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Preliminary recovery study of a commercial molecularly imprinted polymer for the extraction of glyphosate and AMPA in different environmental waters using MS

Bérendère Claude¹ · Catherine Berho² · Sami Bayoudh³ · Laurence Amalric² · Emeline Coisy² · Reine Nehmé¹ · Philippe Morin¹

Abstract A commercial molecularly imprinted polymer (MIP) dedicated to glyphosate (GLY) and its main metabolite, aminomethylphosphonic acid (AMPA), was lately assessed as “POCIS-like” sampler on mineral water. The obtained results were encouraging with 111 and 122 mL day⁻¹ as sampling rates for GLY and AMPA, respectively. Therefore, before applying this passive sampler to environmental waters, the commercial phase was tested on different water matrices as a solid-phase extraction (SPE) device. The SPE protocol was carried on 250 mg of MIP with the following three steps: conditioning by Milli-Q water, loading of the sample (15 mL), and elution of the analytes by 4 mL 0.1 M HCl that were evaporated to dryness and recovered in 15 mL of the suitable solvent for analysis. This protocol was first applied to mineral water spiked by GLY and AMPA at environmental concentration levels (25–750 ng L⁻¹). Analyses were carried out by ultra-performance liquid chromatography hyphenated to tandem mass after derivatization of GLY and AMPA by 9-fluorenylmethylchloroformate. The linear correlation between concentrations measured with and without SPE on MIP was proved.

Furthermore, other extractions showed that high concentrations of metal ion interferents (lead(II), cadmium(II), and zinc(II)) in groundwaters did not reduce SPE performance of the MIP.

Then, concentration assays were undertaken and brought noteworthy results, such as the recovery of 80% GLY and AMPA from groundwater spiked at 10 ng L⁻¹ and concentrated 100 times. For this purpose, ion exclusion chromatography hyphenated to mass was applied without previous derivatization of the analytes. The same concentration factor and analytical method were applied to 100 ng L⁻¹ spiked sea water with recoveries of 96% for GLY and 121% for AMPA.

Keywords AMPA · Glyphosate · Molecularly imprinted polymers · UPLC-MS/MS · IEC-MS · Metal ion interference · Groundwater · Sea water

Introduction

Among the broad-spectrum herbicides extensively used in agriculture, glyphosate [*(N*-phosphonomethyl)glycine] is one of the most widely encountered. Glyphosate (GLY) affects the enzymatic mechanism of the plant, hence the inability of the plant to produce amino acids and metabolites required for its growth. GLY is converted into aminomethylphosphonic acid (AMPA) by biodegradation and both molecules are easily adsorbed by most of soils. However, the water saturation of soil can lead to ground and surface water pollution (Borggaard and Gimsing 2008). Thus, a thorough monitoring of GLY and AMPA in environmental waters is necessary despite the very low concentrations of these analytes (about 0.5 µg L⁻¹).

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The aim of our work was to test a new commercially available sorbent as a receiving phase in passive sampling device to specifically bind in situ GLY and AMPA in environmental waters. It appears that the commonly used passive sampler for polar compounds, i.e., Polar Organic Chemical Integrative Sampler (POCIS) that contains HLB® hydrophilic-lipophilic-balanced sorbent (Oasis, Waters), failed to trap organic or inorganic anions in environmental waters because of the hydrophilic or ionic properties of the analytes.

Therefore, anion-exchange sorbents seem to be an alternative to this lack of retention of anionic analytes. Indeed, GLY and AMPA were quantitatively bound by strong anion-exchange sorbents, such as styrene-divinylbenzene resins (Amberlite®IRA-410, Amberlite®IRA-900, OH⁻ form) with benzyltrimethyl(2-hydroxyethyl) ammonium functional groups (Mallat and Barceló 1998; Corbera et al. 2005) or a silica-based support (Strata® SAX) with propyltrimethylammonium functional groups (Wagner et al. 2012). Despite these positive results obtained in laboratory, to our knowledge, these anionic exchange phases have not yet been tested as integrative passive sampler sorbents for GLY and AMPA.

Several weak anion-exchange sorbents such as silica-based dimethylaminopropyl or diethylaminopropyl supports were tested (Pastias et al. 2001) and also displayed efficient retention of GLY and AMPA in pure water without ionic matrix.

