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Automated trace level determination of glyphosate and aminomethyl phosphonic acid in water by on-line anion-exchange solid-phase extraction followed by cation-exchange liquid chromatography and post-column derivatization

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Abstract

An automated method based on the on-line coupling of anion-exchange solid-phase extraction (SPE) and cation-exchange liquid chromatography followed by post-column derivatization and fluorescence detection has been developed for the trace level determination of glyphosate and its primary conversion product aminomethyl phosphonic acid (AMPA) in water. PRP-X100 poly(styrene–divinylbenzene)-trimethylammonium anion-exchange cartridges (20×2 mm, 10 μm) were selected for the SPE of glyphosate and AMPA. The ionic compounds present in the samples strongly influenced the extraction of both analytes; however, when an on-line ion-exchange clean-up step was introduced before sample SPE, the problem was largely solved. By processing 100-ml samples detection limits better than 0.02 μg/l for glyphosate and 0.1 μg/l for AMPA were achieved in river water. Both analytes were unstable in solution and the approach of storing samples on the PRP-X100 SPE cartridges was evaluated for a period of 1 month under three different storage conditions (deep freeze, refrigeration and 20°C). © 2001 Published by Elsevier Science B.V.

Keywords: Water analysis; Glyphosate; Aminomethylphosphonic acid; Pesticides

1. Introduction

Glyphosate [*N*-(phosphonomethyl) glycine] is a nonselective postemergence herbicide used for the control of a wide range of weeds [1]. Because of its wide application range, its low mammalian toxicity and its use in glyphosate-resistant genetically modified plants, it has become one of the most widely used herbicides. It is also used for vegetation control in non-crop areas and for weed control in aquatic systems. Glyphosate is retained strongly by various soil components [1,2] and is metabolized by soil microorganisms [1,3–5]; chemical degradation is negligible and photodegradation occurs to a small extent [1]. Aminomethyl phosphonic acid (AMPA) is the major conversion product of glyphosate in soil systems [1]. Despite its strong retention on soil components, traces of glyphosate have been detected in ground water near treated areas [6,7].

Both gas (GC) and liquid chromatography (LC) have been employed for the analysis of glyphosate and AMPA in environmental water. GC analysis is

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performed after a derivatization procedure that converts glyphosate and AMPA to sufficiently volatile derivatives [8–11]. In LC methods both pre-column and post-column derivatization procedures producing fluorescent derivatives have been employed. Pre-column procedures are based mainly on derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) [12–15], while in post-column procedures the most commonly used derivatization reaction is with *o*-phthalaldehyde and mercaptoethanol after oxidation of glyphosate to glycine [16–19].

All analytical methods developed so far involve off-line preconcentration and/or derivatization of glyphosate and AMPA and subsequent chromatographic analysis. In recent years, however, automation of micropollutant monitoring devices for on-line in-situ quality control of aquatic systems is gaining preference and the purpose of this work was to develop such a fully automated method for the trace level determination of glyphosate and AMPA in water. The method was based on the on-line coupling of anion-exchange solid-phase extraction (SPE) and cation-exchange LC analysis. Analytes were detected by fluorescence detection after post-column oxidation of glyphosate to glycine and derivatization of both analytes with *o*-phthalaldehyde and *N*,*N*-dimethyl-2-mercaptoethylamine hydrochloride. Emphasis was given on the evaluation of a sample storage protocol based on analyte stabilization onto SPE cartridges.

2. Experimental

2.1. Reagents and solvents

Glyphosate (99.6%) was donated by Zeneca (Zeneca Ellas, Athens, Greece) and AMPA (99%) was purchased from Sigma (Deisenhofen, Germany).

Potassium dihydrogenphosphate, potassium hydroxide, sodium chloride, sodium hydroxide, boric acid and concentrated sodium hypochlorite solution (12.5% available chlorine) were of reagent grade and purchased from Riedel-de Haën (Seelze, Germany). Orthophosphoric acid was purchased from Carlo Erba (Milan, Italy). Thiofluor (*N*,*N*-dimethyl-2-mercaptoethylamine hydrochloride) and *o*-phthalaldehyde were of chromatographic grade and purchased from Pickering Labs. (Mountain View, CA, USA).

