Journal of Chromatography A 1687 (2023) 463697

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Direct, automated and sensitive determination of glyphosate and related anionic pesticides in environmental water samples using solid-phase extraction on-line combined with liquid chromatography tandem mass spectrometry

J. López-Vázquez, L. Pérez-Mayán, V. Fernández-Fernández, R. Cela, I. Rodríguez*

Department of Analytical Chemistry, Nutrition and Food Sciences. Research Institute on Chemical and Biological Analysis (IAQBUS). Universidade de Santiago de Compostela, 15782-Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 10 October 2022
Revised 29 November 2022
Accepted 30 November 2022
Available online 5 December 2022

Keywords:
Glyphosate
Zwitterionic pesticides
On-line solid-phase extraction
Liquid chromatography mass spectrometry

ABSTRACT

An automated procedure for the simultaneous determination of six anionic pesticides, including glyphosate (GLY) and its transformation product aminomethylphosphonic acid (AMPA), was developed and applied to the analysis of environmental water samples. The proposed method combines on-line concentration of water samples (0.160 mL), with compounds separation in an anion-exchange liquid chromatography (LC) column, followed by their selective determination by tandem mass spectrometry (MS/MS). The global procedure was completed in 25 min, providing limits of quantification (LOQs) between 5 ng L^{-1} and 20 ng L^{-1}, with reduced effect of the surface water matrix in the efficiency of process (SPE and ionization yields). The method was applied to the analysis of grab samples obtained from three watersheds, in two rural and one residential area, in Galicia (Northwest Spain). Out of six investigated compounds, Fosetyl, AMPA and GLY were noticed in the set of processed samples. Their detection frequencies increased from 12% (Fosetyl) to 88% (AMPA). Median concentrations followed the same trend varying from 9 ng L^{-1} (Fosetyl) to 44 ng L^{-1} (AMPA). The higher levels and the large seasonal variations in the residues of the latter species were noticed in small rivers affected by discharges of municipal sewage treatment plants (STPs).

1. Introduction

Glyphosate (GLY) is a non-selective herbicide impairing the synthesis of aromatic aminoacids by plants. It is widely employed to control the development of weeds in permanent and transgenic crops and to homogenize the harvest of GLY sensitive plants. Additionally to agriculture uses, GLY is also applied to destroy vegetation growing in the limits of roads, as well as in forestry, to control the development of water and nutrients competing plants [1].

After application, GLY is assumed to remain bonded to cations existing in soil, particularly to iron, copper and aluminum containing minerals. This behavior, combined with an estimated soil half-life of a few days [2], turns in a low groundwater ubiquity score (GUS index 0.21) [3], pointing out to a reduced risk of leaching to groundwater and/or surface water. Aminomethylphosphonic acid (AMPA) is the main transformation product of GLY in soils. AMPA is also a Zwitterionic species, with a slightly higher half-life in soil than the parent herbicide. Despite direct migration of both compounds to the aquatic media is unlikely, the misuse of the parent herbicide, runoff transport associated to soil particles during heavy rain events, wind erosion and atmospheric drift might result in the contamination of surface waters with GLY and/or AMPA [4]. Furthermore, phosphate fertilizers increase the release of GLY, and AMPA, from soil to the water phase, due to displacement of both compounds from their metallic quelates [5]. In line with these comments, several studies have reported the presence of GLY and AMPA in surface water from agriculture impacted basins [6] and, particularly, in streams draining transgenic crops [7–9]. A national scale survey carried out in USA (more than 3000 samples were taken from 70 rivers and streams for two years) has reported detection frequencies and median concentrations of 74% and 50 ng L^{-1} for GLY, with even higher figures for AMPA [9]. Residues of these pesticides are not limited to intensive agriculture areas. In fact, in Germany, the occurrence and the average concentrations of

* Corresponding author.
E-mail address: isaac.rodriguez@usc.es (I. Rodríguez).

https://doi.org/10.1016/j.chroma.2022.463697

GLY and AMPA were higher for water samples obtained in the surrounding of urban areas than in rural environments [6].

The most often employed methodology to determine GLY and AMPA in aqueous matrices involves compounds derivatization using 9-fluorenylmethylchloroformate (FMOC–Cl) before extraction and liquid chromatography (LC) determination [10–12]. FMOC derivatization decreases the polarity of both compounds allowing their effective extraction and concentration using reversed-phase sorbents (or liquid-liquid extraction) and their further analysis by LC under the same separation mode. However, the reaction of these compounds with FMOC–Cl shows a slow kinetics, it requires removing the excess of derivatization reagent and/or the reaction by-products before LC analysis. Thus, other analytical methods have been proposed. Although some of them have explored alternative derivatization reactions, in some cases combined with gas chromatography-based techniques [13,14], the major stream considers direct analysis of native compounds, exploring different LC stationary phases. Among them, hydrophilic interaction, mixed-mode and anionic exchange columns have been already tested for the separation of both species, and other anionic and/or Zwitterion pesticides, either in food or in water samples [15–22]. Other compounds with similar features (anionic character and high polarity) to GLY include the fungicide Fosetyl-Al [23], the herbicide glufosinate (GLU) and the environmental transformation products of the latter compound N-acetyl glufosinate (NAG) and 3-(methylphosphinic) propionic acid (MPPA) [24]. It is worth noting that neither Fosetyl, nor MPPA can be determined using the FMOC–Cl derivatization approach. Advances in the determination of these anionic, metal complexing compounds are not only related with evaluation of new stationary phases, but also with the testing of different additives (i.e. medronic acid) [25] and/or PEEK lined columns preventing non-reversible interactions between analytes and metal cations, either coming from samples, column walls and/or the LC instrument itself [17].

Another limitation for direct analysis of Zwitterionic species is the difficulty to extract and concentrate these compounds from water samples. Direct injection of large sample volumes, use of anion-exchange solid-phase extraction (SPE) sorbents, or selective concentration of pre-defined compounds (i.e. GLY) with molecularly imprinted polymers (MIP), are some of the solutions reported in the literature [18,21,26]. To the best of our knowledge, neither these extraction procedures have been on-line combined with LC and tandem mass spectrometry (MS/MS) detection, nor they have reached similar LOQs to those reported for the FMOC–Cl protocol [11,12].

This manuscript pursuits two aims. The first was assessing the performance of an automated, direct LC-MS/MS methodology for the simultaneous determination of GLY, AMPA and two additional anionic pesticides (Fosetyl-aluminum; GLU) and also the environmental transformation products of the later: NAG and MPPA in environmental water samples. The second aim was to evaluate their occurrence and possible seasonal variations, in samples obtained from three different watersheds in Galicia (Northwest Spain).