Other investigations were achieved by using alumina-coated iron oxide nanoparticles to concentrate GLY in washing waters of fruits (Hsu and Whang 2009); the authors underlined the strong and specific interactions occurring between the phosphonate functional group of the analytes and the alumina sorbent. However, the nanoparticles are not conceived for in situ apparatus. Titanium dioxide was also used as binding phase for passive sampling of GLY and AMPA in Diffusive Gradients in Thin films (DGT) configuration by Fauvelle et al. (2015). Nevertheless, anions present in the environmental water matrices decreased the adsorption capacity of this sorbent previously observed with pure water.

In this context, we assessed the performance of a new commercial sorbent as receiving phase in passive sampling of GLY and AMPA in natural waters. This work was incorporated in the framework of ORIGAMI project financed by French National Agency for Research (ANR). The sorbent used was developed by the ANR industrial partner, AFFINISEP (Petit-Couronne, France), as a molecularly imprinted polymer (MIP), which means a specific sorbent of the target analytes, GLY and AMPA. MIP materials contain specific cavities that are complementary to the target molecules in size, shape, and position of the functional groups. A first MIP dedicated to GLY and AMPA was synthesized in our laboratory (Puzio et al. 2014) from phenylphosphonic acid (template) and allylthiourea (functional monomer) which are able to interact

with each other by hydrogen bonding in the polymerization solvent (acetonitrile). During polymerization of all monomers (cross-linker: ethylene glycol dimethacrylate and functional monomer: allylthiourea), the [template—functional monomer] complexes constituted anchor points for making cavities in the finally obtained polymer. These cavities, also called imprints, were able to retain GLY and AMPA not only by hydrogen bonds and electrostatic interactions but also by shape recognition (Fig. S-1) in aqueous matrices. With this homemade allylthiourea MIP, the recoveries of GLY were equal to 84% in mineral waters and to 96% in groundwaters. However, this polymer failed to trap AMPA with satisfactory yields in environmental waters because of the ionic competitive effect of the matrix as proved by thorough experiments reported in Puzio et al.'s article.

In order to overcome this barrier regarding AMPA binding in natural waters, the recently AFFINISEP-marketed sorbent (AFFINIMIP® SPE Glyphosate-AMPA) has been tested, especially as it produced good sampling rates, 111 mL day⁻¹ for GLY and 122 mL day⁻¹ for AMPA, in mineral water during laboratory calibration as a “POCIS-like” sampler (Berho et al. 2017).

The aim of the present work was firstly to develop an efficient and quick extraction protocol of GLY and AMPA in mineral water by AFFINIMIP® SPE cartridges (250 mg/3 mL). Then, the SPE protocol was tested on groundwaters where the presence of cations at either high concentrations (i.e., calcium, magnesium) or trace levels (i.e., metal cations such as cadmium, lead, and zinc) could compromise GLY and AMPA retention by the sorbent. Finally, two ways of improving the sensitivity of the analysis were studied either by increasing the sample loading volume on the MIP cartridge (concentration factors 50–100) or by using a new analytical technique, ion exclusion chromatography hyphenated to mass spectrometry (IEC-MS) which does not require a derivatization step, in contrast to ultra-performance liquid chromatography hyphenated to tandem mass spectrometry (UPLC-MS/MS), and therefore decreases the final recovering volume after evaporation to dryness of the elution fraction.

The determination of GLY and AMPA were performed either by capillary electrophoresis (CE) for the development of the SPE protocol or by UPLC-MS/MS or IEC-MS for trace analysis. UPLC-MS/MS requires a pre-column derivatization of GLY and AMPA with 9-fluorenylmethylchloroformate (Fmoc) to decrease the hydrophilic nature of the analytes and promote their retention on the chromatographic column. IEC-MS, recently developed by several authors (Bauer et al. 1999; Hao et al. 2011; Chen et al. 2013) for GLY and AMPA analysis, was applied to analyze SPE extracts of groundwaters and sea waters whose saline matrix prevents any derivatization, even after extraction by MIP extraction.

Material and methods

Chemicals and solutions

MIP cartridges (250 mg/3 mL) for SPE (AFFINIMIP® SPE Glyphosate-AMPA) were provided by AFFINISEP (Petit-Couronne, France). Milli-Q water was provided by an Elgastat UHQ PS Water Purifier (Apeldoorn, The Netherlands). Ammonium acetate (NH₄Ac), Fmoc and glyphosate PESTANAL analytical standard (GLY) used for SPE of spiked samples were from Fluka (Saint-Quentin Fallavier, France).