Water used in LC eluent was laboratory-distilled and filtered through a 0.2-μm membrane filter (Gelman Science, Ann Arbor, MI, USA).

2.2. SPE materials

Silica-based trimethylaminopropyl and diethylaminopropyl anion-exchange SPE cartridges (10×3 mm, 40–70 μm) were purchased from Spark Holland (Emmen, The Netherlands). PRP-X100 poly-(styrene–divinylbenzene) based trimethylammonium anion-exchange SPE cartridges (20×2 mm, 10 μm) were purchased from Hamilton (Reno, NV, USA). A special holder was used for mounting these cartridges onto the PROSPEKT system.

A 60:40 (w/w) mixture of the A-510 (polystyrene – divinylbenzene) - dimethylethylammonium anion-exchange (14–52 mesh) and the C-100H (polystyrene–divinylbenzene)-sulfonate cation-exchange (16–45 mesh) resins (Purolite, Wales, USA) was donated by Bacakos (Thessaloniki, Greece). Empty 6-ml polypropylene columns were purchased from Merck (Darmstadt, Germany) and were dry-packed manually with the resin mixture.

2.3. Instrumentation

The solid-phase extraction of the aqueous samples was performed on-line using the automated PROSPEKT system (Spark Holland). The LC analysis was performed using the Marathon IV HPLC pump (Rigas Labs, Thessaloniki, Greece). For the injection of standard solutions the Basic Marathon autosampler (Spark Holland) equipped with a 20-μl injection loop was employed. The Prometheus 300 derivatization system (Rigas Labs) equipped with two reaction coils of 500 μl and 300 μl volumes, respectively, was used for analyte post-column derivatization. The derivatization reagents were introduced by two Marathon I pumps (Rigas Labs). The detector employed was the 980 Spectroflow fluorescence detector (Kratos Analytical, Ramsen, NJ, USA) equipped with 5 μl flow cell. The acquired signal was recorded by a personal computer operated under the Millenium software (Waters, Milford, MA, USA).

The analytical system was fully automated and could be operated unattended.

2.4. Analytical procedure

Surface and ground water samples were collected in polypropylene plastic bottles, because of the possible adsorption of glyphosate onto glass surfaces. Samples were filtered through a 0.2-μm membrane filter (Gelman Science) and aliquots of 100 ml were automatically processed onto PRP-X100 (20×2 mm, 10 μm) anion-exchange SPE cartridges at a flow-rate of 5 ml/min. Prior to sample loading the SPE cartridges were conditioned with 10 ml of distilled water at a flow-rate of 5 ml/min. A transparent polypropylene cartridge packed with 300 mg of the 60:40 (w/w) mixture of the A-510 and C-100H ion-exchange resins was installed in the sample transfer line of the PROSPEKT system. Before installation it was conditioned with 10 ml of distilled water. After sample loading the SPE cartridges were flushed with 2 ml of distilled water at a flow-rate of 2 ml/min and the solutes were subsequently eluted on-line onto the analytical column by the flow of the mobile phase in the forward flush mode.

Isocratic LC was performed on a PRP-X400 poly(styrene–divinylbenzene)-sulfonate cation-exchange (250×4.1 mm, 7 μm) column (Hamilton) at 36°C. The eluent was a 5 m*M* KH_2PO_4 solution in distilled water and the pH was adjusted to 1.9 with orthophosphoric acid. The eluent flow-rate was 0.5 ml/min.

The first derivatization reagent was prepared by adding 100 μl of the concentrated sodium hypochlorite solution in 1 l of a 0.1% KH_2PO_4, 0.1% NaOH and 1% NaCl buffer solution at pH 11.6. The second derivatization reagent was a 0.01% *o*-phthalaldehyde and 0.2% *N,N*-dimethyl-2-mercaptoethylamine hydrochloride solution made in a 3% boric acid and 3% KOH buffer solution at pH 10.4. The flow-rate of the first reagent was 0.25 ml/min and of the second reagent 0.3 ml/min. The first reaction coil was thermostated at 36°C, while the second was operated at ambient temperature. The excitation/emission wavelengths of the fluorescence detector were 330/465 nm and data acquisition rate was 2 measurements/s.