2. Material and methods

2.1. Standards, solvents and sorbents

The standards of GLY, AMPA, Fosetyl-aluminum, GLU and MPPA were acquired from Sigma-Aldrich (St. Louis, MO, USA). NAG was provided by LGC standards (London, UK). Native parent pesticides and their transformation products were analytical grade quality, with a purity above 98%. Their chemical structures are given as supplementary information, Fig. S1. Isotopically labelled analogues of GLY (1,2–$^{13}C_2$, ^{15}N; 99%), AMPA (^{13}C,^{15}N, 97%), Fosetyl-aluminum-d_{15} (95%) and GLU-d_3 (98%) were provided by Toronto Research Chemicals (North York, Canada). Individual solutions of each compound were prepared in ultrapure water, containing 0.1% of formic acid. Further dilutions were made in ultrapure water. Calibration standards containing increasing concentrations of native compounds (from 5 ng L^{-1} to 5000 ng L^{-1}), and a constant level of labelled species (500 ng L^{-1}), were also prepared in ultrapure water. All the standard solutions were stored in polypropylene tubes to prevent sorption of analytes on the surface of glass vials.

Acetonitrile (ACN) and methanol (MeOH), both LC-MS grade, were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Genie U system (Rephile, Shanghai, China). Ammonium bicarbonate and formic acid, both LC-MS purity, were supplied by Honeywell Fluka (Seelz, Germany) and Fisher scientific (Portsmouth, NH, USA), respectively.

2.2. Samples

Samples employed during method development and validation include ultrapure water, surface water obtained from streams and rivers, mineral water (commercially available bottled water), tap water and well water. Regarding environmental studies, 17 sampling points were selected from three areas in Galicia (Norwest Spain), Fig. 1A. Points S1 to S13 correspond to a hilly rural area, with a low population density distributed in tiny villages and dispersed farms, devoted to vineyard production. In this region, samples were obtained from the two main rivers draining vineyards: Avia (S2, S3, S4) and Miño (S5, S6, S9 and S11), as well as some tributary streams and groundwater springs, Fig. 1B. Points S14 and S15 correspond to a small river (Tinto), flowing through a residential, peri–urban area, with a low impact of agriculture activities. Sampling sites were placed upstream (S14) and downstream (S15) the discharge point of a STP serving a population of 13,000 inhabitants, Fig. 1C. In both areas, four sampling campaigns were carried out from the beginning of spring to summer.

Points S16 and S17 were placed in a medium size river (Limia) flowing through a rural flat area of arable fields, devoted to production of cereals (maize and wheat) and potatoes, Fig. 1D. The river also receives the discharge of treated water from a STP serving a population of 12,000 inhabitants. In this area, both sampling points were placed after the municipal STP. Three sampling campaigns were carried out to detect potential agriculture uses of herbicides before tillage of agriculture fields (spring), and as driers of wheat and potato crops, in the middle of summer and the beginning of autumn, respectively.

Table S1 summarizes the exact position of each point and the sampling dates. With the exception of the surface water reservoir in Miño river, significant variations in the flow of the rest of rivers and streams were noticed during the sampling period. Particularly, low flows were observed in the latter campaign in the three considered areas. Available data for major rivers, obtained from regional water management authorities, are compiled in Table S2.

Samples were taken in polypropylene flasks and transported to the laboratory at room temperature, within 4 h. Thereafter, they were either analyzed in the next 24 h, or stored at −20 ºC until analysis. Tap water was collected in the laboratory when needed and mineral water was purchased in local markets.

2.3. Sample preparation

Sample preparation involved filtration (case of environmental water samples), using 0.22 µm hydrophilic polytetrafluoroethylene (PTFE) syringe filters acquired from Phenomenex (Torrance, CA, USA), addition of the mixture of surrogate standards (SSs) at 500 ng L^{-1}, and analysis by SPE on-line connected with the LC-MS/MS system under conditions reported in the next section.

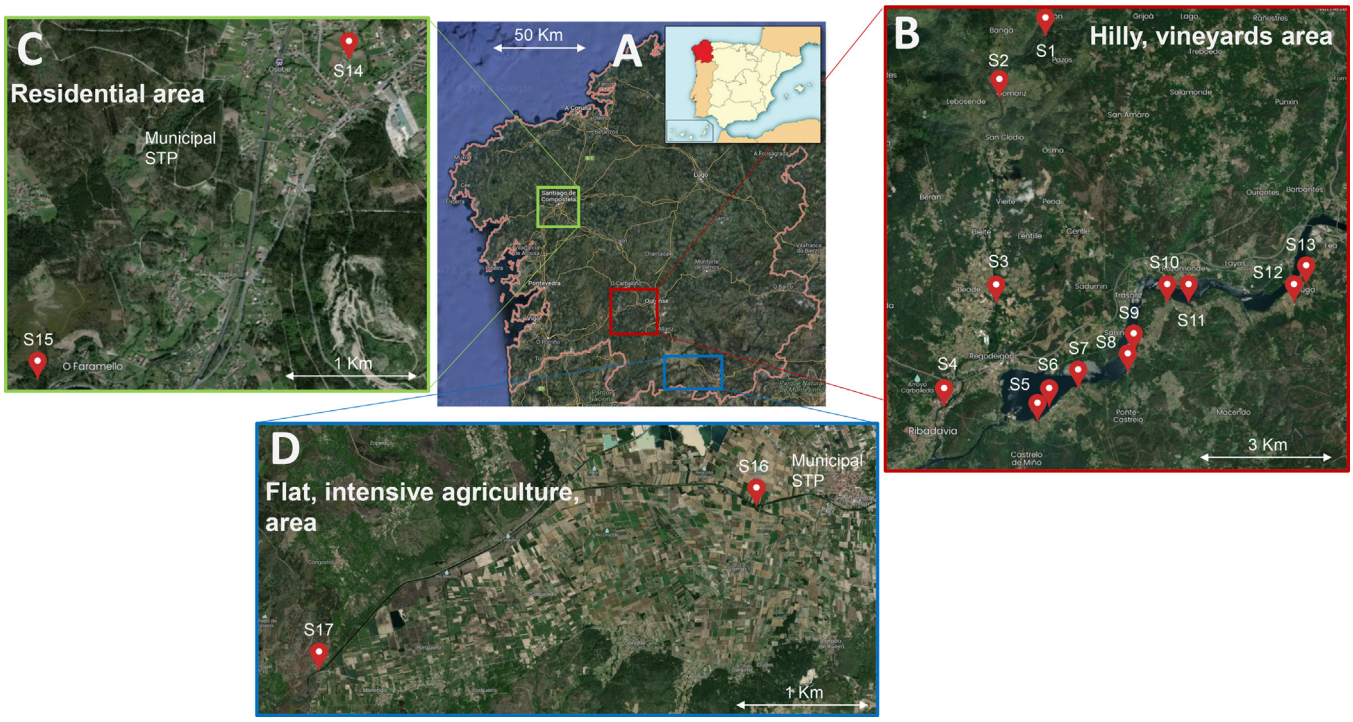

Fig. 1. Map of sampling points (S1-S17) in the three different areas in Galicia (Northwest Spain).

