Talanta 161 (2016) 700–706

Contents lists available at ScienceDirect

Talanta

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

A simple method for the determination of glyphosate and aminomethylphosphonic acid in seawater matrix with high performance liquid chromatography and fluorescence detection

Shu Wang, Baomin Liu, Dongxing Yuan, Jian Ma*

State Key Laboratory of Marine Environmental Science, College of the Environment and Ecology, Xiamen University, 361102 Xiamen, Fujian, China

ARTICLE INFO

Keywords:
Glyphosate
Aminomethylphosphonic acid
9-fluorenylmethylchloroformate derivatization
Seawater
High performance liquid chromatography

ABSTRACT

Glyphosate (GLYP) is an important herbicide which is also used as the phosphorus source for marine organisms. The wide applications of GLYP can lead to its accumulation in oceans and coastal waters, thus creating environmental issues. However, there is limited methods for detection of GLYP and its degradation product, aminomethylphosphonic acid (AMPA) in saline samples. Therefore, a simple and fast method for the quantification of GLYP and AMPA in seawater matrix has been developed based on the derivatization with 9-fluorenylmethylchloroformate (FMOC-Cl), separation with high performance liquid chromatography (HPLC) and detection with fluorescence detector (FLD). In order to maximize sensitivity, the derivatization procedure was carefully optimized regarding concentration of FMOC-Cl, volume of borate buffer, pH of borate buffer, mixing and derivatization time. The derivatization reaction could be completed within 30 min in seawater samples without any additional clean-up or desalting steps. Under the optimized conditions, the developed HPLC method showed a wide linear response (up to several mg/L, $R^2 > 0.99$). The limits of detection were 0.60 μg/L and 0.30 μg/L for GLYP and AMPA in seawater matrix, respectively. The relative standard deviation was 14.0% for GLYP (1.00 mg/L) and 3.1% for AMPA (100 μg/L) in saline samples with three different operators (n=24). This method was applied to determine the concentration of GLYP and AMPA in seawater culture media and the recovery data indicated minimal matrix interference. Due to its simplicity, high reproducibility and successful application in seawater culture media analysis, this method is a potentially useful analytical technique for both marine research and environmental science.

1. Introduction

Phosphorus (P) is an essential macronutrient for all living organisms, and therefore P recycling is an important focus of biogeochemical researches in the marine science [1]. The analysis, formation, distribution, and turnover of P have been widely studied and reviewed [1–5]. P is present in seawater in both inorganic and organic forms: the inorganic P fraction consists mostly of orthophosphates, as well as other small part of pyrophosphates, and condensed metaphosphates and polyphosphates; while the organic form of P mainly includes monomeric and polymeric esters, phosphonates and organic condensed phosphates [3].

Normally, inorganic P compounds (mainly refer to phosphate) are the preferred source of phosphorus for most organisms. However, while in phosphate-depleted regions of the open ocean, various organic phosphorus derivatives (mostly dissolved form, DOP) could serve as alternative P sources [6]. For example, phosphonates, which contains a stable C–P bond and contributes 25% of DOP content in marine systems, could be utilized by *Trichodesmium* as a P source [7]. As there is limited information available on the concentrations or distributions of specific dissolved phosphonate compounds in seawater, different kinds of phosphonates have been tested as P source in marine biological research [8–13].

Glyphosate [N-(phosphonomethyl) glycine; GLYP] is a widely used broad-spectrum, non-selective, post-emergence herbicide [14], and its main metabolite is aminomethylphosphonic acid (AMPA). GLYP is of low toxicity and considered to be less harmful than that of other herbicides [15]. However, the wide applications of GLYP and its relatively long half-life (7–315 days, most commonly 45–60 days) can lead to its accumulation and persistence in coastal waters [16]. Since GLYP is prone to accumulation in coastal waters and even in open oceans, biological incubation experiments have investigated GLYP as a P source nutrient or growth inhibitor of phytoplankton [16]. Selective culture studies have indicated that several alkylphosphonates, including GLYP and AMPA, can serve as a sole source of P for microbial growth [17]. Very recently, Wang et al. [18] studied the

* Corresponding author.
E-mail address: jma@xmu.edu.cn (J. Ma).

http://dx.doi.org/10.1016/j.talanta.2016.09.023
Received 11 July 2016; Received in revised form 1 September 2016; Accepted 8 September 2016
Available online 13 September 2016

physiological effects of GLYP on 14 species of five major coastal phytoplankton phyla and found that GLYP could be used as P source by some species. However, it was toxic to some species and yet had no effects on some others. Therefore, the continued use of this herbicide will likely exert significant impact on the coastal marine phytoplankton community structure [18]. However, there are no available data on the GLYP concentration variation during these incubation experiments due to the lack of appropriate analytical methods, which limits the further studies of these biological processes.

