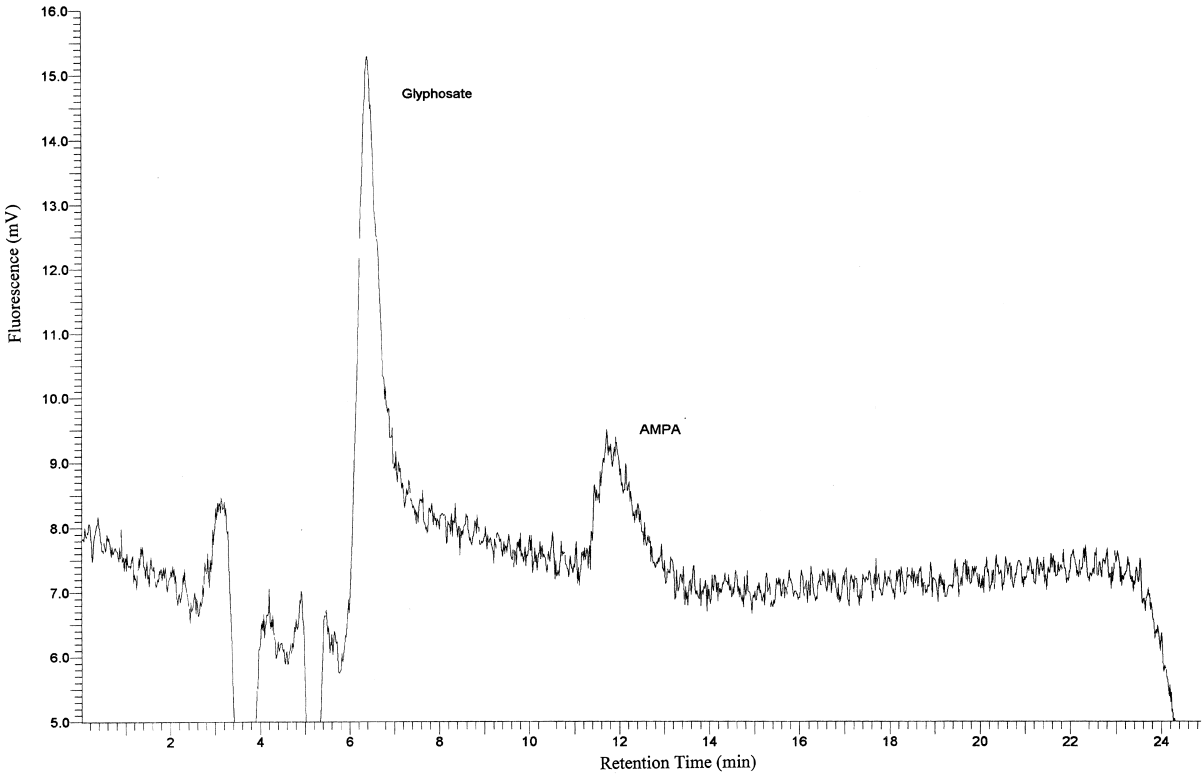

Fig. 1. Chromatogram corresponding to the extraction of 50 ml of a lake water sample and analysis by ion-exchange chromatography with post-column reaction coupled to a fluorimetric detector. The sample contains glyphosate at 15 ppb and AMPA at 6 ppb. For extraction and chromatographic conditions see Section 2.

bility of the method to isolate glyphosate and AMPA from complex matrices such as industrial waste waters. Furthermore, this method employs ion-exchange sorbents that can be regenerated by replacing the counter-ion of the resin, indefinitely. The inclusion of this step can also enlarge the lifetime of the column, which can be seriously damaged by the presence of metal ions and organic compounds. This constitutes a clear advantage to guarantee the performance of the chromatographic column and ensure the capability of the method.

3.5. Precision and accuracy

Repeatability and reproducibility were tested by extraction of three replicated ground water samples spiked with 20 $\mu g\ l^{-1}$ of glyphosate and AMPA during three consecutive days.

The repeatability ranged from 5 to 18% (R.S.D.), indicating a slight lack of accuracy probably due to the sample pretreatment. Although the extraction step was automated using an automatic SPE device, the inclusion of two preconcentration steps involves an increase of the measurement dispersion.