Isotope-labeled glyphosate (1,2-¹³C, ¹⁵N; 13C-GLY) 100 mg L⁻¹ in water and isotope-labeled AMPA (¹³C, ¹⁵N; 13C-AMPA) 100 mg L⁻¹ in water, used for calibration, were provided by Dr. Ehrenstorfer (CIL Cluzeau, Sainte Foy la Grande, France).

Aminomethylphosphonic acid (99%, AMPA) used for SPE of spiked samples, hexadecyltrimethylammonium bromide (98%, CTAB), 4-morpholine-ethanesulfonic acid (≥99%, MES), L-histidine (≥99%) and phthalic acid (≥99.5%) used for CE analyses, disodium tetraborate decahydrate, glyphosate (98%) used for calibration control of UPLC-MS/MS analyses, and all solvents used for MIP synthesis were from Sigma-Aldrich (Saint-Quentin Fallavier, France).

The salts of cadmium(II), lead(II), and zinc(II) in 2–5% HNO₃ matrix used to spike groundwaters were bought as Inorganic Reference Standard 1000 µg L⁻¹ solutions to Techlab (Metz, France).

Other chemicals and solvents were of analytical grade and used without further purification.

For SPE and UPLC-MS/MS analyses, standard stock solutions of GLY and AMPA were prepared dissolving about 10 mg powder, accurately weighed, in 100 mL of water to get a final concentration of approximately 100 mg L⁻¹. They were stored at 4 °C and renewed every week. These stock solutions were used for spiking mineral waters at a concentration level of 25 mg L⁻¹ for SPE development with CE-indirect UV detection as analytical control tool.

For UPLC-MS/MS analyses, a 10 mg L⁻¹ composite standard was prepared in water by mixing and diluting the individual 100 mg L⁻¹ standard stock solutions. A solution of 13C-GLY and 13C-AMPA (internal standards, IS) was prepared at 10 mg L⁻¹ by diluting commercial standards in water. The standard working solutions (25–750 ng L⁻¹) were prepared by successive dilutions of the 10 mg L⁻¹ composite standard in water. Each working solution was then spiked with the IS solution to obtain a final concentration of 13C-GLY and 13C-AMPA at 1250 ng L⁻¹.

The mineral water was purchased from a local supermarket. For IEC-MS, all standard and sample solutions were prepared in mineral water acidified with 12.5% formic acid.

Apparatus and analytical conditions

Instrumentation and analytical conditions for CE analyses

CE analyses were performed using a P/ACE™ MDQ Capillary Electrophoresis System (Sciex, Villepinte, France). The detection system was equipped with a UV-DAD system, and the signal was monitored at 240 nm (indirect UV detection). Data were collected by the software provided by Beckman (Gold version 2.3). A fused-silica capillary of 60.2 cm length (effective length, 50 cm) and 50 µm of inner diameter was used for CE separations conducted at 25 °C.

Before the first analysis, the capillary was activated by NaOH 1 M flushing during 30 min. Then, capillary was rinsed with water (20 min) and conditioned with a 7.5 mM phthalic acid-51.3 mM L-histidine running buffer (pH 6.5, ionic strength of 21.8 mM, buffer capacity of 25 mM/pH unit) containing 1 mM CTAB for 45 min. All rinse cycles were done at 30 psi. Solutions were filtered with a syringe filter having a 0.22 µm porosity polypropylene membrane (VWR Paris, France).

Samples were hydrodynamically injected at the cathode using a pressure of 0.5 psi for 30 s. Then, the CE separation was performed by applying a negative voltage of -25 kV (current ≈ 7 µA) during 10 min. Between two consecutive analyses, the capillary was rinsed with the running buffer at 35 psi for 5 min.

Instrumentation and analytical conditions for UPLC-MS/MS

UPLC-MS/MS analyses were performed on a Waters UPLC system coupled to a Waters Micromass MS-MS (Waters Quattro-Premier XE/Q) equipped with online SPE precolumn (Oasis HLB 2.1 × 20 mm, 25 µm particle size, Waters) and a chromatographic column ACQUITY UPLC HSS T3 (2.1 mm × 100 mm, particle size 1.8 µm, Waters) maintained at 30 °C. The compounds were separated using a gradient separation and analyzed by tandem MS with electrospray ionization in positive-ion mode using multiple reaction monitoring (MRM). This analytical method was described in details by Puzio et al. (2014).

