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Abstract.

A new green method for trace level quantification of four herbicides, glyphosate (GLYP), glufosinate (GLUF), and their main metabolites, aminomethylphosphonic acid (AMPA) and 3-(methyl-phosphinico)- propionic acid (MPPA), was developed. The purification step without any derivatization was conducted by solid-phase extraction using Chelex-100 resin in the Fe (III) form, followed by elution with 5% NH 4 OH. The four analytes were quantified by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. The developed extraction method was validated on five fresh and sea water matrices with mean recoveries ranging from 80.1% to 109.4% (relative standard deviation < 20%). The extraction conditions were evaluated and certified for the high applicability of the extraction method too. The limits of detection (ng/L) in the five water matrices were in ranges 0.70 –4.0, 2.4 –3.9, 1.8 –4.7, and 1.6 –4.0 for GLYP, AMPA, GLUF, and MPPA, respectively. The method was successfully applied to detect the four compounds in surface waters sampled along the Red River Delta region in July 2019. The highest concen- trations were detected at 565, 1,330, 234, and 871 ng/L for GLYP, AMPA, GLUF, and MPPA, respectively. These results showed the potential capacity of this new method for convenient monitoring of herbicides and their metabolites in the diverse natural water system.

1. Introduction

Non-selective herbicides, glyphosate [N-(phosphonomethyl) glycine] (GLYP) and glufosinate [ammonium-DL-homoalanine- 4-yl(methyl)-phosphinate] (GLUF), and their metabolites, aminomethylphosphonic acid (AMPA) and 3-(methyl-phosphinico)- propionic acid (MPPA) (**Table S1**), represent significant threats for the animal, human health and the environment [1–3 , 4–8]. The amount of GLYP used in agriculture rose 13.3-fold, from 43,0 0 0 t in 1994 to 747,0 0 0 t in 2014 in the world [4] . Nowadays, GLYP is used in more than 130 countries, with a total global consumption estimated greater than 825,0 0 0 t [9 , 10]. In Vietnam, GLYP (registered for use since 1994) became the most popular herbicide and is present in around 104 commercial products. GLUF was also authorized in the 1990s but it stays less popular and less sold than GLYP [11] . In 2019, Vietnam became one of the first countries to ban GLYP (decision No.1186/QD-BNN-BVTV-April 10th, 2019), intending to protect human health, animals, ecosystems, and the environment. However, it is estimated that around 5.10 6 L of GLYP has been still circulating during the last past year in Vietnam [12] . This raises the possibilities of herbicide residues maintaining and occurring in the natural water environment after they were applied for weed treatment [13–15] . To access very low concen- tration especially for drinking water (0.1 μg/L) [16] following the maximum residue thresholds of GLYP in the European Union, it is therefore essential to conduct research studies based on an accurate and sensitive analytical method to detect and quantify these compound in the water environment.

To date, from around 1 0 0 0 publications, liquid chromatogra- phy coupled to tandem mass spectrometry (LC-MS/MS) and inte- grated pre-column derivatization using 9-fluorenylmethyl chloro- formate has been the most popular method for the determina- tion of GLYP, GLUF, and their main metabolites in water due to its high selectivity and sensitivity [17–24]. Consequently, this deriva- tization extraction method has been applying for analyte herbi- cide compounds for diverse water sample types collected (see for examples [11 , 21 , 25–28]). Nevertheless, this sample clean-up pro- cedure is rather time-consuming, needs heavy preparation. More- over, it is costly and shows low accuracy as well as sensitivity for the quantification at sub-μg/L level of analytes [29 , 30]. It is, thus, necessary to develop a direct and efficient quantitative method for these compounds such as the one recently proposed by Car- retta et al. [31] , which have used a kit containing the derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (from Waters Corporation, Milford, MA, USA). Some other authors [6 , 32–34] have also optimized a series of two-steps enrichment method by using Chelex-100 resin in combination with AG1-X8 resin to ex- tract these herbicides in surface, waste, and sea waters with high recovery, reproducibility, and less time-consuming procedure than the other ones (**Figure S1**). However, this method still needs a very high concentration of 6 M acid and/or 10 M HCl solutions that may destruct and corrode metal parts in the extraction and evaporation systems. Therefore, other methods avoiding the use of high con- centration of strong acid during pretreatment steps need to be de- veloped. Hence, the present work aimed firstly to develop and validate a new, fast, simple, and safe one-step purification/extraction proce- dure to directly determine concentrations of GLYP, GLUF, and their metabolites in surface river waters, without tedious derivatization by using Fe(III) forms in Chelex-100 resin. This procedure is as- sociated with a quantification method by ultra-performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) in different typical water samples (ultra-pure or tap water, fresh river water, estuary, and sea waters). This method was then applied to determine the concentrations of the four compounds in natural surface waters of the Red River Delta (RRD), Vietnam, from Hanoi city to the end of Ba Lat estuary (see **Figure S2** and **Table S2**).