2.4. LC-MS/MS equipment and determination conditions

The LC-MS/MS system was an Agilent 1290 Infinity II liquid chromatograph connected to an Agilent 6495, triple quadrupole (QqQ) mass spectrometer, equipped with a jet stream ESI ionization source. In addition to the binary analytical pump, the LC-MS platform included an auxiliary pump to deliver calibration standards and samples through the SPE cartridge, on-line coupled to the analytical column. Both, cartridge and column, were connected using a 10-port, 2-possition valve. Fig. S2 shows a scheme of the valve during on-line SPE concentration and desorption steps. The LC instrument included an autosampler, with a 100 µL needle loop, and an extended injector seat permitting to accommodate up to 0.5 mL samples before being transferred to the SPE cartridge.

Compounds were separated using a Metrosep A Supp 6, strong anionic exchange column (150 mm x 2 mm, 5 µm), acquired from Metrohm (Herisau, Switzerland). The mobile phase used in the analytical column consisted of a mixture of ACN:ultrapure water (1:1) with a 45 mM concentration of bicarbonate ammonium (phase A); and ultrapure water, 50 mM in bicarbonate ammonium (phase B). Its composition was programmed as follows: 0–3 min, 0% B; 7.5 min, 35% B; 10 min, 60% B; 11–18 min, 100% B; 18.1–25 min, 0% B. The flowrate of mobile phase and the column temperature were 0.3 mL min^{-1} and 30 ºC, respectively. In the auxiliary pump, ultrapure water (phase C) and MeOH (phase D) were used. As SPE sorbent, we employed an anionic exchange pre-column (5 mm x 4 mm, 5 µm) from Metrohm, with the same stationary phase as the analytical column, and a larger internal diameter.

Under final working conditions, 0.160 mL samples were loaded in the pre-column using ultrapure water (phase C), as carrier at 0.5 mL min^{-1} for 1 min, then the flowrate of ultrapure was increased to 1 mL min^{-1}, and maintained until 2.5 min. In this step, anionic species were retained in the on-line connected pre-column, whilst other components flowed to waste. After 2.5 min, the 10-port valve switched to elution position, with compounds being transferred from the pre-column to the analytical column. The valve returned to its initial position (loading mode) after 15 min, and the SPE sorbent was conditioned using MeOH (15–18 min, 1 mL min^{-1}) followed by ultrapure water (18.1–25 min, 0.5 mL min^{-1}).

Nitrogen was employed as drying (11 L min^{-1}, 150 ºC), sheath (12 L min^{-1}, 400 ºC) and nebulizing gas (55 psi) in the ESI source. The needle voltages were 3000 V and 1500 V for ESI (+) and ESI (-) modes, respectively. The fragmentor voltage was 166 V. Table 1 gathers the *m/z* values for precursor and product ions for native pesticides and SSs. Retention times and ratios between qualification (Q2 and Q3) and quantification (Q1) transitions of each compound are also given in Table 1.

2.5. Extraction efficiency and samples quantification

The efficiency of the SPE on-line process was assessed comparing the slope of calibration curves obtained for spiked aliquots of river and mineral water (50 ng L^{-1} to 2000 ng L^{-1}, $n = 6$ different concentration levels) with those corresponding to standards in ultrapure water with same concentration levels. Responses (peak areas) obtained for the Q1 transition of each compound, without any correction with SSs, were plotted against added concentrations. Slope ratios above the unit correspond to increased apparent extraction efficiencies for real samples compared to standards in ultrapure water, while values below the unit have the opposite meaning. Changes in the slopes of calibration curves can be related to variations in the efficiency of the SPE process itself, and/or to variable yields of ESI ionization depending on the sample matrix.

The accuracy of the method was estimated as the difference between concentrations measured for spiked and non-spiked fractions of different water samples divided by the added value and multiplied by 100. Experimental concentrations were determined against calibration standards prepared in ultrapure water (5 ng L^{-1} to 5000 ng L^{-1}), containing same level of SSs as water samples. Peak areas for each compound were corrected with that measured for the corresponding SS, Table 1.

With each set of environmental water samples (15 to 20 samples, plus calibration standards were analyzed in duplicate per

Table 1
Summary of retention times, precursor and product ions for each compound using SPE on-line connected to LC-QqQ-MS.

Compound	Retention time (min)	ESI mode	Precursor ion (*m/z*)	Q1 (CE, eV)	Q2 (CE, eV)	Q3 (CE, eV)	Q2/Q1 ratio	Q3/Q1 ratio
[a]Fosetyl	5.14	–	109	81 (12)	79 (28)	63 (36)	0.33	0.70
[b]AMPA	5.64	–	110	63 (20)	79 (36)		1.09	
[c]GLU	5.95	+	182	56 (28)	136 (12)		0.91	
[d]GLY	8.23	+	170	88 (8)	60 (18)	42 (32)	0.33	0.60
[d]NAG	8.21	+	224	56 (44)	164 (10)	136 (20)	0.29	0.51
[d]MPPA	8.61	+	153	135 (8)	79 (24)		0.79	
[a]Fosetyl-d_5	5.13	–	114	83 (12)	81 (28)	63 (36)		
[b]AMPA-^{13}C,^{15}N	5.52	–	112	63 (20)	79 (36)			
[c]GLU-d_3	5.94	+	185	56 (28)				
[d]GLY-$^{13}C_2$,^{15}N	8.22	+	173	91 (8)	62 (17)			

a to d, denote the surrogate standard assigned to each pesticide.
CE, collision energy (eV).

batch), one procedural blank and one spiked sample (addition level 100 ng L^{-1}) were processed. Acceptable data correspond to concentration levels below method LOQs (from 5 to 20 ng L^{-1}, depending on the compound) in procedural blanks, and recoveries in the range from 80% to 120%. LOQs were calculated as the concentration of each compound providing a signal to noise (S/N) ratio of 10 for the Q1 transition while ratios between qualification transitions (Q2 and Q3 when available) and Q1 remain with ± 30% of average values obtained within the calibration range of the procedure, Table 1. Compounds identification in non-spiked samples was based on retention time match with calibration standards (maximum variation ± 0.1 min) and qualification (Q2, and Q3 when available) to quantification (Q1) ions response ratios showing differences lower than ± 30% compared to those obtained for calibration standards, Table 1.