Briefly, samples were spiked with 13C-GLY and 13C-AMPA surrogate standards before addition of borate-Na buffer (pH 9). Then, Fmoc derivatization was carried out for 15 h in darkness at room temperature. Finally, derivatized samples were submitted to online SPE and UPLC-MS/MS analysis. Analyses were carried on with a 5 mM ammonium acetate solution/acetonitrile elution gradient in order to minimize the elution of excess Fmoc reagent from the SPE cartridge.

For each sample, the compounds were identified by comparing their retention times to those of internal standards and also the ratio of the quantitation MRM daughter-ion to the confirming MRM daughter-ion for each compound. The

concentration of each identified compound was calculated by determining the ratio of the area response produced by the quantitation daughter-ion of the analyte to the area response produced by the quantitation daughter-ion of the corresponding internal standard. The same ratios previously obtained from the analyses of the standard working solutions (25–750 ng L⁻¹) were used as reference for the calculation of GLY and AMPA concentrations in real samples as well as in the molecularly imprinted solid-phase extraction (MISPE) elution fractions.

Instrumentation and analytical conditions for IEC-MS analyses

IEC-MS analyses were performed in an external laboratory on an ACQUITY LC chromatography system hyphenated to a Quattro Premier MS mass spectrometry (Waters). Chromatography was performed using an IC-PAK™ ion-exclusion column (Waters, 150 mm × 7.8 mm, 7 μm particle size). The pH of the volatile mobile phase was 2.5. All samples were spiked with 13C-GLY and 13C-AMPA surrogate standards dissolved in mineral water acidified by 12.5% formic acid. In these conditions, the LOQ of GLY and AMPA analyzed in complex matrices such as sea waters was equal to 5 μg L⁻¹ without previous concentration.

MISPE

MISPE protocol development

The AFFINIMIP® SPE Glyphosate-AMPA cartridge (250 mg/3 mL) was conditioned with 6 mL of mineral water.

For the preliminary MISPE protocol, 3 mL of mineral water spiked with GLY and AMPA (25 mg L⁻¹) was percolated through the cartridge. The analytes were eluted twice with 3 mL of 0.1 M HCl solution. All elution fractions were evaporated until dryness under a gentle nitrogen flow, recovered in 3 mL of Milli-Q water, and injected into the CE system.

In order to improve MISPE recoveries by varying the elution volume, three different volumes (4, 5, and 6 mL) of 0.1 M HCl were tested after loading 15 mL of samples of GLY and AMPA (500 ng L⁻¹) dissolved in mineral water. The elution fraction was evaporated to dryness under a gentle nitrogen flow. Then, the residue was dissolved in 15 mL of mineral water, derivatized, and analyzed by UPLC-MS/MS. Each assay was done in duplicate.

Linearity MISPE performance on mineral water

For the linearity assessment of MISPE followed by derivatization and UPLC-MS/MS analysis, the protocol was the same as the one described in the last section, except the concentrations of GLY and AMPA. Four concentration levels (100, 250,

500, and 750 ng L⁻¹) were obtained by spiking 50 mL of mineral water with suitable volumes of an intermediate stock solution of GLY and AMPA (10 mg L⁻¹) prepared in mineral water by mixing and diluting 100 mg L⁻¹ stock solutions. All samples were extracted by MISPE in duplicate. The loading volume was 15 mL, and the elution was performed with 4 mL of 0.1 M HCl. The elution fraction was evaporated until dryness and dissolved in 15 mL of mineral water. A 5-mL aliquot was derivatized and analyzed by UPLC-MS/MS as described in the “Instrumentation and analytical conditions for UPLC-MS/MS” section, and another 5 mL aliquot was stored at -20 °C as reference. The concentrations of GLY and AMPA in mineral water samples were measured by UPLC-MS/MS after derivatization.

Determination of metal interferences in real matrices

The divalent metal cations such as zinc(II) (Zn), lead(II) (Pb), and cadmium(II) (Cd) were studied as potential interferences for GLY and AMPA retention on MIP. For that, a groundwater was spiked with the stock intermediate solution of GLY and AMPA 10 mg L⁻¹ to get a final concentration of 300 ng L⁻¹. Then, the sample was spiked with suitable volumes of Zn, Pd, and Cd stock solutions (100 mg L⁻¹) in order to reach four different levels of metal concentrations (Table 1). Finally, four samples were obtained and each of them was extracted following the same MISPE protocol as the one described in the “Linearity MISPE performance on mineral water” section. The elution fractions were analyzed by UPLC-MS/MS after evaporation to dryness and derivatization.