2. Material and methods

2.1. Standards and reagents

Glyphosate, glufosinate, aminomethylphosphonic acid, 3- (methyl phosphinico) propionic acid were purchased from Sigma Aldrich (Singapore). The isotopically labeled internal standards, [13 C 3 , 15 N]- *N* -(phosphonomethyl)-glycine (labeled GLYP) and [13 C 3 , 15 N, methylene-D 2] aminomethylphosphonic acid (labeled AMPA) were obtained from Cambridge Isotope Laboratories (Saint-Aubin, France). HPLC-grade formic acid (FA) (≥98%), hydrochloric acid (HCl) (36.5 –38%), ammonia (25%), and acetonitrile (ACN) (HPLC grade) were purchased from Merck (Singapore). Chelex-100 sodium form resin (50 – 100 mesh, dry) were also obtained from Sigma-Aldrich (Singapore). FeCl 3 hexahydrate (99%) from Sigma- Aldrich was used for conditioning Chelex resin after swelling in ultra-pure water (UPW) (Milli-Q, Merck, Singapore, 18.2 M _.cm, TOC < 2 μg/L). The stock solutions of GLYP, GLUF, AMPA and MPPA were prepared at 1 0 0 0 mg/L in UPW then stored –20 °C in 15 mL tubes (Eppendorf, Germany). Standard solutions were prepared at 1 0 0 0 μg/L concentration and stored at –20 °C in Eppendorf tubes. The concentration of the internal standards (IS) in the working solution was 100.0 μg/L, and prepared from initial IS solution 10.0 mg/L, which was stored at 4 °C in dark during the experimental period.

2.2. Instrumentation

Liquid chromatography analysis was performed on an ACQUYTY Ultra Performance LC system (Waters, Milford, MA, USA). The analyte separation was conducted on a Dionex ionPAC CS 12A IC column (4 mm x 250 mm x 5 μm, Thermo Scientific, Waltham, MA, USA), since the four compounds were well separated by using cation-exchange column combining acidic mobile phase compatible with mass spectrometry in a direct analysis [30 , 35]. The mobile phase was a 95:5 (v:v) mixture of UPW and ACN (containing 0.5% formic acid in both solvents). The pH value of mobile phases normally was 2.2 ±0.1. The column was operated at a flow rate of 0.45 mL/min with isocratic elution mode. The column temperature was constantly kept at 30 °C. The auto-sampler temperature was set at 4 °C and the injection volume was 10.0 μL. The target compounds were then detected by tandem mass spectrometry (MS/MS) using triple quadrupole mass spectrometry (Xevo TQD, Waters, Manchester, UK) with an orthogonal Z-spray-electrospray interface. The mass spectrometer was operated in negative electrospray ionization mode and data were acquired using multiple reaction monitoring (MRM). The performance of the mass spectrometer was checked by the tune and accurate mass calibration solutions (Waters, Manchester, UK). The optimized ionization source parameters were the same for both periods: source temperature, 150 °C; ionization voltage, –3 kV; desolvation temperature, 550 °C; curtain gas, 900 L/h; solvate pressure, 7 bar. N 2 was used as desolvation gas and extracted from room air by an N 2 generator (N2–14, Parker Hannifin Corp, Haverhill, MA, USA). Optimum ionization of the TQD for each target was determined by direct injection of the 1.0 mg/L standard solutions in the multiple reaction monitoring (MRM) mode. In MRM mode, the two most important parameters are collision energy and production ions. For each analyte, the two best transitions were used as quantitative and confirmation transition pairs. The most fragment ions for the quantitative four compounds were 168 → 81 (GLYP), 110 → 81 (AMPA), 180 → 95 (GLUF), and 151 → 78 (MPPA). Besides, the transitions 170 → 81 and 114 → 81 were found for GLYP-IS and AMPA-IS, respectively (**Figure S3** and **Table S3)** . Dwell times of 0.050 and 0.025 s were chosen for the corresponding GLYP and other analytes transitions. The MassLynx v 4.1 (Waters, Manchester, UK) was used for control, acquisition, and data evaluation.