The R.S.D. values corresponding to the reproducibility of the method varied between 13 and 18%. It should be pointed out that the fluorimetric response reproducibility strongly depends on the stability of the post-column system, which is in turn function of the degree of cleanliness and the pressure stability of the post-column reagent pumps.

3.6. Degradation of glyphosate

Earlier studies concerning the degradation of glyphosate reported the importance of the microorganism activity in the metabolism of this compound [15], chemical degradation and photodecomposition seemed to be minor routes for glyphosate breakdown. Glyphosate is completely degraded to CO_2 by microorganisms in water [15].

The experiments performed in this study enabled one to ascertain the influence of temperature, sunlight exposition, pH, presence of humic substances and microorganisms and the matrix effect on the degradation of glyphosate. Table 5 shows the calculated first-order rate constants for the degradation of glyphosate under the above mentioned conditions. Regarding pH effect it can be clearly seen that acid conditions favors the stability of glyphosate as a result of a diminution of the microbial activity. Another important factor in the degradation process is the temperature, as it was expected an increase on the temperature enhances the breakdown of glyphosate owing to the mainly microbial degradation of this compound. Although it seems that the photolysis of glyphosate tends to be negligible, some authors [15] reported its occurrence in a significant extent in water. In this case the degradation of glyphosate in environmental waters could be explained by the sum of the microbial activity, the temperature and in less extension by photolytic processes. We should also take into account that experiments were performed with closed glass bottles, therefore losses due to evaporation were minimized. Although water samples from Ebro river seemed to have higher microbial activity and humic content, half-lives obtained were higher than the ones exhibited by the corresponding ground water sample. The differences can be explained in terms of the presence of an indirect photolysis mechanism. For those substances that do not absorb sunlight, like glyphosate, indirect photo-

Table 5
Half-lives, $t_{1/2}$, constant rates, k, and correlation constants, r^2, of glyphosate in water under different storage conditions ($n=7$)

Water type	k (h^{-1})	$t_{1/2}$ (h)	r^2
Ground water, pH 3 exposed to sunlight	$3\cdot10^{-3}$	230	0.93
Ground water, pH 7 exposed to sunlight	$1\cdot10^{-2}$	60	0.97
Ground water, pH 3, dark conditions (4°C)	$9\cdot10^{-4}$	730	0.94
Ground water, pH 7, dark conditions (4°C)	$9\cdot10^{-4}$	770	0.97
Ground water, pH 7, 4 mg/l HS, exposed to sunlight	n.c.	n.c.	n.c.
River water, pH 3 exposed to sunlight	$2\cdot10^{-3}$	345	0.98
River water, pH 7 exposed to sunlight	$7\cdot10^{-3}$	100	0.99

n.c.=Not calculated (low data fitting).

lysis through a photosensitizer is the only possible photochemical transformation mechanism. Taking into account the high content of salts of the ground water sample used (see Section 2), it is possible to consider the presence of a photoinduced mechanism through a sensitizer like the nitrate group. The oxidation of compounds in water in presence of nitrates was previously reported by Zepp et al. [16]. Fig. 2 shows the chromatograms from an extraction of glyphosate in two different natural water matrices. The effect of the interferences due to the water matrix can be clearly seen from the chromatograms, ground water sample (Fig. 2B) presents more interferences than river water sample (Fig. 2A), due to the high amounts of salt present.

The presence of humic substances enhances the rate of decomposition of glyphosate, after 90 h no signal due to this compound was obtained. The first-order degradation constant for the sample containing humic substances was not reported due to the lack of regression fitting. As mentioned above, the presence of indirect photolysis is an important factor