Concentration factor

A concentration factor of 50 and 100 was investigated by loading 500 and 1000 mL, respectively, of mineral water spiked with the stock intermediate solution of GLY and AMPA (10 mg L⁻¹) to get a final concentration of 10 ng L⁻¹. The MISPE protocol was the same as the one applied for linearity study (“Linearity MISPE performance on mineral water” section) except for the sample volume loaded. The residue obtained after evaporation of the elution

Table 1 Metal composition of the spiked groundwater

Level of metal spiking	Metal concentration (μg L ⁻¹)		
	Cd	Pb	Zn
0	0.01	0.05	0.36
1	0.02	0.2	0.49
2	0.1	3.5	16.4
3	4.5	90.9	180

fraction was dissolved in 10 mL of mineral water, divided into two 5 mL aliquots which were derivatized and analyzed by UPLC-MS/MS. Moreover, the extraction was tested in duplicate.

For the assessment of the sensitivity obtained by IEC-MS, a concentration factor of 100 was applied by loading 100 mL of groundwater (the same groundwater as the one used for metal interference study; “Determination of metal interferences in real matrices” section) spiked with 10 ng L⁻¹ of GLY and AMPA, and 100 mL of sea water spiked with 100 ng L⁻¹ of GLY and AMPA. The dry residue obtained by evaporation of the elution fraction was recovered in 1 mL of acidified (12.5% formic acid) mineral water.

All MISPE protocols have been recapped in Fig. S-2.

Results and discussion

MISPE protocol optimization

The present study is part of the ORIGAMI ANR project dedicated to the development of two MIPs of GLY and AMPA used as binding phase for passive sampling. The first one was a homemade allylthiourea-based MIP (Puzio et al. 2014) with high recoveries for GLY in environmental waters but inefficient for AMPA. The second sorbent, AFFINIMIP® SPE Glyphosate-AMPA sorbent, was synthesized in order to solve the problem of AMPA trapping in natural waters. According to the manufacturer, this material was prepared from a functional monomer more reactive than thiourea function in order to exchange stronger electrostatic interactions with the phosphonic function of GLY and AMPA. Moreover, the ethylene glycol dimethacrylate cross-linker was replaced by an ionic and hydrophilic material in order to both strengthen interactions with AMPA and decrease nonspecific hydrophobic interactions between MIP and organic pollutants of medium and low polarity present in environmental waters and also to improve the MIP wetting by water matrices. Therefore, the MISPE protocol required an elution solution with strong ionic competitors such as basic (NH₄OH) or acidic (HCl) solutes. Among these two options, only acidic solutions were compatible with the analyses of samples containing traces of GLY and AMPA by UPLC-MS/MS. Indeed, basic solutions have caused low repeatability of peak areas. Therefore, 0.1 M HCl aqueous solution was used as eluent.

AFFINIMIP® SPE Glyphosate-AMPA sorbent was first assessed according to the MISPE protocol described in the “MISPE protocol development” section. Briefly, after a conditioning step by mineral water, 3 mL of mineral water spiked with GLY and AMPA (25 mg L⁻¹) was loaded on the cartridge. The analytes were eluted twice with 3 mL of 0.1 M HCl aqueous solution. Both elution fractions were evaporated until dryness under a gentle nitrogen flow, recovered in 3 mL

volume of Milli-Q water, and injected into the CE system. The fraction recovered after sample loading was straight analyzed by CE. This CE analytical control, initially developed by Cikalo et al. in 1996, is very fast (less than 6 min), economical, and does not require any derivatization. A standard solution prepared from Milli-Q water spiked with GLY and AMPA (25 mg L⁻¹) was analyzed in order to identify and quantify the analytes in all MISPE fractions.

The electropherogram of the recovered loading MISPE fraction displayed no peak corresponding to the migration times of the target analytes which proved that GLY and AMPA were totally retained by the sorbent during the loading step. As expected, both analytes were recovered in the elution fractions in this way: 68% of GLY/67% of AMPA in the first elution fraction and 17% of GLY/20% of AMPA in the second elution fraction.