Analyte quantification was performed via external calibration. Stock standard solutions of glyphosate and AMPA were prepared at a concentration of 200 μg/ml in distilled water and stored at ~4°C. Mixed working solutions at concentrations of 50–0.05 μg/ml were prepared weekly in distilled water and stored also at ~4°C. Using these solutions 10-point calibration curves (1–1000 ng injected amounts) were constructed for each analyte.

3. Results and discussion

3.1. Sample SPE

3.1.1. SPE cartridge selection

Anion-exchange SPE was selected for the extraction of glyphosate and AMPA from water, since at the pH values of most environmental water samples both analytes have a net negative charge. PRP-X100 poly(styrene–divinylbenzene) based trimethylammonium (20×2 mm, 10 μm) and silica based trimethylaminopropyl and diethylaminopropyl (10×3 mm, 40–70 μm) anion-exchange SPE cartridges were tested. Data of analyte recovery vs. sample volume were acquired for each cartridge type by sequentially extracting in triplicate 2–150 ml sample volumes of distilled water spiked at the level of 5 μg/l without a clean-up cartridge installed in the sample transfer line (Fig. 1). Glyphosate was recovered quantitatively up to 150 ml, while AMPA showed breakthrough already at less than 5 ml on all cartridge types tested. Other anion-exchange resins (like AG 1-X8) have been reported to perform better for AMPA [10,11]; the reported experiments, however, were performed in the off-line mode and higher resin amounts were employed.

Narrower analyte peaks were obtained when the PRP-X100 (20×2 mm, 10 μm) cartridges were coupled on-line with LC than other cartridge types tested (data not shown). Next to cartridge characteristics (dimensions, particle size, particle chemical structure, etc.) the elution power of the eluent also affects the analyte elution profiles; however, given the satisfactory performance of the PRP-X100 cartridges, no further studies were performed and the PRP-X100 cartridges were selected for further use.

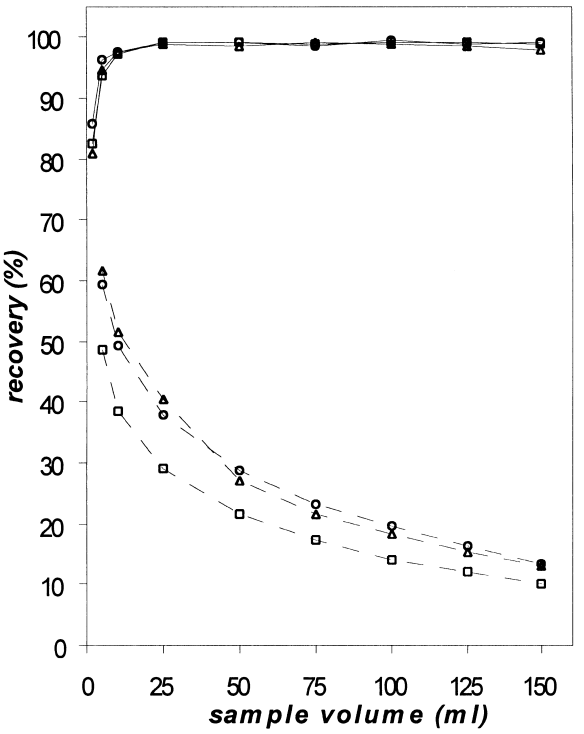

Fig. 1. Mean glyphosate (solid lines) and AMPA (dashed lines) recoveries (%, $n=3$) vs. sample volume, when distilled water spiked at the level of 5 μg/l was processed on the PRP-X100 (20×2 mm, 10 μm) (○), diethylaminopropyl (10×3 mm, 40–70 μm) (△), and trimethylaminopropyl (10×3 mm, 40–70 μm) (□) SPE cartridges. All other experimental conditions are given in the text.