2.3. One-step purification of water sample

Generally, 250 mL of collected or stored sample was used for herbicide extraction. Firstly, the pH of each sample was adjusted to 2.0 ±0.1 by adding 6 M HCl in order to prevent possible interactions of herbicide compounds with matrix and bottle components. Samples were then filtrated by 0.45 μm cellulose nitrate membrane (Whatman), which was washed several times before and after filtering with 0.01 M HCl solution. Subsequently, the sample was spiked with 100.0 μL of the internal standard (IS) mixture solution (to achieve 100 μg/L IS concentration in the final extracted solution) and put at room temperature for at least 1 h. For preparing the SPE column, 2 mL swell Chelex-100 resin was poured into an 8 mL empty PP tube fitted with 20 μm polyethylene (PE) frit and a stop cock. Then, the one-step extraction procedure for herbicides extraction was followed the main extraction steps summarized in **Fig. 1** as: (i) **column activation** : the Chelex-100 resin was coated continuously with acidic 0.033 M FeCl 3 and subsequently washed with acid HCl solution (0.02 M) as described by Popp et al. [6] (**Figure S1**); (ii) **sample loading** : the acidic water sample was injected into the column of Chelex resin in the formed Fe(III) at an approximate flow rate of 5 mL/min using Visiprep vacuum manifolds from Supelco (Sigma-Aldrich, Singapore). The sample bottle was rinsed with HCl solution (0.01 M) after sample injection into the column; (iii) **washing column** : the column was washed with 10 mL HCl (0.02 M) and 10 mL UPW to discard all previous fractions. At these steps, insignificant leached ion Fe(III) was measured in waste solutions after loading samples by using the standard colorimetric method for iron analysis [36] . At the same time, the elimination of significant interference was also checked and confirmed based on the relative mass balances of Ca 2 + , Mg 2 + , and Na + or salinity, etc., found in the loaded water sample and leached waste solution from the SPE column; and (iv) **analytes elution** : analytes were then slowly eluted through the column with 8 mL of 5% NH 4 OH solution (volumetric concentration in UPW). The eluent solution was collected in a 25 mL PE flask which was pre-rinsed with UPW. In a final step, the eluent solution was completely evaporated at 60 °C with low pressure using a Speed *vac* Concentrator (Thermo Fischer, Massachusetts, USA) and reconstituted with 1 mL UPW. The aqueous solution was filtered via a 0.22 μm syringe filter and transferred to a 1.5 mL amber glass LC vial. The clear solution was then injected triplicated into UPLC-MS/MS for each extraction. Globally, this extraction procedure, for which separation steps are minimized and less toxic chemicals are consumed with respect to their amounts and volumes, follows at least three (3 to 5) of the twelve principles of green engineering and might be considered as a green method [37] . All the sample extraction procedures are summarized in **Fig. 1**.

2.4. In-house validation of the developed method

Validation of the method was performed according to European guidelines on analytical quantity control and validation procedures for pesticide analysis in the laboratory [38 , 39]. Thus, the extraction yield and matrix effect were evaluated to validate the one- step purification method for further water monitoring from different water matrices: UPW, tap water, Red River estuary and sea waters. River water samples were collected in the Red River stream close to Hanoi, whereas estuary and sea waters were collected at Balat estuary and East Sea of Nam Dinh city, respectively. Tap water from the water supply system and UPW were sampled in our free-contamination laboratory (Water-Environment-Oceanography (WEO) department, USTH, Hanoi) at room temperature using PP bottles in the same manner collection of natural water. Tap water was collected underneath from clean faucets after flushing thoroughly at least 2 to 3 min after getting a constant temperature. The main parameters of the different water samples for these tests are reported in **Table S4.** To evaluate the extraction performance and fixing the extraction conditions, the sample extraction volumes (from 250 to 1 0 0 0 mL) as well as the concentrations (from 0.5% to 10%) and volumes (from 3.0 mL to 10.0 mL) of elution solution NH 4 OH were tested following the

mean recovery values. Also, to investigate the matrix effect, spiking tests were performed in these water matrices at three different concentrations (160.0, 400.0, and 600.0 ng/L) including internal standards (IS). The following injection sequence was implemented on LC-MS/MS in order to perform
natural water blank subtraction for each water type: UPW, blank, and spiked sample (each solution was injected triplicated). In this sequence, UPW was used to check the reference of the analysis system. For each different water type, samples were spiked and analyzed in triplicate. Then, the linear curves were plotted as a linear function of the peak area ratio *vs.* the concentration of each target analyte. Corresponding ISs were used for GLYP and AMPA, whereas AMPA-IS was used for GLUF as they are both primary amines, and for secondary amines, GLYP-IS was also used for MPPA [17 , 40]. The standard deviation of slopes and intercepts for the different curves were compared as well. The limits of detection (LOD) and the limits of quantification (LOQ) of the method were determined from spiked samples of five matrices surface water samples as the lowest concentrations, showing a signal/noise (S/N) ratio equal to or above 3 and 10, respectively [41 , 42]. These determinations consisting in the previous detection of the instrument LOQs for different compounds were set at the lowest standard concentrations, which showed the S/N ratios of analyte transitions greater than the threshold after injecting the series of standard solutions with decreasing concentration from 100.0 to 0.1 μg/L. The LODs and LOQs of the four analytes were then spiked in different environmental water samples at levels of instrument LOQs. Consequently, the matrix effect, extraction efficiency, and overall recovery were taken into account for LOD and LOQ calculations. The accuracy and precision of the extraction method were assessed and evaluated in all water matrices based on the recoveries of spiked concentrations at 80.0, 320.0, and 800.0 ng/L using linear fitting established in natural Red River water samples at ten different spiked concentrations varying from 40.0 to 800.0 ng/L (corresponding to spiked concentrations from 10.0 to 200.0 μg/L in 1.0 mL of final extracted solutions). Standard calibration curves built from natural river samples were also applied for the quantification of environmental samples. Besides, the method repeatability was assessed by calculating standard deviations of responses from thirty spiked river water samples at each of the three concentrations (40.0, 400.0, and 720.0 ng/L) within three months.