In order to get maximum recoveries, the volume of the acidic elution solution (0.1 M HCl) was adjusted by supplementary MISPE assays that were carried out with 500 ng L⁻¹ spiked mineral waters (15 mL) as described in the “MISPE protocol development” section. Three volumes of 0.1 M HCl (4, 5, and 6 mL) were tested in duplicate. Each elution fraction was evaporated until dryness and recovered in 15 mL mineral water, derivatized by FMOC, and analyzed according to UPLC-MS/MS method as reported in the “Instrumentation and analytical conditions for UPLC-MS/MS” section. The results showed that high and repeatable recoveries (137 ± 9% for GLY and 93 ± 1% for AMPA, *n* = 2) were obtained by using 4 mL elution volume of acidic solution. However, a poor repeatability of recoveries was noticed with elution volumes higher than 4 mL. Thus, with 5 mL elution volume, the standard deviation of GLY recovery was ±25% and, with 6 mL elution volume, the standard deviations of both GLY and AMPA recoveries were ±30%.

No loss of analyte occurred during the evaporation step as it was quantitatively checked by evaporating 4 mL of a solution of 0.1 M HCl spiked with GLY and AMPA 500 ng L⁻¹ (data not shown).

Linearity MISPE performance on mineral water

MISPE protocol was further applied to 15 mL of mineral water samples spiked at usual concentration levels of GLY and AMPA in environmental waters (100, 250, 500, and 750 ng L⁻¹). For each level, the MISPE was duplicated. All samples and MISPE elution fractions were analyzed by UPLC-MS/MS after FMOC derivatization. The results were reported as a curve which relates MISPE elution concentration of GLY and AMPA versus their concentration in sample. Two blanks constituted of mineral water without GLY and AMPA were submitted to the same protocol. As expected, the concentrations measured for these blanks were lower than the limits of quantification of UPLC-MS/MS.

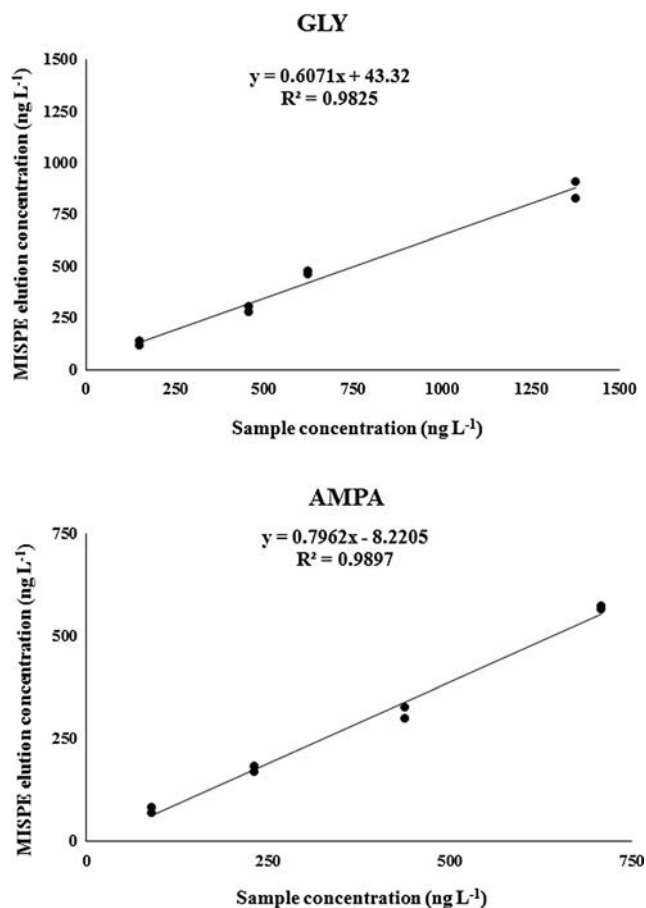


Fig. 1 Graphs of MISPE elution concentration of GLY and AMPA as functions of the sample concentration. Determination by FMOc derivatization and UPLC-MS/MS. Samples: mineral water spiked at four levels of concentration: 100, 250, 500, and 750 ng L⁻¹ of GLY and AMPA. MISPE protocol described in the “Linearity MISPE performance on mineral water” section

The results obtained for spiked mineral waters showed a linear relationship between the MISPE elution concentrations and the concentrations in samples with determination coefficients of 0.9825 and 0.9897 for GLY and AMPA, respectively (Fig. 1). The slopes (about 0.61 for GLY and 0.80 for AMPA) lower than one revealed a constant underestimation of the concentrations by the protocol including MISPE. This was noteworthy for GLY.

Moreover, considering the results obtained for the extraction of AMPA in spiked mineral water (500 ng L⁻¹) by the homemade allylthiourea-based MIP (Puzio et al. 2014), the recovery was significantly improved with the use of the AFFINIMIP® SPE Glyphosate-AMPA sorbent ($72 \pm 4.5\%$ $n = 2$) compared to the one obtained with the allylthiourea-based MIP ($13.4 \pm 2.9\%$, $n = 3$).