3.1.2. Sample clean-up

Both analytes were very poorly recovered when analyzing real instead of distilled water samples. Recoveries were as low as 3.2% for glyphosate and 0.5% for AMPA, when 50 ml of ground water spiked at the level of 10 μg/l was processed, while the respective recoveries from distilled water were 99 and 29% (Fig. 1). Sample pH was found to have an influence on the recovery of both analytes, however no significant differences were observed for pH 4–8 (data not shown). Inorganic ions (and organic compounds as well) present in water have been reported to interfere with the retention mechanisms of ion exchangers [19]. To examine the influence of the ionic compounds present in the samples on SPE performance, a clean-up involving the use of a mixed ion exchanger was introduced before sample SPE for their removal. A 6-ml polypropylene column was packed with 300 mg of the 60:40 (w/w) mixture of the A-510 and C-100H ion exchange resins and 100 ml of ground water was percolated in triplicate through the column in the off-line mode. The clean-up column was previously conditioned with 10 ml of distilled water. The percolated samples were then spiked at the level of 10 μg/l and 50-ml portions were subsequently analyzed. The recoveries of glyphosate and AMPA were increased up to 83 and 26%, respectively, approaching thus the levels achieved when the analytes were dissolved in distilled water. To examine if glyphosate and AMPA were retained on the clean-up column, the ground water was first spiked at the level of 10 μg/l and then 100 ml aliquots were percolated in triplicate through the clean-up column, while 50-ml portions of them were finally processed. Glyphosate was completely unretained, while 14% of AMPA was retained on the clean-up column and its total recovery was reduced to 22%. To maintain the automation of the analytical method a polypropylene cartridge packed with 300 mg of the resin mixture was installed on the sample transfer line of the PROSPEKT system. This on-line clean-up configuration performed as effectively as the off-line clean-up experiments. The colour of the resin mixture was gradually turned to brown when overloaded with ionic compounds thus designating the timing for replacement. The time of replacement was highly depending on the amount of the ionic substances present in the processed water samples. Adequate SPE cartridge performance could be extended with proper clean-up cartridge replacement and more than fifty samples could be analyzed using the same SPE cartridge; longer analytical column life was also achieved.

3.1.3. Sample volume optimization

Glyphosate was recovered quantitatively and its detection limit was improved correspondingly with increasing sample volume up to 150 ml. AMPA, however, showed breakthrough already at less than 5 ml (Fig. 1) and the sample volume had to be optimized with respect to this compound. The extracted amounts of AMPA on the PRP-X100 (20×2 mm, 10 μm) cartridges, when distilled water spiked at the level of 5 μg/l was processed, are plotted

against the respective sample volumes in Fig. 2. The lower sample volume to be processed in order to achieve the best detection limit of AMPA is about 125 ml. However, in order to reduce the overall analysis time, a sample volume of 100 ml was selected. Processing of sample volumes higher than the analyte breakthrough volumes, in order to improve the detection limits, is well documented [20] especially for selective analyte detection.

3.2. LC determination

Cation-exchange LC of glyphosate and AMPA is well documented [16–19] and similar LC conditions were adopted in this work. The analytical column was thermostated at the same temperature as the first reaction coil (36°C). Under the selected conditions glyphosate and AMPA were fully separated and eluted as slightly tailed peaks at 7.2 and 9.5 min, respectively.

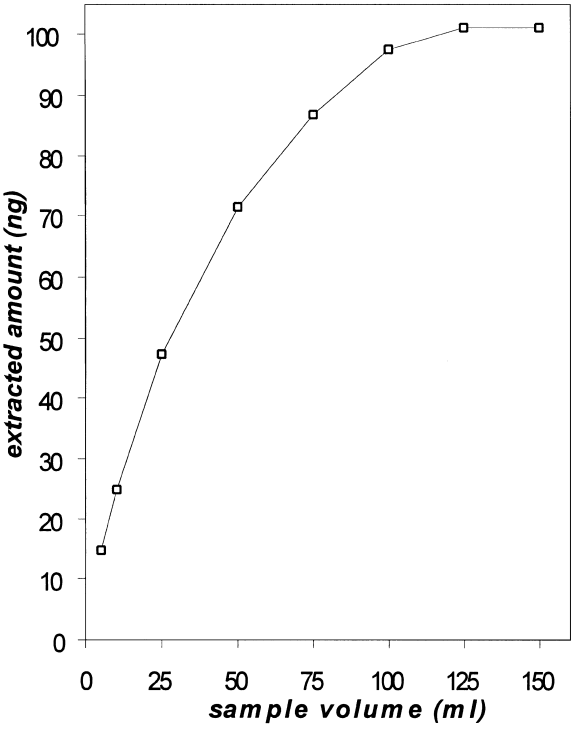

Fig. 2. Extracted amounts of AMPA (ng) on the PRP-X100 (20×2 mm, 10 μm) cartridges vs. sample volume, when distilled water spiked at the level of 5 μg/l was processed. All other experimental conditions are given in the text.