3. Results and discussion

3.1. Optimization of SPE on-line connected to LC-QqQ-MS

Table 1 summarizes retention times, ionization mode, and *m/z* values for precursor and product ions of target compounds and SSs. Although both ionization modes were evaluated, ESI (+) produced higher signal to noise (S/N) response ratios for all compounds except in case of Fosetyl and AMPA.

At least two MRM transitions were selected per compound. The dwell time per transition was set at 100 ms for AMPA and GLY (to enhance the detectability of both pesticides) and 20 ms for the rest of compounds. The gradient of mobile phase was adapted from our previous study dealing with the determination of AMPA, GLY and Fosetyl in vegetal origin samples [17], considering the delay of retention times induced by the on-line SPE extraction-desorption steps. Although alternative gradients to that reported in Section 2.4 can be considered, baseline separation between the peaks of AMPA and Fosetyl is mandatory since both compounds share several product ions and the *m/z* of their precursors ($[M-H]^-$ ions) differ only in 1 unit, Table 1. So, the cluster of signals associated to the $[M-H]^-$ ion of Fosetyl (*m/z* values 109 and 110, the latter due to the natural abundance of ^{13}C) might lead to false positives for AMPA, whose precursor ion ($[M-H]^-$) has a *m/z* ratio of 110, unless both compounds are baseline separated.

As regards the on-line SPE concentration step, the flowrate of water (phase C, from 0.5 to 2 mL min^{-1}, during 2.5 min) employed to load standards and/or samples (up to 0.45 mL aliquots) in the on-line connected SPE sorbent showed a little effect in their MRM responses. Values below 0.5 mL min^{-1} turned in a poor repeatability; whilst compound losses can occur at loading flowrates above 2 mL min^{-1} as a result of too low equilibration times. Eventually, flowrates of 0.5 mL min^{-1} (0–1 min) and 1 mL min^{-1} (1–2.5 min) were employed. During this step, target compounds are retained by the anionic-exchange sorbent, whilst neutrals and cationic species flow through to waste. The above flowrates led to pressure values of 30 and 60 PSI in the on-line cartridge.

For standards prepared in ultrapure water, responses of all the compounds (peak areas without SS corrections) increased steady for volumes of sample from 0.05 mL to 0.45 mL (data not shown); however, a different behavior was noticed for environmental samples. Fig. 2 shows the slopes of calibration curves obtained for spiked aliquots of river and a commercial, bottled mineral water normalized to those measured for ultrapure water. These two matrices were selected as representative of soft (river water, Ca^{2+} 5 mg L^{-1}, Mg^{2+} 3 mg L^{-1}) and hard (mineral water, Ca^{2+} 86 mg L^{-1}, Mg^{2+} 30 mg L^{-1}) waters. For sample volumes of 0.080 and 0.160 mL, determination coefficients (R^2) above 0.999 were obtained for the plots of response versus concentration, with similar slopes for the 3 types of water. However, when 0.240 mL of sample are loaded in the on-line cartridge, significant reductions in the normalized slopes of several compounds, except NAG and MPPA, were found, Fig. 2. Direct injection (0.05 mL volume aliquots) of same spiked samples in the anionic exchange column, avoiding the SPE step, reflected important variations in the slopes of calibration curves corresponding to the river and the mineral water matrix compared to those obtained for ultrapure water. Particularly, the responses of GLU were enhanced significantly in both water matrices compared to ultrapure water; moreover, GLY could not be detected in hard mineral water and the efficiencies of MPPA and AMPA detection were reduced in more than 90%, Fig. S3. This information, in combination with data shown in Fig. 2, supports the fact that the on-line SPE step, contributes to reduce the complexity of the sample and to improve the performance of compounds determination, avoiding the entrance of neutrals and basic species in the chromatographic column.

3.2. Performance of SPE on-line combined with LC-MS/MS

The linearity of the method was assessed with calibration standards prepared in the range from 5 ng L^{-1} to 5000 ng L^{-1}, at nine different concentration levels (5, 10, 20, 50, 100, 250, 500, 2000 and 5000 ng L^{-1}, injected in duplicate). After correction of MRM responses with those of isotopically labelled standards, R^2 values above 0.998 were obtained, Table 2. Despite NAG and MPPA are structurally related to GLU, their retention times were closer to that of GLY; thus, the labelled analogue of GLY was used as SS of these two compounds. The reproducibility of responses (peak area without SSs correction) for a 40 ng L^{-1} standard in ultrapure water varied from 2% for NAG to 8% for AMPA (n = 9 extraction-determination cycles within a 24-h sequence). Fig. 3 shows the chromatogram for a low-level calibration standard (20 ng L^{-1} per compound).

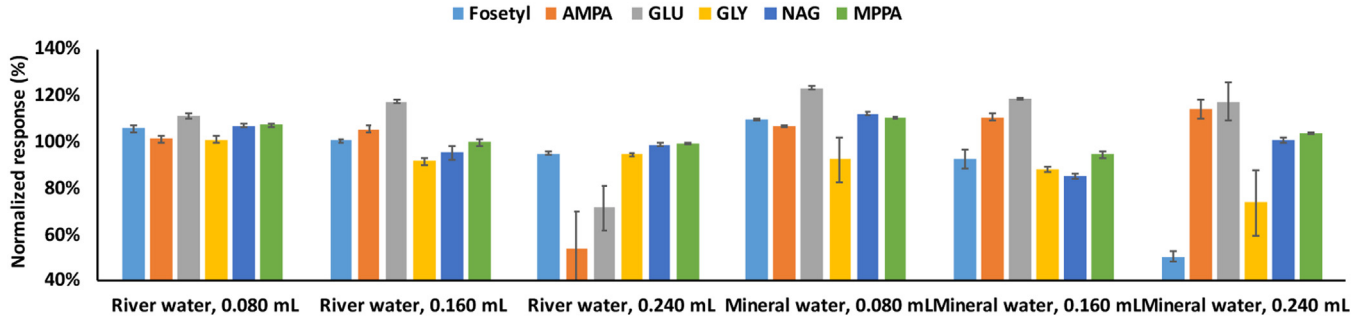

Fig. 2. Slopes of calibration curves obtained for spiked aliquots of two different water samples normalized to those corresponding to ultrapure water. Calibration range: 50 ng L^{-1} to 2000 ng L^{-1}. Error bars reflect standard deviations for the slope of calibration curves corresponding to each matrix.

Table 2
Linearity, accuracy (recoveries for spiked samples, 80, 200 and 500 ng L^{-1}, with SD,%) and LOQs of the SPE on-line LC-QqQ-MS direct analysis method.