Influence of complexing metal interferences

All groundwaters analyzed contained two main mineral components at milligrams per liter concentration level,

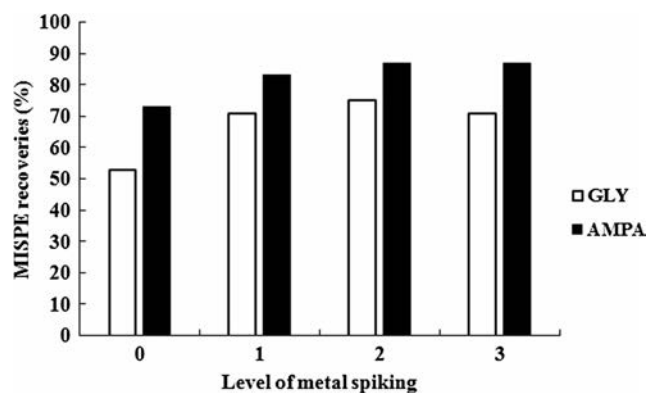


Fig. 2 Elution recoveries ($n = 1$) of GLY and AMPA in groundwater spiked at four levels of metal concentration (Table 1). Experimental conditions described in the “Determination of metal interferences in real matrices” section

calcium(II), and magnesium(II) ions, which are well known for forming strong coordination complexes with GLY and AMPA (Madsen et al. 1978). However, some metal cations (Cd, Pb, Zn...) present in natural waters at trace concentration ($0.1 \mu\text{g L}^{-1}$) form also very stable complexes with GLY for pH higher than 5 (Kobylecka et al. 2000). No result has been reported concerning the complexation of AMPA. However, its phosphonic acid function, in both mono- and bi-anionic forms at pH 7 (Fig. S-3), leads one to believe that AMPA may also complex with metal cations in environmental waters.

We investigated herein if the extraction yield of GLY and AMPA (300 ng L^{-1}) from groundwaters could be reduced by the complexation of GLY and AMPA with the metal cations. Several sample solutions were prepared by spiking a groundwater containing initially low amounts of metal ions ($0.01 \mu\text{g L}^{-1}$ Cd, $0.05 \mu\text{g L}^{-1}$ Pb, and $0.36 \mu\text{g L}^{-1}$ Zn) at three levels of metal ion concentration (see Table 1). Each level of metal spiking was carefully selected according to data obtained from a French database about groundwaters (ADES) in which GLY and AMPA were identified. It was also noteworthy that the selected groundwaters had the same pH (7.1) as the mineral water (7.2) previously used for the linearity study and quite similar ionic strength (8.6 versus 9.2 mmol L^{-1} for mineral water). Furthermore, Ca and Mg concentrations were also in the same order of magnitude for both waters (Ca, 2 mmol L^{-1} in mineral water versus 2.7 mmol L^{-1} in groundwater; Mg, 1.1 mmol L^{-1} in mineral water versus 0.3 mmol L^{-1} in groundwater).

Thus, four water samples were spiked with GLY ($225 \pm 14 \text{ ng L}^{-1}$) and AMPA ($237 \pm 19 \text{ ng L}^{-1}$) as indicated by their subsequent UPLC-MS/MS analyses. Each sample was submitted to MISPE following the same protocol as that applied for the linearity study. Recoveries were calculated from the recovered concentrations of GLY and AMPA divided by the concentrations obtained by direct analysis of spiked groundwater. The results were reported as a histogram in Fig. 2. In pure groundwater, AMPA was recovered with a

yield of 75% that is almost equal to the value obtained in mineral water (76%, AMPA spiked at 250 ng L^{-1}), whereas GLY displayed a lower recovery (51%) than in mineral water (64%). On the other hand, recovery yields of GLY and AMPA were not significantly modified by the addition of metal ions in the studied concentration range, with mean values ($n = 3$) close to $68 \pm 10\%$ for GLY and $82 \pm 6\%$ for AMPA. Thus, the presence of these metal ions at these levels of concentration in water was not a hindrance to the binding of these pesticides by the MIP sorbent.