The reaction conditions for the first derivatization step (conversion of glyphosate to glycine) were slightly modified from those recommended by Pickering Labs. [21]. Reaction conditions which strongly influence the response of both analytes are the hypochlorite concentration, the reagent flow-rate and the reaction volume and temperature [18,19]. The response of glyphosate and AMPA was calculated against the hypochlorite concentration at various flow-rate settings of the oxidative reagent (Fig. 3). At low hypochlorite concentrations glyphosate exhibited reduced response due to the rate limiting effect of the oxidative reagent. Increasing hypochlorite concentration the response of glyphosate was rapidly increased reaching gradually a maximum value. AMPA was not unreactive towards hypochlorite and its response was decreased in the presence of excess of hypochlorite (Fig. 3). Similar results were reported also in the literature [18]. A dilution factor of $1/10^4$ (100 μl added in 1 l) of the concentrated sodium hypochlorite solution and a reagent flow-rate of 0.3 ml/min were adequate for glyphosate to approach its maximum response; however, a flow-rate of 0.25 ml/min was selected in order to accommodate for a better AMPA response (Fig. 3). Because concentrated sodium hypochlorite solution is unstable, reoptimizing of the sodium hypochlorite concentration and of the respective reagent flow-rate was necessary after prolonged storage or when a new batch of the solution was purchased. The coil temperature for the oxidation reaction was set at 36°C [19,21].

The reaction conditions for the second derivatization step (formation of the fluorescent derivative) and the settings of the fluorescence detector were those recommended by Pickering Labs. [21]. In the absence of a 100 μl reaction coil a 300 μl one was used instead.

Using the selected post-column derivatization conditions glyphosate and AMPA could be readily detected in calibration solutions corresponding to 1 ng injected amount for each analyte. In the official method proposed by the AOAC [16], which is based on the same derivatization reactions, the most dilute calibration solution corresponds to 5 ng injected amount.

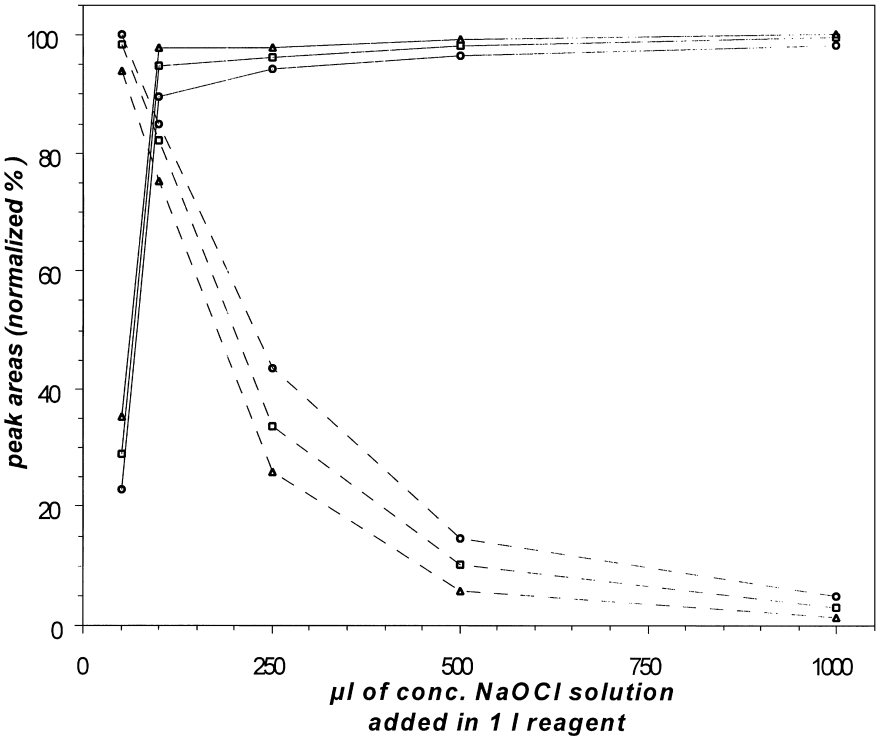

Fig. 3. Relative glyphosate (solid lines) and AMPA (dashed lines) response vs. hypochlorite concentration at oxidative reagent flow-rates of 0.2 (○), 0.25 (□) and 0.3 ml/min (△). Relative responses are calculated as analyte peak areas (when 20 μl of a 5 μg/ml calibration solution were injected) normalized % by the maximum reading observed for each analyte. All other experimental conditions are given in the text.