2.5. Application to natural water analysis

The validated methodology was applied to monitor the four analytes in surface waters of the main Red River stream crossing the RRD region. Nearby riverbanks, country farmers are employed in agriculture to produce rice, the national vegetables, fruits, and crops [43]. Natural water samples were collected at eight different sites including seven samples, RR-1 to RR-7, collected along the RRD transect around Hanoi city, one estuarine, and one sea waters sampled at Ba Lat mouth (RR-8 and RR-9) in July 2019 (**Figure S2** and **Table S2**). The about 1-L surface water samples were transferred to high-density polypropylene (PP) bottles, which were pre-washed with UPW and rinsed thoroughly with sampled water. At each sampling site, the sample was collected using a grab sampler and stored for the later LC-MS/MS analysis. Other physical parameters were measured and recorded directly on the field by using Multi-parameter Waterproof Meter HI98196 (Hanna Instruments, Woonsocket, Rhode Island, USA). The representative on-site parameters were as follows: pH, water temperature, conductivity, hardness, and total organic carbon (see **Table S4**).

3. Results and discussion

3.1. One-step extraction procedure development

Usually, the four analytes, GLYP, GLUF, AMPA, and MPPA, which are highly soluble in water, require cleanup procedures with ionexchange mechanisms for the direct extraction

procedures [44] . For instance, the Fe(III) coating formed in the Chelex-100 resin at pH 2.0 can complex organic ligands having carboxylic, amine, and phosphonate groups to create the complexation with herbicides molecules [45 , 46]. Speciation calculation based on stability constants determined by Motekaitis and Martell [46] showed that glyphosate is mainly present as Fe(III)-complexes at low pH (**Fig. 2**). This supports the assumption of an efficient and selective pre-concentration of analytes even from matrix-rich samples. However, this complexation Fe(III)-GLYP decreases when pH increases above 3 with the formation of other aqueous GLYP species due to the formation Fe(III) hydroxo complexes. Additionally, it has been shown less adsorption ability of analytes on Fe(III) (hydr)oxides such as ferrihydrite or goethite for pH greater than pH 5.6 [47] . From speciation calculation (**Fig. 2**), desorption of GLYP and other ligands will occur by increasing pH. Different aqueous solutions such as NH 4 OH, NH 4 HCO 3 , (NH 4) 2 CO 3, or (NH 4) 2 SO 4 , could be possible but the presence of anions such as sulfate, carbonate, or hydroxyl could lead to Fe(III) precipitate interfering with ligand desorption. Moreover, ammonium ion can also suppress the broad peaks of GLYP and other analytes on chromatography coupled mass spectrometry analysis [48] . NH 4 OH sample solution was finally chosen to directly elute the 4 compounds after their preconcentration in the Chelex-100 resin in the Fe(III) form. Indeed NH 4 OH has the advantage both not to change Fe(III) (hydr)oxide precipitate and to be completely evaporated under the nitrogen flow at 60 °C before reconstitution in UPW for target compounds measurement on LC-MS/MS system. The effect of the NH 4 OH ion components and the enrichment factor were tested in five repeated experiments using sea water spiked at 80.0 ng/L for all target compounds following the previously described procedure (see **Section 2.3**) since sea water represents the most complex water matrix including organic matter, metal ions, and high salinity (**Table S4**). After analysis, the results reported in **Fig. 3** and **Table S5** showed that the different extraction conditions had a significant effect on the extraction yields and were consistent with speciation calculation (**Fig. 2**). Briefly, the extraction efficiencies for the four compounds increased when the volume of the 5% NH 4 OH solution increased from 3 to 8 mL and then were stable relatively at 10 mL (**Fig. 3 A**). Similarly, the whole extraction procedure provided high recovery values in the range 85.6% –105.5% for all compounds at testing concentrations of 5% and 10% NH 4 OH (**Fig. 3 B**). However, at different sample volumes, all analytes achieved high recovery ranged from 71.1% to 107.9%, which evidenced for less effect of the sample volume on the variety of the extraction performance (**Fig. 3 C**). Therefore, these results confirmed reliability for the high extraction performance as the procedure schemed in **Fig. 1** .