Concentration factor

a) Increased loading volume of environmental water

Up to now, GLY and AMPA have been analyzed in groundwaters by FMOc derivatization and UPLC-MS/MS analysis with a LOQ of 50 ng L^{-1} (Arkan and Molnár-Perl 2015). However, a detection limit of 10 ng L^{-1} is required to assess their monitoring in environmental waters. In this context, the MISPE concentration of GLY and AMPA in groundwater samples would be a significant improvement to get the detection limit of 10 ng L^{-1} in natural waters. Thus, larger volumes of water (500 and 1000 mL) have been extracted, knowing that at least several hundreds of milliliters of water would normally pass through the sorbent during its in situ exposure as a passive integrative sampler.

In this context, we tried to concentrate 50 times the sample by loading 500 mL of mineral water spiked with 10 ng L^{-1} of GLY and AMPA on the MIP cartridge and recovering the dry residue in 10 mL of mineral water after evaporation of the elution fraction. The quantification by UPLC-MS/MS after FMOc derivatization gave final concentrations of $6.7 \pm 0.7 \text{ ng L}^{-1}$ for GLY and $5.6 \pm 0.2 \text{ ng L}^{-1}$ for AMPA ($n = 2$). Then, the same experiments were carried out with a larger volume (1000 mL) of 10 ng L^{-1} spiked mineral water with final concentrations of 5.5 ± 0.8 and $3.5 \pm 0.3 \text{ ng L}^{-1}$ ($n = 2$) for GLY and AMPA, respectively. Thus, recoveries of extraction decreased as the loading volume increased, and a 500 mL volume of mineral water was retained as the maximum volume of sample loaded. The influence of the loaded sample volume on GLY and AMPA recoveries had already been studied by Corbera et al. (2005) who used a strong anionic-exchange resin to extract these two pesticides from river waters. This author also concluded to an important increase on recoveries with smaller sample volumes. However, we must be careful to compare the recoveries obtained by MISPE with those obtained by ionic-exchange resins since many experimental parameters differ such as water matrix, analyte concentration, and sorbent amount.

b) IEC-MS analysis

Subsequently, the groundwater used for the study of metal interferences ("Influence of complexing metal interferences" section) was spiked with GLY and AMPA solution (10 ng L^{-1})

and submitted to 100 times concentration by MISPE. The spiked groundwater (100 mL) was loaded on the MIP cartridge. After elution and evaporation to dryness, the dry fraction was recovered by 1 mL of mineral water acidified by 12.5% formic acid for analysis by IEC-MS. As FMOc derivatization step requires a minimum volume of 4.75 mL, it was difficult to get concentration factors higher than 50 (loading volume = 500 mL and recovering volume = 10 mL as done with groundwater in the previous section) unlike IEC-MS that did not require any derivatization. Data obtained by MISPE-IEC-MS concluded that GLY and AMPA were present in groundwater at a concentration ($8 \pm 1 \text{ ng L}^{-1}$, $n = 2$) close to the expected value.

The experiments were then conducted with sea water spiked by 100 ng L^{-1} of GLY and AMPA. Like before, MISPE was carried out with 100 times concentration of sample and IEC-MS analyses concluded to 96 ng L^{-1} of GLY and 121 ng L^{-1} of AMPA. These results proved that, thanks to MISPE concentration, the LOQ of $5 \text{ } \mu\text{g L}^{-1}$ ("Instrumentation and analytical conditions for IEC-MS analyses" section) obtained by direct IEC-MS can be revised to a lower value estimated at 100 ng L^{-1} .

Conclusions

In this work, a commercial MIP dedicated to the extraction of GLY and AMPA was applied to various kinds of natural waters (mineral, ground, and seawaters) as SPE sorbent, before subsequent analysis by UPLC-MS/MS with derivatization or by IEC-MS. After optimization of the elution step, the performances of the analytical system (MISPE followed by UPLC-MS/MS) were investigated according to different criteria: linearity, interference of metal ions, and concentration factor. The linearity of this analysis was demonstrated in a concentration range up to 750 ng L^{-1} with correlation coefficients (r^2) higher than 0.98. The presence Pb, Cd, and Zn metal ions in the sample matrix did not significantly modify the MISPE performance, and mean recoveries of 68% for GLY and 82% for AMPA were obtained.

The detection limits of GLY and AMPA in mineral and ground waters have been improved (10 ng L^{-1}) by means of 50 or 100 times MISPE concentration factor and UPLC-MS/MS or IEC-MS. Moreover, MISPE applied to seawater, with a concentration factor of 100, and IEC-MS enabled to decrease the determination level of GLY and AMPA to 100 ng L^{-1} .

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