3.3. Validation data

The present method was used to analyze glyphosate and AMPA in surface and ground water. It was proved highly selective for the target analytes and no matrix interference peaks appeared in the chromatograms. Chromatograms from the analysis of an Axios river water sample unspiked and spiked at the level of 0.1 μg/l are shown in Fig. 4.

By processing 100 ml samples, detection limits (calculated as the lower fortification levels where analytes were detected with a signal-to-noise ratio above three) better than 0.02 μg/l for glyphosate and 0.1 μg/l for AMPA were achieved in Axios river water. From the other LC methods based on the same post-column derivatization reactions detection limits of 0.5 μg/l for both analytes have been reported for the official AOAC method employing sample evaporation for analyte preconcentration [16], while among LC methods based on pre-column derivatization with FMOC-Cl detection limits at the level of 0.03 μg/l have been reported for a method employing on-line SPE of the FMOC derivatives and subsequent LC–MS–MS analysis [15]. Among the GC methods detection limits at the level of 0.05 μg/l for both analytes have been recently reported for a method based on a two-step ion-exchange sample preconcentration followed by derivatization with trifluoroacetic anhydrite/trifluoroethanol and subsequent GC–MS analysis [11]. All the above mentioned methods, however, lack the level of automation of the proposed method.

The recoveries of glyphosate and AMPA from Axios river water at the level of 2 μg/l were 84 and 15%, respectively. The repeatability was good with RSD values of the analyte recoveries being less than 15% ($n=3$), even at the lower fortification levels considered. When a new water matrix was to be

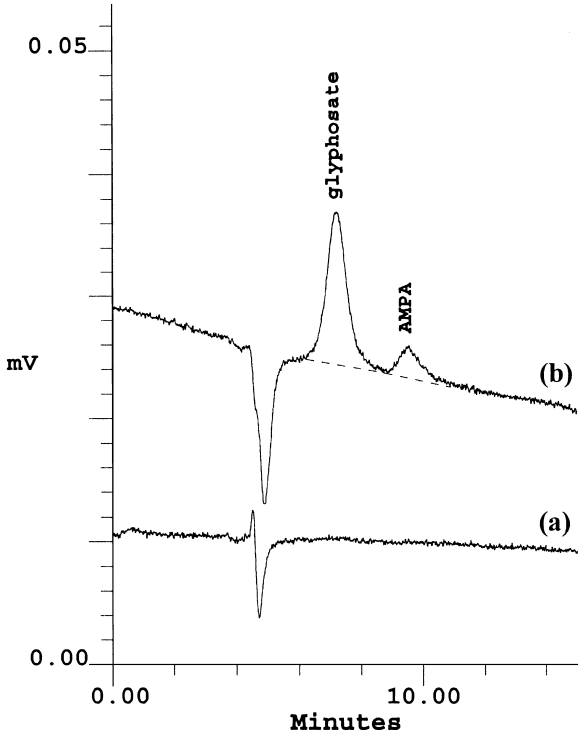

Fig. 4. Chromatograms from the analysis of an Axios river water sample not spiked (trace a) and spiked at the level of 0.1 μg/l (trace b). The experimental conditions are given in the text.

analyzed, analyte recoveries and detection limits were recalculated; however, after the introduction of the clean-up step, we did not observe any significant variations in method performance among different water matrices.

Calibration data for glyphosate and AMPA in the range of 1 to 1000 ng injected amounts could be fitted linearly with r^2 values better than 0.999; however, quadratic fitting was more precise giving r^2 values better than 0.9999. The detector response for both analytes was slightly dependent on the age of the derivatization reagents. Fresh derivatization reagents were therefore prepared daily and full recalibration of the analytical method was performed.