The determination of GLYP and AMPA in environmental and biological samples are of importance to both environmental scientists and analytical chemists, therefore, various modern instrumental analysis methods have been utilized for this purpose. Different analysis methods, including high performance liquid chromatography (HPLC) coupled with fluorescence detector (FLD) or mass spectrometry (MS), ion chromatography, capillary electrophoresis, gas chromatography, enzyme-linked immunosorbent assay, electrochemical and biological sensors, optical devices, etc., as comprehensively reviewed by Stalikas and Konidari [19] and very recently by Koskinen et al. [20]. Among these methods, HPLC separation, either before or after a derivatization step is generally preferred. The lack of a chromophore or fluorophore in the GLYP molecule prevents its direct detection with conventional systems such as ultraviolet-visible or fluorescence detectors [21,22]. In most cases, pre-column derivatization followed by FLD is utilized, and the most commonly used derivatization reagent for this purpose is 9-fluorenylmethylchloroformate (FMOC-Cl) [23,24], which reacts with both primary and secondary amine groups to produce stable and highly fluorescent derivatives [25].

Pre-column derivatization with FMOC-Cl was initially proposed by Moye and Boning [26]. Since then, various modifications and improvements have been proposed in order to improve the method performance, including clean-up and concentration steps. However, there is no report to date on the simple, efficient and simultaneous quantification of GLYP and AMPA in saline samples, such as coastal water with varied salinity or open seawater with high ionic strength (e.g. salinity 35). The effects of high salt concentrations of seawater samples on the sensitivity and accuracy of this derivatization and determination method are unknown. There are very limited reports about the determination and persistence study of GLYP and AMPA in seawater utilizing MS analysis [27,28]. While electrospray tandem mass spectrometry detection coupled with on-line SPE and liquid chromatography (LC) may have achieved the highest level of selectivity so far [29,30], it requires labor intensive pretreatment steps, addition of ion-pair reagents, more specialized training for operators and higher costs per sample, thus limiting their widespread use, especially in the non-analytical chemistry community.

Therefore, the objective of this study is to develop an optimized FMOC-derivatization based HPLC method for the simple, rapid, and simultaneous determination of GLYP and AMPA, which can be used for biologists in their incubation experiments. The effect of salinity on the effectiveness of the method has been comprehensively evaluated. The established method was successfully applied for the quantification of GLYP and AMPA in seawater culture media and indicated minimal matrix interference in various aqueous samples.

2. Experimental

2.1. Reagents and standards

The GLYP (99.7%) standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). AMPA (99.0%) standard and FMOC-Cl (98.0%) were purchased from J & K Scientific Co., Ltd., China. Glyphosate-FMOC (99.0%) and AMPA-FMOC (98.0%) were obtained from Dr. Ehrenstorfer (Augsburg, Germany). HPLC-grade methanol (MeOH) and acetonitrile (ACN) were obtained from TEDIA Co., USA. The other chemicals, purchased from Sinopharm Chemical Reagent Co., China, were reagent grade or better. Ultra-pure water, collected from a Millipore water purification system (Millipore Co., MA, USA, 18.2 ΩM), was used during the experiment.

Standard stock solutions of GLYP and AMPA at 5.00 g/L were prepared by dissolving accurately weighed 50.0 mg of the each of the powders separately in 10.0 mL water. Working standard solutions in the range of 0.0100–5.00 mg/L were prepared by appropriate stepwise dilutions of the stock solution. These working standard solutions were kept for no more than one week. Standard stock solutions of 500 mg/L of the FMOC-derivatives were prepared in water. For derivatization, FMOC-Cl stock solution of 6.0 mmol/L and borate buffer of 0.50 mol/L were used. The FMOC-Cl solution was prepared daily by dissolving 77.6 mg of FMOC-Cl in 50.0 mL ACN. To obtain the borate buffer of 0.20 mol/L at pH 8.85, 3.05 g sodium tetraborate and 0.49 g boric acid were dissolved in 200 mL water at 50 °C in a water bath. Stock solution of ammonium acetate (0.50 mol/L) was prepared in water, which was diluted with water to 5.0 mmol/L and adjusted with aqueous NH_3 to pH 9.0, to be used as the mobile phase for HPLC. All the solutions were stored at 4 °C in a refrigerator. Artificial seawater of salinity 35 was prepared by dissolving 31 g NaCl and 10 g $MgSO_4 \cdot 7H_2O$ in 1 L water, and stored in polyethylene bottles. Different salinity levels of the artificial seawater were obtained by dilution.

All glassware used in the experiments, except for glass tubes, were washed three times with tap water and then rinsed with ultra-pure water in an ultrasonic bath, oven dried at 100 °C, and finally baked in a muffle furnace at 450 °C for 4 h. After being washed six times with ultra-pure water and MeOH successively in an ultrasonic bath, the glass tubes were then thoroughly rinsed with pure water, and finally, dried naturally in a clean box. The clean glassware were covered with aluminum foil until use.