Compound	R^2 (5–5000 ng L^{-1})	Recovery (average with SD, $n = 3$ replicates)									LOQs (ng L^{-1})
		Ground water			River water			Tap water			
		80 ng L^{-1}	200 ng L^{-1}	500 ng L^{-1}	80 ng L^{-1}	200 ng L^{-1}	500 ng L^{-1}	80 ng L^{-1}	200 ng L^{-1}	500 ng L^{-1}	
Fosetyl	0.9994	71 (4)	77 (7)	122 (6)	115 (2)	91 (5)	95 (7)	118 (2)	92 (3)	126 (1)	5
AMPA	0.9993	102 (2)	88 (5)	112 (3)	83 (3)	80 (3)	86 (6)	101 (2)	89 (4)	106 (8)	5
GLU	0.9997	99 (3)	85 (3)	111 (1)	98 (1)	84 (5)	95 (8)	104 (3)	87 (1)	105 (5)	20
GLY	0.9999	93 (1)	85 (4)	106 (2)	103 (4)	87 (4)	88 (9)	101 (4)	86 (3)	114 (4)	10
NAG	0.998	87 (3)	99 (6)	101 (27)	79 (1)	92 (4)	97 (8)	83 (3)	93 (4)	141 (3)	5
MPPA	0.999	90 (2)	102 (6)	102 (14)	88 (3)	97 (5)	101 (9)	91 (2)	99 (4)	111 (2)	5

The accuracy of the method was investigated considering three water matrices and three addition levels (80 ng L^{-1}, 200 ng L^{-1} and 500 ng L^{-1}). Obtained data are summarized in Table 2. In general, recoveries varied in the range from 80% to 105%, with associated standard deviations (SDs) below 8%. In the particular case of Fosetyl, recoveries for the different matrices showed average percentages between 71% and 126%, with similar SDs to those reported for the rest of compounds, Table 2. Finally, a recovery around 140% was observed for NAG in one of the samples spiked at 500 ng L^{-1}. The LOQs of the procedure, calculated as described in Section 2.5, varied between 5 and 20 ng L^{-1}. GLY and GLU were the species displaying the higher LOQs (10 and 20 ng L^{-1}, respectively), due to the relatively low intensity of the qualification transitions (Q2 and Q3) for the first pesticide (Fig. 3D), and the presence of an interfering peak in the Q2 transition of GLU (Fig. 3C). In order to get these values, the LC instrument was daily conditioned using a solution of citric acid (5 mM) at a flow of 1 mL min^{-1}, for 20 min, before installing the anionic exchange column. No modifications were made in the hardware of the LC-QqQ-MS system apart from (1) using an extended injector seat (0.5 mL volume, made of PEEK) to accommodate the sample before being loaded in the SPE cartridge and (2) connecting the outlet of the column directly to the ESI source (avoiding the six-port valve of the MS spectrometer). The analytical column was used for more than 1000 injections without losses of performance, and the on-line cartridge was replaced when increased pressure values were noticed (c.a. every 300 extraction-desorption cycles).

The previous application of anionic exchange chromatography, considering similar LC-MS/MS conditions to those applied in the current research, achieved LOQs of 100 ng L^{-1} for AMPA, GLY and GLU for direct injection of 0.05 mL samples [18]. Thus, the on-line SPE step shows a significant impact in the sensitivity of the analytical procedure, maintaining the simplicity of the direct injection method, at the expense of a little increase in the duration of the concentration-determination step. LOQs attained in this research are also lower than those attained for GLY and AMPA considering SPE on-line connected to cationic exchange LC, post-column derivatization and fluorescence detection [21]. To the best of our knowledge, the lowest LOQs achieved for GLY, AMPA and GLU in surface water corresponded to the combination of FMOC–Cl compounds derivatization followed by concentration of samples in reversed-phase type sorbents, either in the off-line [11], or on-line modes [10,12]. The above approaches reported LOQs in the range between 0.7 to 5 ng L^{-1}. On the other hand, compounds derivatization was time-consuming and these methods do not cover the determination of Fosetyl and MPPA, since they do not react with FMOC–Cl.

Table 3
Summary of concentrations (ng L^{-1}) for compounds above method LOQs in the set of 66 water samples.

Value	AMPA	GLY	Fosetyl
Maximum	1505.6	3027.5	141.1
Median	44.2	26.9	8.8
Average	110.7	204.7	26.3
Positive samples (%)	88	38	12
Samples above 100 ng L^{-1} (%)	20	6	2

3.3. Occurrence of pesticides in surface water samples

Levels of target compounds in processed samples are given as supplementary information, Table S3. Out of six investigated species, only AMPA, GLY and Fosetyl were noticed. Table 3 summarizes their maximum, median and average concentrations, together with the percentage of positive samples and those above the environmental threshold of 100 ng L^{-1}. Fig. S4 shows the chromatograms corresponding to quantification and qualification transitions of Fosetyl, AMPA and GLY for a non-spiked sample (sampling point S9, 2nd campaign, Table S2) containing concentrations of these compounds in the range from 8.6 ng L^{-1} (Fosetyl) to 61.1 ng L^{-1} (AMPA). AMPA was the compound showing the highest prevalence, with a detection frequency of 88% and a median concentration of 44.2 ng L^{-1}. Although relatively low, this value is similar to those affecting the embryogenic development of amphibians [27]. GLY showed the highest maximum concentration, with a