3.2. Matrix effect

In LC-MS/MS analysis, the sample matrix effect, one of the most important factors which directly influence the analytical method performance (sensitivity, reliability), is often evaluated through several experiments such as post-extraction spiking experiment, matrix-match calibration curves, and isotopic labeled IS spiking tests [49] . In this study, following the European guideline [38 , 39], the matrix effect was evaluated base on the linearity of the spiking test in real water samples at three different concentrations of target compounds. In each water matrix, the response factors, *i.e.* , the relationship between the ratio sample response area/IS area and the concentration, can be assumed linear for the four compounds (**Fig. 4**) with determination coefficient values (R 2) all above 0.90 and RSD (%) values always lower than 20%. The slopes were varying between 0.021 and 0.024 for AMPA, 0.059 and 0.072 for GLYP, 0.029 and 0.037 for MPPA, and between 0.032 and 0.059 for GLUF. Both slope and intercept values were not significantly different between the different sample matrices for four compounds (**Table S6**). Interestingly, the extraction procedures for estuary and sea waters showed purification performance similar to that of other water sources (river,

tap, and UPW), although these water types have more complex matrices with higher values of hardness and electro-conductivity (**Table S4**).
These results, therefore, confirmed the elimination of the water matrix effect during purification steps. Those results showed that this method is applicable for a large range of water matrices and is thus relevant for monitoring water samples following river streams from inland down to the sea, as done here for the Red River stream flowing across the RRD region. Moreover, they confirmed the suitability of river water as a calibration matrix: river water indeed showed similar slopes and interferences to that of environmental matrices and natural water sources, making it suitable for building calibration curves.

3.3. Limits of detection and quantification

The LODs and LOQs values of the four analytes were determined experimentally by analyzing different water matrices spiked at 4.0 or 20.0 ng/L (corresponding 1.0 µg/L and 5.0 µg/L spiked concentrations in the final 1 mL extracted solutions, based on the instrument quantification limits of the four compounds in the standard solutions found at 0.5 µg/L (for GLYP and AMPA) and 1.0 µg/L (for GLUF and MPPA). Five replicates of spiked matrices were prepared and analyzed for each analyte. According to results of S/N ratios (equal and higher than 3) obtained from different water matrices spiked at 4.0 ng/L (**Table S7**), the LOD (ng/L) were investigated in the following ranges: 0.70 –4.0, 2.4 –3.9, 1.8 –4.7, and 1.6 –4.0, for GLYP, AMPA, GLUF, and MPPA, respectively. On the other hand, estuary and sea water matrices spiked at higher concentration (20.0 ng/L) showed S/N ratios generally close to 10 for all compounds while this ratio was often much higher than 10 for freshwater types (**Table S7**). Thus, higher scales of LOQ (ng/L) of the introduced method was achieved in the estuary and sea water, which ranged: 8.7 –20.2, 13.0 –21.7, 14.6 –19.3, and 9.4 –22.4 for GLYP, GLUF, AMPA, and MPPA, respectively, compared to those quantifications in UPW, tap, and river waters with lower LOQs values ranged in 2.2 –9.2 (ng/L) for all analytes. The sensitivity of the developed method is consistent concerning recent works (**Table S8)** , in which analytical method processes without sample pre-concentration, clean up or non-derivatization steps are shown to increase the detection capacity of the method. For instance, Coutinho et al. [50] described LODs values of 35.0 and 240.0 ng/L for GLYP and AMPA respectively in mineral water by using anion exchange chromatography and coulometric detection. Guo et al. [30] reported the LODs for both GLYP and AMPA in six water matrices (drinking, tap, lake, creek, stream, and ground waters) were determined to be 100.0 ng/L whereas the LODs values ranged from 20.0 to 50.0 ng/L for both analytes. In surface water, Guo et al. [51] used HLB cartridges and HIILIC column to detect directly GLYP, GLUF, AMPA and MPPA in surface water with very low LODs and LOQs ranged in 100.0 –150.0 ng/L and 300.0 –500.0 ng/L, respectively. However, direct analysis methods with non-enrichment procedures are less effective on water matrices with complex components. Indeed, the ion suppression in the different water matrices increases in the following order: groundwater < tap water < surface water [52] . Besides, river water can contain a high amount of mud, organic matter and colloids while estuary while sea waters can contain more salt [52] . All these water components need to be washed or removed during extraction in the period of sample preparation before injecting water into analysis instruments. Finally, we could assume that removal of almost water matrix effects was achieved by one-step extraction as shown in this study, and therefore enhanced the limit of detection of the analytical method with very low LODs and LOQs compared to other studies (see **Table S8**).