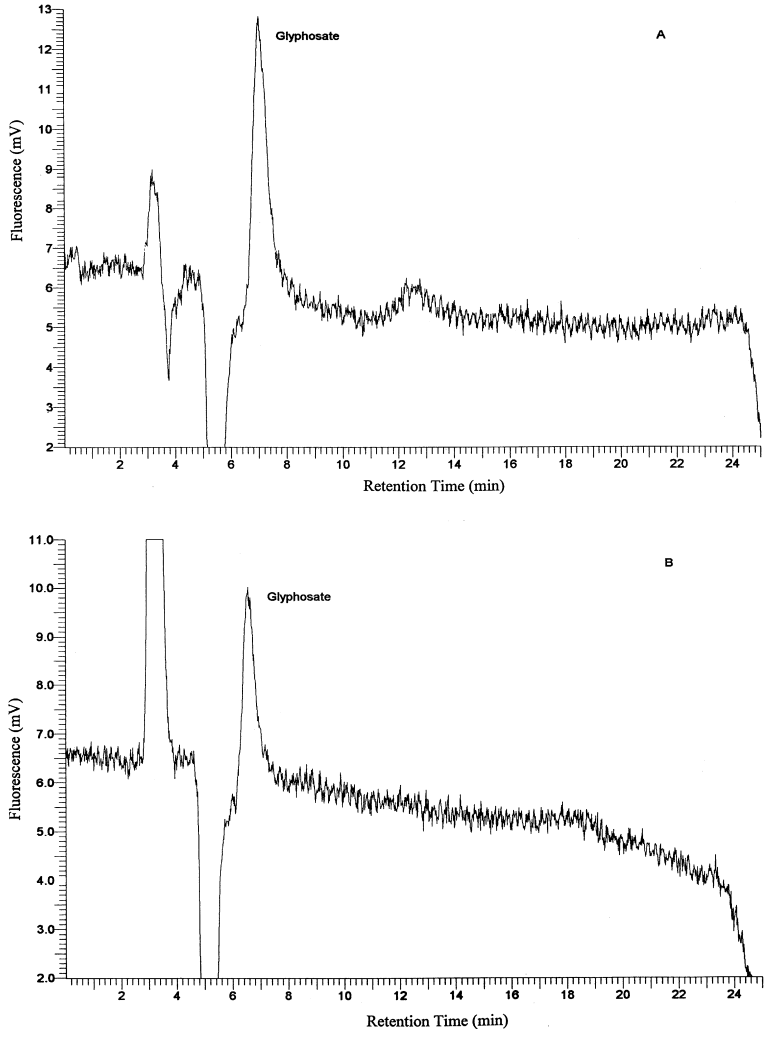

Fig. 2. Chromatograms corresponding to the degradation study of glyphosate in (A) river water and (B) ground water, at time 0 h. Fifty-ml water samples spiked at 20 ppb were extracted and analyzed by ion-exchange chromatography with post-column reaction coupled to a fluorimetric detector. For extraction and chromatographic conditions see Section 2.

to consider, since humic substances were reported to act as photosensitizers and in this case can explain the fast degradation of glyphosate.

The main metabolite of glyphosate, aminophosphonic acid, was detected in small amounts after 300 h in the acidic samples, whilst at pH 7 no evidence of this metabolite was noticed, owing to a complete decomposition of glyphosate.

4. Conclusions

A preconcentration method was described to extract the compounds under study with recoveries ranging from 83 to 90%. Even though the preconcentration process involves two steps the use of an automatic SPE device for off-line extractions is appropriate to reduce sample manipulation. An optimization of the preconcentration step was accomplished obtaining higher recoveries using a combination of LiChrolut EN and anion-exchange resin, working at sample pH values between 6 and 9, and using 10 ml as an elution volume.

The separation and derivatization of the compounds was achieved by means of ion-exchange chromatography and post-column derivatization. The method showed a linear calibration range between 5 and 200 ppb, and limits of detection over 2 and 4 ppb for glyphosate and AMPA, respectively. The results obtained showed an improvement over the reported US EPA method, since a two-step extraction procedure, coupled to a cation-exchange separation followed by post-column reaction and fluorimetric detection, provides a method capable to separate and detect glyphosate and AMPA at ppb level in complex water matrices. The inclusion of an ion-exchange preconcentration step could enlarge the life time of the column, since 800 samples were analyzed without any considerable loss of column efficiency.

The main factors affecting the degradation of glyphosate can be summarized as a combination of microbial activity, temperature and, to a lesser extent, photolysis. The degradation of glyphosate is relatively fast under natural conditions, for ground water and river water samples half-lives were 60 h and 100 h, respectively. The complexity of the water matrix is an important factor to take into account since it can change the degradation rate of glyphosate.

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