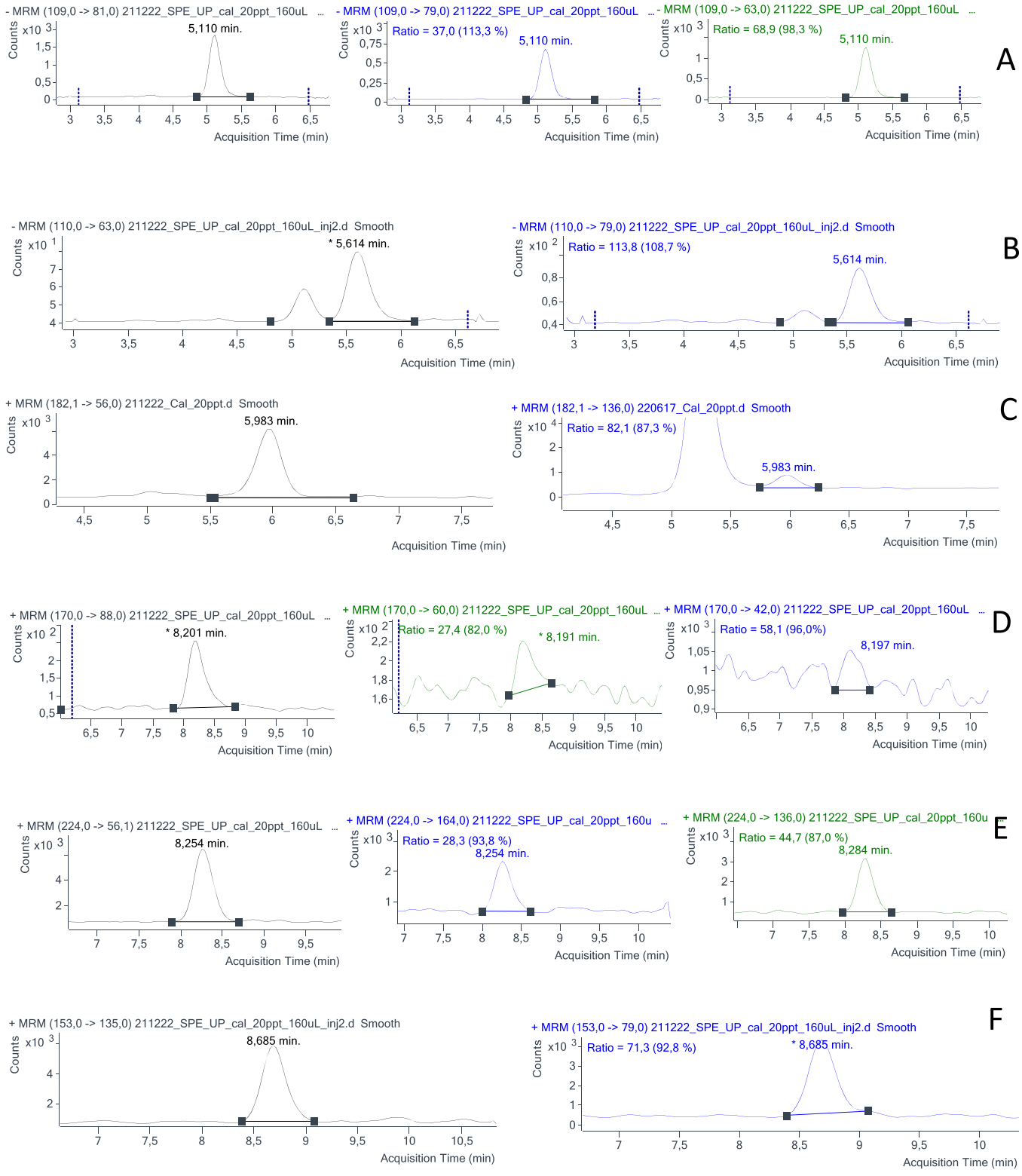

Fig. 3. MRM chromatograms for quantification (Q1, left) and qualification transitions (Q2 to Q3, center to right) of target compounds for a 20 ng L^{-1} standard prepared in ultrapure water. A. Fosetyl. B. AMPA. C. GLU. D. GLY. E. NAG. F. MPPA.

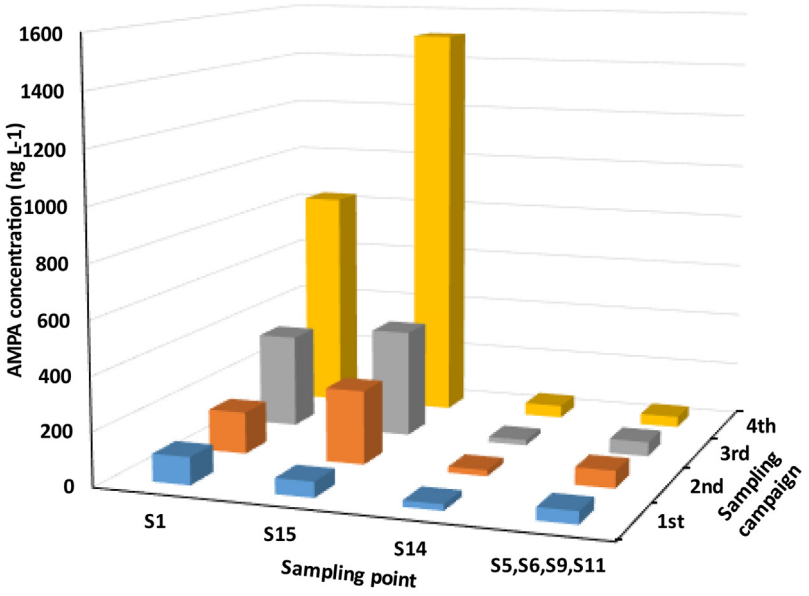

Fig. 4. Seasonal variations in the concentrations of AMPA in sampling points affected (S1, S15) and not affected (S14 and average of S5, S6, S9 and S11) by municipal STPs discharges of treated wastewater.

level above 3000 ng L^{-1} in one of the streams draining the hilly vineyard area; however, the median value (26.9 ng L^{-1}) and the percentage of positive samples for this compound (38%) remained below those obtained for AMPA. This trend is coherent with the higher environmental stability of the later species versus the parent herbicide [7], and potential formation of AMPA from additional precursor molecules. Finally, Fosetyl was the pesticide showing the lowest median concentration (8.8 ng L^{-1}) as well as the smaller percentage of samples above methodś LOQs (12%). Globally, residues of AMPA and GLY found in the set of processed samples stay 1–2 orders of magnitude below those reported in geographic areas, such as USA and South America, where GLY resistant crops are authorized [8,9]. On the other hand, the detection frequencies and average concentrations of both compounds are higher than those corresponding to time-average samples of surface water from agriculture and residential areas in Germany [6].

As regards their geographic distribution, AMPA and GLY were ubiquitous in samples obtained from Limia river (sampling points S16 and S17), whereas Fosetyl remained below method LOQs in this area. Concentrations of the parent pesticide and its degradation product decreased from points S16 to S17 (Table S3), likely due to dilution with the tributary channel joining the main river downstream point S16, Fig. 1. AMPA was also noticed in most samples from the two other investigated areas, except in those obtained from a ground water spring (S10), where all compounds remained undetected. GLY was measured in some surface water samples from the vineyard and the residential areas, with the higher concentrations found in small streams.

The residues of AMPA quantified in sampling points affected by discharges of treated wastewater (codes S1 and S15) were higher than in the rest of surface and spring water from the vineyard and the residential areas, Table S2. Moreover, they increased from spring (1st sampling campaign) to summer (4th campaign), as the flow of streams receiving the discharges from STP decreased significantly, Fig. 4. On the other hand, the average residues of AMPA at sampling points S5, S6, S9 and S11, placed in a large dam containing a practically constant volume of 52 cubic hectometers of water, remained constant during the four sampling campaigns, Fig. 4. This temporal profile of concentrations is coherent with the formation of AMPA not only from GLY, but also from phosphate compounds used in the formulation of detergents during treatment of municipal wastewater, as it has been already pointed out by other authors [11]. Comparison of AMPA levels in samples from the same river (Tinto river), downstream and upstream the discharge of the municipal STP (sampling points S15 and S14), in a residential area, point out again to the contribution of these facilities to the release of AMPA in the aquatic environment, Fig. 4.