3.4. Linearity, method precision, and repeatability

In order to simultaneously analyze the four compounds in all water types by using one calibration curve, the linearity range for the four analytes was established from 10-points spiked river water samples collected in the RRD in ranges 40 –800 ng/L (according to the

final concentrations in 1 mL of analysis solution ranging from 10 to 200 µg/L, including the enrichment factor (250 folds)) for the four analytes. The straight lines were obtained by plotting the peak area ratios between analytes and ISs as a linear function of the concentration of each target analyte at IS spiked concentration fixed at 100 µg/L. For each compound, calibration was established in triplicate. The peak area ratios of the target analyte were taken into account in the linear range. The precision and accuracy of linearity were evaluated based on RSD (%) values of slope and intercepts from repeated experiments (**Table S9**). A good correlation of all target analytes was obtained (the R 2 values are greater than 0.98 for all target analytes). RSD (%) of both slope and intercept of all target analytes were below 12.6%. Recovery tests were conducted to evaluate the precision and accuracy of the analytical method in nine replicated spiked samples within a day at respective 80, 320, and 800 ng/L in five water types to evaluate the accuracy of the method (see the optimized protocol in **Section 2.3**). For all compounds and water matrices, recovery was evaluated by analyzing spiked samples at three levels of concentration in nine repeated experiments ranging between 80.1 and 109.4% with RSD almost always lower than 20% at respective LOQ levels (**Table 1)** . In such conditions, we could assume that the extraction method shows highly effective in directly analyzing simultaneously GLYP, GLUF, and their metabolites for a large range of water origins. Then, the method repeatability or inter-day precision was evaluated by calculating the RSD (%) of responses from three spiked concentrations (40.0, 400.0, and 720.0 ng/L) with thirty triplicated experiments conducted for each spike at different times within three months, following the calibration curves established from spiked river water samples. Variations of responses showed good repeatability with high recovery values varying from 83.5% to 100.4% for the four compounds, corresponding to RSD (%) always lower than 15% (**Table S10)** . Mean yields were substantially increased at the highest concentrations (400.0 and 720.0 ng/L), which can be related to several factors such as the ionization efficiency and/or the influences of the carry-over effect of the analytes in the chromatographic column or sample introduction devices in mass spectrometer [53].

3.5. Natural water analysis

The results reported in **Table 2** showed the successful application of this new analytical method to detect the four compounds in RRD water samples from the river stream to the estuary. Noticeably, the river sample collected at RR-1 location exhibited the maximum concentrations (ng/L) for all compounds, which were 565 ±90, 234.0 ±4.4, 1,330 ±11, and 871 ±61 for GLYP, GLUF, AMPA, and MPPA, respectively. The four-compound concentrations were found higher at the riverside around Hanoi city than at the seaside. Globally, near the river watershed, lands are used for agricultural activities, *e.g.* flood rice fields, vegetable and fruit farms, which require the high application of herbicides [43] . The measured GLYP concentrations in the river stream region (RR-1, 2, and 7) were significantly over the European regulation for its limitation in drinking water [16] . No GLYP was detected in water samples collected at the river mouth (sampling RR-8 and RR- 9). Besides, GLYP metabolite, AMPA was found in five samples from Hanoi city to Balat estuary (RR-1, 2, 3, 7, 8), and mostly higher than GLYP. On the other hand, GLUF was only detected in one sample at site RR-1 while its metabolite, MPPA, was detected in two samples (RR-1 and RR-5) and at higher concentrations (max. 871 ±61 ng/L at RR-1).

4. Conclusions

The new method of one-step extraction analysis was successfully developed and validated for directly detecting herbicides, glyphosate, glufosinate, and their metabolites, AMPA, and MPPA in different water sam ple types (tap, river, estuary, and sea waters). This simple method, which is greener than almost the published ones, meets strictly all the EU, USA,

Canadian, and Australia regulations for analytical method requirements and herbicide limitations in water with very low achieved LOQs (ng/L) values in ranges of 2.7 –20.2, 8.1 – 21.7, 6.4 –19.3, and 5.0 –22.4 for GLYP, GLUF, AMPA, and MPPA analytes in the five water matrices, respectively. Therefore, it has a high potential to be applied to the developed analysis method for the quantification of their concentrations at trace levels in natural water. Consequently, this study provided the short and beginning observation of herbicide fate in natural Red River waters. This opens prospects for next studies focusing on the water monitoring in the whole Red River basin from upstream to sea water and in other river systems in Vietnam as well as other countries. Also, this method, developed for water samples, could be applied to other studies focusing on the fate of the four compounds and/or their interactions with other water components, such as colloids, organic matters, metals ions, etc. This should be an important starting point to provide answers to the crucial question of the behavior and fate of these herbicide family compounds in aquatic systems not only in Vietnam but worldwide too.

Acknowledgment
This study was funded by the University of Science and Technology (USTH), Vietnam (PENROSE project, no. USTH.WEO. 01/18- 19), and benefited from the support of the LOTUS international joint laboratory funded by the Institute of Research of Environment (IRD) (https://lotus.usth.edu.vn). The authors wish to thank the collaboration of our colleagues at WEO-Dept, USTH (httpss://usth.edu.vn). We would like to thank Laboratoire Mixte International - "Drug Resistance in South East Asia"(LMI-DRISA) for supporting the repair fee of UPLC-MS/MS. Last but not least, we warmly thank the supports of the French USTH consortium for the CTV grant to come to France and of the French Embassy in Vietnam (GLAIVE project).