3.4. Stability studies

Using the present method a degradation study was performed for both analytes in filtered ground water; solutions were stored under refrigeration (~4°C) and 20°C. Aliquots of 1 l of ground water filtered through a 0.2-μm membrane filter and spiked with both analytes at the level of 5 μg/l were stored in polypropylene plastic bottles under the selected storage conditions for a period of 1 month. The recoveries of both analytes were recorded weekly by withdrawing and processing 50-ml portions of each sample in triplicate (Fig. 5). Losses of 21 and 32% were observed for glyphosate after 1 month of storage under refrigeration and 20°C, respectively; the corresponding losses of AMPA were 22 and 72%. A lag phase in the degradation curve of AMPA was observed, when solutions were stored under refrigeration (Fig. 5). Biological [19] and chemical factors such as chlorine [17] have been reported to

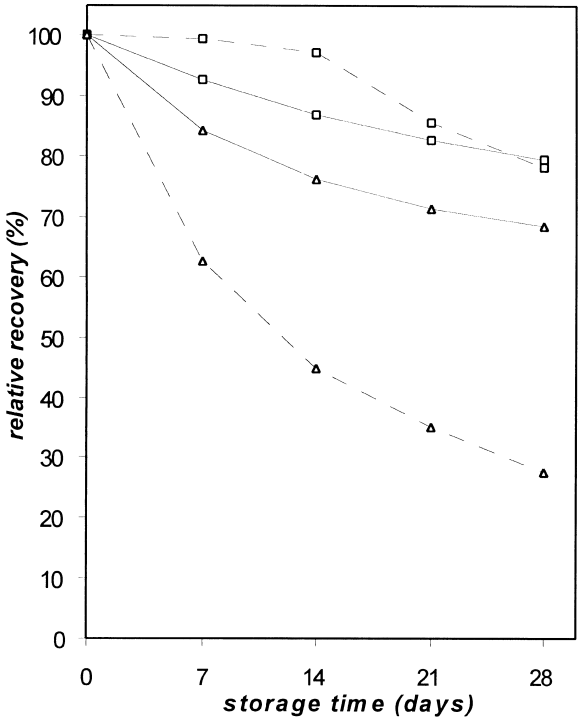

Fig. 5. Mean glyphosate (solid lines) and AMPA (dashed lines) relative recoveries (%, $n=3$) from filtered ground water vs. storage time, when the solutions were stored under refrigeration (~4°C) (□) and 20°C (△). Relative recoveries are calculated by normalization % with the initial recovery. The spiking level was 5 μg/l. All other experimental conditions are given in the text.

enhance glyphosate degradation in water. In this study the bulk amount of microorganisms was removed during filtration and only chemical factors should be considered.

The stability of glyphosate and AMPA was also evaluated in the sorbed state onto PRP-X100 cartridges; cartridges were stored under deep freeze (−24°C), refrigeration (~4°C) and 20°C. Aliquots of 50 ml of distilled water spiked at the level of 5 μg/l were loaded onto PRP-X100 cartridges and the cartridges were immediately stored under the selected storage conditions. The recoveries for both analytes were recorded weekly during 1 month in triplicate. Both analytes were found to be stable under deep freeze and refrigeration conditions, while losses of 11% for glyphosate and 7% for AMPA were observed after 1 month, when cartridges were stored at 20°C. This approach can be used therefore effectively for sample storage in monitoring programs concerning glyphosate and AMPA analysis in the aquatic environment.

4. Conclusions

The developed method provides an automated analytical tool for the trace level determination of glyphosate and AMPA in the aquatic environment. Even though AMPA is not recovered quantitatively, the proposed method can be used effectively for screening its presence along with glyphosate. Detection limits better than 0.02 μg/l for glyphosate and 0.1 μg/l for AMPA can be achieved. Future work is needed, however, to improve AMPA recovery and correspondingly the detection limit for that compound. The ionic compounds present in environmental water affect quantitatively the performance of the anion-exchange SPE and a clean-up step is therefore required prior to sample SPE. Both glyphosate and AMPA were unstable in solution even under refrigeration conditions (~4°C). They were stable, however, at the sorbed state on the PRP-X100 anion-exchange SPE cartridges and the respective technique has been therefore adopted for sample storage.

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