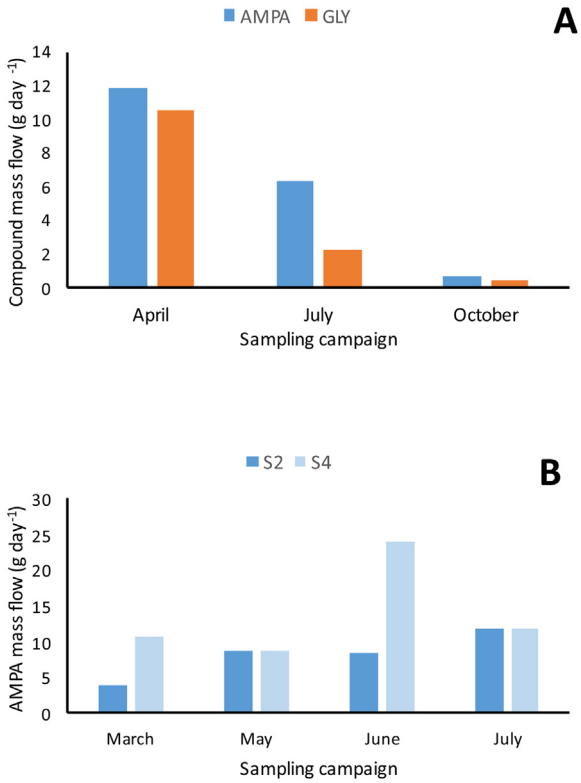

Fig. 5. A, mass flows (g day^{-1}) of GLY and AMPA in sampling point S17 (Limia river). B, mass flow of AMPA (g day^{-1}) in sampling points S2 and S4 (Avia river).

Fig. 5A shows the mass flow (g day^{-1}) of AMPA and GLY at point S17, during the three sampling campaigns. Those for AMPA in points S2 and S4, during four campaigns, are presented in Fig. 5B.

Depicted data reflect the total release of compounds in the aquatic environment, independently of the flow of water courses. In Limia river (point S17), draining the flat, intensive agriculture production area, the release of GLY and AMPA clearly decreased from spring to autumn. On the other hand, the mass flows of AMPA in Avia river (sampling points S2 and S4) were more homogeneous, with the highest value observed in June.

4. Conclusions

The on-line combination of SPE, using a PEEK-lined strong anionic exchange cartridge, with an analytical column containing same stationary phase permitted the sensitive, automated determination of six Zwitterionic pesticides in environmental water samples, without any previous sample pretreatment, except filtration. The procedure achieved LOQs between 5 and 20 ng L^{-1}, with acceptable accuracy values (calculated recoveries from 71% to 126% in all samples, but one), and limited effect of the sample matrix in the responses of target compounds. Thus, it represents a significant improvement compared to direct injection methods using same LC separation mechanism, and a much faster option than FMOC–Cl based derivatization approaches. Analysis of surface water samples, obtained in a residential area and two agriculture basins with different types of crops, showed the presence of AMPA, GLY and, less often, Fosetyl in this environmental compartment. The lower levels of AMPA and GLY were noticed in springs of groundwater, whilst the higher concentrations of AMPA were associated to STPs affected streams and rivers. Further studies should assess the origin of AMPA noticed in this kind of water courses, including the search of additional precursors to GLY.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

J. López-Vázquez: Investigation, Methodology, Writing – review & editing. **L. Pérez-Mayán:** Investigation, Methodology, Writing – review & editing. **V. Fernández-Fernández:** Formal analysis, Writing – review & editing. **R. Cela:** Conceptualization, Funding acquisition. **I. Rodríguez:** Conceptualization, Supervision, Funding acquisition, Writing – original draft.

Data availability

Data will be made available on request.

Acknowledgments

L.P.M thanks a FPU grant to the Spanish Ministry of Science. This study was supported by Spanish Government and Xunta de Galicia through grants *PGC2018–094613-B-I00* and *ED431C 2021/06*, respectively. Both projects are co-funded by the EU FEDER program. We acknowledge Agilent for providing access to LC-ESI-MS/MS instrumentation and technical assistance with anionic exchange LC separations.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.463697.

References

[1] F. Veiga, J.M. Zapata, M.L. Fernandez Marcos, E. Alvarez, Dynamics of glyphosate and aminomethylphosphonic acid in a forest soil in Galicia, north-west Spain, Sci. Total Environ. 271 (2001) 135–144, doi:10.1016/S0048-9697(00)00839-1.

[2] O. Delhomme, A. Rodrigues, A. Hernandez, S. Chimjarn, C. Bertrand, M. Bourdat-Deschamps, C. Fritsch, C. Pelosi, S. Nélieu, M. Millet, A method to assess glyphosate, glufosinate and aminomethylphosphonic acid in soil and earthworms, J. Chromatogr. A. 1651 (2021) 462339, doi:10.1016/j.chroma.2021.462339.

[3] PPDB: pesticide properties DataBase, (n.d.). https://sitem.herts.ac.uk/aeru/ppdb/en/Reports/373.htm (accessed October 9, 2022).

[4] O.K. Borggaard, A.L. Gimsing, Fate of glyphosate in soil and the possibility of leaching to ground and surface waters : a review, Pestic. Manag. Sci. 456 (2008) 441–456, doi:10.1002/ps.1512.

[5] L. Simonsen, I.S. Fomsgaard, B. Svensmark, N.H. Spliid, Fate and availability of glyphosate and AMPA in agricultural soil, J. Environ. Sci. Heal. Part B. 43 (2008) 365–375, doi:10.1080/03601230802062000.

[6] N. Tauchnitz, F. Kurzius, H. Rupp, G. Schmidt, B. Hauser, M. Schrödter, R. Meissner, Assessment of pesticide inputs into surface water by agriculture and urban sources- a case study in the Querne/Weida catchment, central Germany, Environ. Pollut. 267 (2020) 115186, doi:10.1016/j.envpol.2020.115186.

[7] V.C. Aparicio, E. De Gerónimo, D. Marino, J. Primost, P. Carriquiriborde, J.L. Costa, Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins, Chemosphere 93 (2013) 1866–1873, doi:10.1016/j.chemosphere.2013.06.041.