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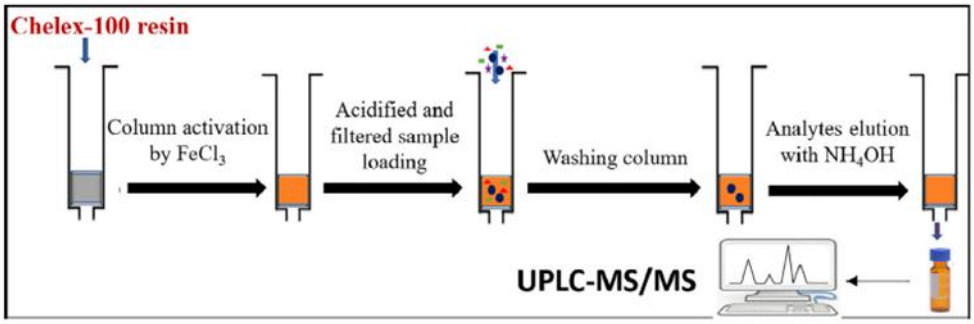

Fig. 1. Schema summarizing the one-step extraction.

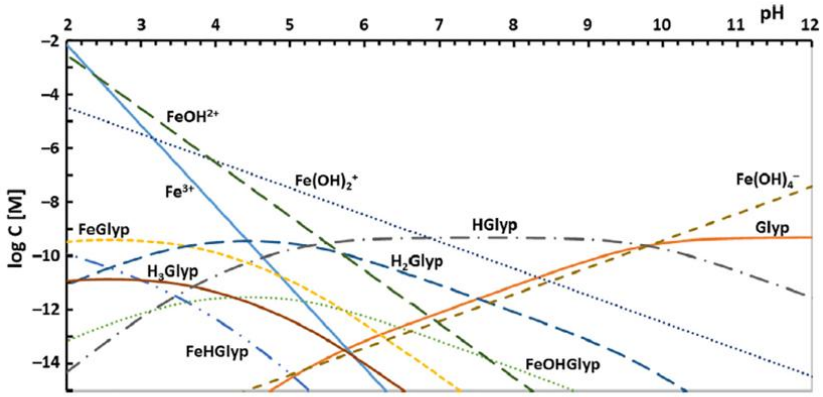

Fig. 2. Calculated speciation of 80 ng/L (0.47 nM) glyphosate in the presence of ferrihydrite, $FeOOH_{(s)}$, at 0.03 M ionic strength using VminteqA2 program (Glyp represents the most deprotonated species of glyphosate). Stability constants for glyphosate complexation with Fe(III) are from Motekaitis and Martell (1985) [46].

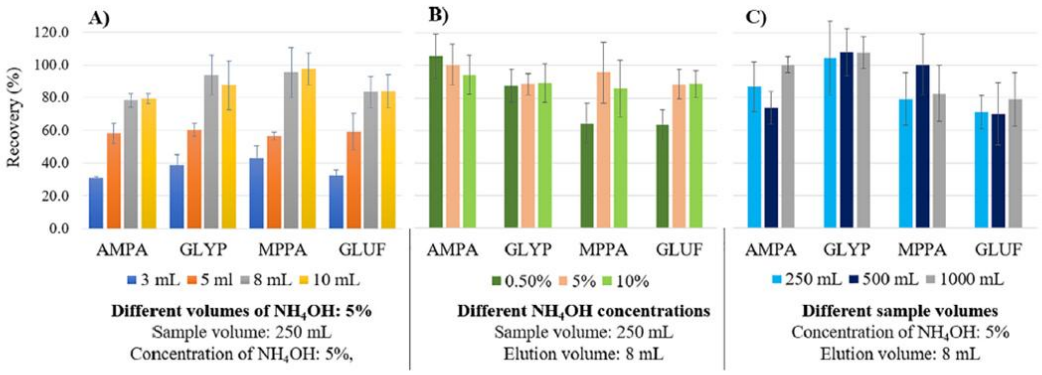

Fig. 3. Evaluation of one-step extraction procedure by determination of mean recoveries of the four target compounds in the function of the differences of ammonium extraction volume (A), ammonium concentration (B), and sample volume (C) at spiked 80 ng/L concentration in sea water. Five replicated experiments were implemented for each condition.