[8] D.J. Pérez, F.G. Iturbure, G. Calderon, L.A.E. Oyesqui, E. De Gerónimo, V.C. Aparicio, Ecological risk assessment of current-use pesticides and biocides in soils, sediments and surface water of a mixed land-use basin of the Pampas region, Argentina, Chemosphere 263 (2021) 128061, doi:10.1016/j.chemosphere.2020.128061.

[9] L. Medalie, N.T. Baker, M.E. Shoda, W.W. Stone, M.T. Meyer, E.G. Stets, M. Wilson, Influence of land use and region on glyphosate and aminomethylphosphonic acid in streams in the USA, Sci. Total Environ. 707 (2020) 136008, doi:10.1016/j.scitotenv.2019.136008.

[10] M. Ibáñez, Ó.J. Pozo, J.V. Sancho, F.J. López, F. Hernández, Residue determination of glyphosate, glufosinate and aminomethylphosphonic acid in water and soil samples by liquid chromatography coupled to electrospray tandem mass spectrometry, J. Chromatogr. A. 1081 (2005) 145–155, doi:10.1016/j.chroma.2005.05.041.

[11] I. Hanke, H. Singer, J. Hollender, Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection, Anal. Bioanal. Chem. 391 (2008) 2265–2276, doi:10.1007/s00216-008-2134-5.

[12] T. Poiger, I.J. Buerge, A. Bächli, M.D. Müller, M.E. Balmer, Occurrence of the herbicide glyphosate and its metabolite AMPA in surface waters in Switzerland determined with on-line solid phase extraction LC-MS/MS, Environ. Sci. Pollut. Res. 24 (2017) 1588–1596, doi:10.1007/s11356-016-7835-2.

[13] T. Arkan, I. Molnár-Perl, Advances in the alkylsilyl derivatization of glyphosate and aminomethylphosphonic acid: a critical comeback to the N-tert.-butyldimethylsilyl-N-methyltrifluoroacetamide reagent, Microchem. J. 132 (2017) 262–267, doi:10.1016/j.microc.2017.02.004.

[14] A. Connolly, S. Koslitz, D. Bury, T. Brüning, A. Conrad, M. Kolossa-Gehring, M.A. Coggins, H.M. Koch, Sensitive and selective quantification of glyphosate and aminomethylphosphonic acid (AMPA) in urine of the general population by gas chromatography-tandem mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1158 (2020) 122348, doi:10.1016/j.jchromb.2020.122348.

[15] H. Guo, L.S. Riter, C.E. Wujcik, D.W. Armstrong, Direct and sensitive determination of glyphosate and aminomethylphosphonic acid in environmental water samples by high performance liquid chromatography coupled to electrospray tandem mass spectrometry, J. Chromatogr. A. 1443 (2016) 93–100, doi:10.1016/j.chroma.2016.03.020.

[16] E. Okada, T. Coggan, T. Anumol, B. Clarke, G. Allinson, A simple and rapid direct injection method for the determination of glyphosate and AMPA in environmental water samples, Anal. Bioanal. Chem. 411 (2019) 715–724, doi:10.1007/s00216-018-1490-z.

[17] L. Pérez-Mayán, G. Castro, M. Ramil, R. Cela, I. Rodríguez, Approaches to liquid chromatography tandem mass spectrometry assessment of glyphosate residues in wine, Anal. Bioanal. Chem. 414 (2021) 1445–1455, doi:10.1007/s00216-021-03775-w.

[18] Y. Geng, L. Jiang, D. Zhang, B. Liu, J. Zhang, H. Cheng, L. Wang, Y. Peng, Y. Wang, Y. Zhao, Y. Xu, X. Liu, Glyphosate, aminomethylphosphonic acid, and glufosinate ammonium in agricultural groundwater and surface water in China from 2017 to 2018: occurrence, main driver, and environmental risk assessment, Sci. Total Environ. 769 (2021) 144396, doi:10.1016/j.scitotenv.2020.144396.

[19] U. Jost, F. Habedank, Two-dimensional hydrophilic interaction and reversed phase liquid chromatography easily extracted pesticides and polar pesticides multi-residue method––a concept, J. Chromatogr. A. 1621 (2020) 461040, doi:10.1016/j.chroma.2020.461040.

[20] S. Dovidauskas, I.A. Okada, F.R. dos Santos, Validation of a simple ion chromatography method for simultaneous determination of glyphosate, aminomethylphosphonic acid and ions of Public Health concern in water in-

tended for human consumption, J. Chromatogr. A. 1632 (2020) 461603, doi:10.1016/j.chroma.2020.461603.
[21] J. Patsias, A. Papadopoulou, E. Papadopoulou-Mourkidou, 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, J. Chromatogr. A. 932 (2001) 83–90, doi:10.1016/S0021-9673(01)01253-5.
[22] C. Hao, D. Morse, F. Morra, X. Zhao, P. Yang, B. Nunn, Direct aqueous determination of glyphosate and related compounds by liquid chromatography/tandem mass spectrometry using reversed-phase and weak anion-exchange mixed-mode column, J. Chromatogr. A. 1218 (2011) 5638–5643, doi:10.1016/j.chroma.2011.06.070.
[23] J.L. Hidalgo-Ruiz, R. Romero-González, J.L. Martínez Vidal, A. Garrido Frenich, Monitoring of polar pesticides and contaminants in edible oils and nuts by liquid chromatography-tandem mass spectrometry, Food Chem 343 (2021), doi:10.1016/j.foodchem.2020.128495.
[24] G. Jia, J. Xu, X. Long, S. Ge, L. Chen, D. Hu, Y. Zhang, Enantioselective degradation and chiral stability of Glufosinate in soil and water samples and formation of 3-Methylphosphinicopropionic acid and N-Acetyl-glufosinate metabolites, J. Agric. Food Chem. 67 (2019) 11312–11321, doi:10.1021/acs.jafc.9b01028.
[25] J.J. Hsiao, O.G. Potter, T. Chu, H. Yin, Improved LC/MS Methods for the Analysis of Metal-Sensitive Analytes Using Medronic Acid as a Mobile Phase Additive, (2018). doi:10.1021/acs.analchem.8b02100.
[26] B. Claude, C. Berho, S. Bayoudh, L. Amalric, E. Coisy, R. Nehmé, P. Morin, Preliminary recovery study of a commercial molecularly imprinted polymer for the extraction of glyphosate and AMPA in different environmental waters using MS, Environ. Sci. Pollut. Res. 24 (2017) 12293–12300, doi:10.1007/s11356-017-8844-5.
[27] M. Cheron, F. Brischoux, Aminomethylphosphonic acid alters amphibian embryonic development at environmental concentrations, Environ. Res. 190 (2020) 109944, doi:10.1016/j.envres.2020.109944.