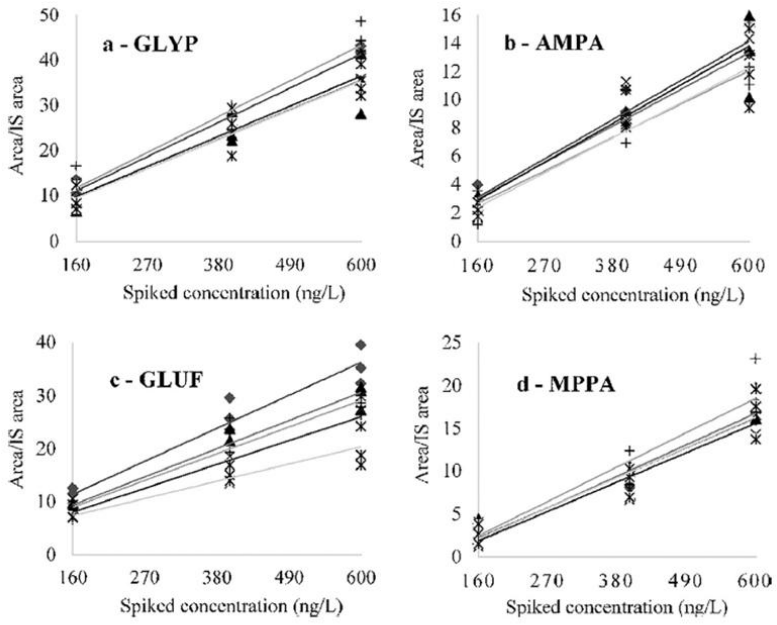

Fig. 4. The linearity of spiked water samples at triplicated three concentrations (160, 400, and 600 ng/L) normalized by internal standards (IS) for a) GLYP; b) AMPA; c) GLUF; and d) MPPA, with different matrices: (◆) Ultrapure-water (UPW), (+) tap water (TW), (▲) river water (RW), (×) estuary water (EW) and (✳) sea water (SW).

Table 1
Mean recoveries of spiked water samples [%, (RSD%)].

Water sample (n = 9)	Spiked concentration (ng/L)	GLYP	AMPA	GLUF	MPPA
	80	91.1 *(14.5)*	87.0 *(15.7)*	88.8 *(13.5)*	85.5 *(10.9)*
UPW	320	95.7 *(10.2)*	109.4 *(7.5)*	102.9 *(13.0)*	94.0 *(9.0)*
	800	101.1 *(14.9)*	93.4 *(10.0)*	90.2 *(8.8)*	92.5 *(10.6)*
	80	86.9 *(17.0)*	81.1 *(13.5)*	81.6 *(12.3)*	85.5 *(18.6)*
Tap water	320	97.6 *(13.6)*	93.7 *(14.3)*	95.8 *(6.6)*	88.2 *(14.7)*
	800	90.1 *(13.3)*	88.6 *(9.5)*	86.8 *(14.6)*	88.4 *(11.3)*
River water	80	87.0 *(14.8)*	89.1 *(16.2)*	83.7 *(19.7)*	80.8 *(10.9)*
	320	93.6 *(12.5)*	91.3 *(10.8)*	92.6 *(8.3)*	92.9 *(9.1)*
	800	90.0 *(13.2)*	96.5 *(11.6)*	91.0 *(5.6)*	90.9 *(10.0)*
	80	88.3 *(14.9)*	81.3 *(17.8)*	82.9 *(19.2)*	80.1 *(18.8)*
Estuary water	320	92.0 *(15.5)*	89.3 *(5.4)*	90.9 *(13.3)*	85.1 *(11.3)*
	800	89.2 *(14.7)*	88.0 *(12.4)*	87.8 *(12.6)*	89.4 *(13.6)*
	80	85.0 *(12.9)*	80.6 *(13.0)*	85.0 *(17.5)*	82.0 *(15.0)*
Sea water	320	90.2 *(14.2)*	90.9 *(8.2)*	89.0 *(16.8)*	88.8 *(15.5)*
	800	90.3 *(8.4)*	88.2 *(9.4)*	84.3 *(16.3)*	85.1 *(12.1)*

Table 2
The concentration of glyphosate, AMPA, glufosinate, and MPPA in nine surface Red River water samples, (Conc. ± SD [ng/L]).

Sample [*]	GLYP	AMPA	GLUF	MPPA
RR-1	565 ± 90	1,330 ± 11	234.0 ± 4.4	871 ± 61
RR-2	501 ± 5	271 ± 28	–	–
RR-3	–	11.0 ± 0.4	ND	ND
RR-4	ND	–	ND	ND
RR-5	–	ND	–	15.0 ± 3.9
RR-6	ND	–	–	ND
RR-7	299.0 ± 1.2	62 ± 12	–	–
RR-8	- -	14.0 ± 2.3	- -	ND
RR-9	ND	ND	ND	ND

SD: Standard deviation was determined from triplicated measurement;.
"-" and "- -": under the limits of detection of analytes in river and estuary matrices respectively; ND: not detected;.

[*] Samples from RR-1 to RR-7 were collected in the main flow of RRD surrounding Hanoi city, while the estuarine and sea waters were sampled at sites RR-8 and RR-9.