2.2. Derivatization procedure

The following general derivatization procedure was used for the sample determination and the optimized steps are described below. In a typical procedure, 500 μL of the sample was transferred to a 1 mL glass tube. Then, 100 μL of 0.20 mol/L borate buffer solution, 100 μL water and 100 μL of 6.0 mmol/L FMOC-Cl stock solution were added to the sample. Subsequently, 200 μL MeOH was added to obtain the initial mobile phase conditions for the injection into HPLC. After mixing for 10–20 s using a vortex mixer, the mixture was filtered through a 0.22 μm syringe type nylon filter into a 1.5 mL sample vial. The recovery of the filtration step was checked using standard solutions and no significant losses occurred during filtration (Fig. S1 in Supplementary materials). After 30 min, the samples were injected and analyzed on the HPLC equipped with an auto-sampler. The seawater samples need to be diluted repeatedly to bring down the salinity, so that the final salinity in the reaction medium is 7. Working standards were freshly prepared in artificial seawater having the same salinity level as the seawater samples. Standard curves were prepared by derivatization of the standards simultaneously using the same reagents for each set of samples.

2.3. HPLC conditions

HPLC analyses were carried out on a modular Shimadzu LC chromatographic system equipped with a binary pump (LC-20AB), an auto-sampler (SIL-20A) and a FLD (RF-20A). Shimadzu CLASS-VP software was employed for recording the chromatograms and calculating peak areas. The separation of FMOC- derivatives was performed on an Agilent reversed-phase ZORBAX SB-C18 column (5 μm particle size, 150×4.6 mm i.d.). The FLD was set at 265 nm (excitation) and 315 nm (emission). Mobile phase components were 5 mmol/L ammonium acetate (adjusted with aqueous NH_3 to pH 9.0) and MeOH. The percentage of MeOH was changed linearly as follows for gradient elution:0 min, 20%;3 min, 20%;6 min, 70%;18 min, 70%;23 min, 20%.

Then the column was re-equilibrated for 3 min, resulting in a total run time of 26 min with the flow rate of 1.0 mL/min. The injection volume for HPLC analysis was 20 μL and the column was maintained at room temperature.

The HPLC components and C18 column are sensitive to presence of large amounts of salts in the samples. To minimize potential instrument damage and detection problems, it is highly recommended to dilute high salinity samples (e.g. bring down salinity from 35 to 7). LC system was washed for about 30 min with high proportion of water after running a batch of seawater samples. The C18 column utilized in this study has been used for one year and continues to perform well even after injecting about 400 saline samples.

2.4. Samples

Different types of aqueous samples were collected and analyzed according to the procedure described above. Tap water and rain water were collected and measured directly without further treatment. Lake water was collected from the lake in Xiamen University and river water was collected from upstream of the Jiulong River. Before analysis, the lake and river water samples were completely mixed and filtered through 0.45 μm syringe type polyether sulfone filter. Reclaimed water was collected from the water outlet of sprinkling irrigation in campus and used directly. Seawater of salinity 35, used as sample matrix, was collected using Niskin bottles during a cruise in the Western Pacific in April 2015. The seawater was frozen at −20 °C immediately after collection and filtered through a 0.45 μm membrane filter before use.

Seawater culture media samples were provided by Prof. S.J. Lin's group in Xiamen University, who studied the differential growth responses of marine phytoplankton to herbicide GLYP. The samples were composed of normal f/2 or L1 medium, spiked with GLYP, and used to evaluate whether the examined *Isochrysis galbana* was able to use GLYP as P source and degrade GLYP to phosphate [18]. These samples were collected on different culture days and stored in a refrigerator at 4 °C before analysis. The detailed experiments could be found elsewhere [18].

3. Results and discussion

3.1. Simultaneous determination of GLYP and AMPA

Most of the previously reported chromatographic separation methods of GLYP-FMOC and AMPA-FMOC derivatives have utilized RP C18 columns along with mobile phases consisting of different mixtures of ACN or MeOH and ammonium acetate buffers at variable pH [29,31–33]. Due to the high polarity of the derivatives, rapid analysis can be achieved on a RP C18 column. However, the GLYP-FMOC and AMPA-FMOC derivatives have similar chemical properties due to their similar structures. Thus, MeOH is a better choice as the organic solvent in the mobile phase to separate these two derivatives, due to its weaker elution ability. At the end of the derivatization reaction, these fluorescent derivatives exist as negatively charged species, which are stable only under basic conditions [34]. Accordingly, the mobile phase composed of MeOH and water buffered with 5 mmol/L ammonium acetate at pH 9.0 can provide better chromatographic signals. Under the optimized gradient elution program, retention times for GLYP-FMOC and AMPA-FMOC were 4.0 and 7.4 min, respectively. Retention times were checked by directly injecting the standard solutions of the FMOC-derivatives, without the derivatization step (Fig. S2).

3.2. Optimization of the derivatization

3.2.1. Effect of FMOC-Cl concentration

The molar ratio of the analytes and the derivatizing reagent affects the formation of the derivatized product. FMOC-Cl reacts not only with the analytes (GLYP and AMPA in this study) but with other amines, amino acids and water. Thus, typically an excess of FMOC-Cl has to be added to the sample to ensure complete derivatization. Although these by-products do not show much interference in the determination of GLYP and AMPA, their formation ought to be minimized. It has been reported that preparing the derivatized sample in the presence of 10% ACN can provide better chromatographic signals [29]. Therefore FMOC-Cl was dissolved in ACN, and 100 μL of the prepared FMOC-Cl solution was added to 1 mL quantitative tubes. The concentration of

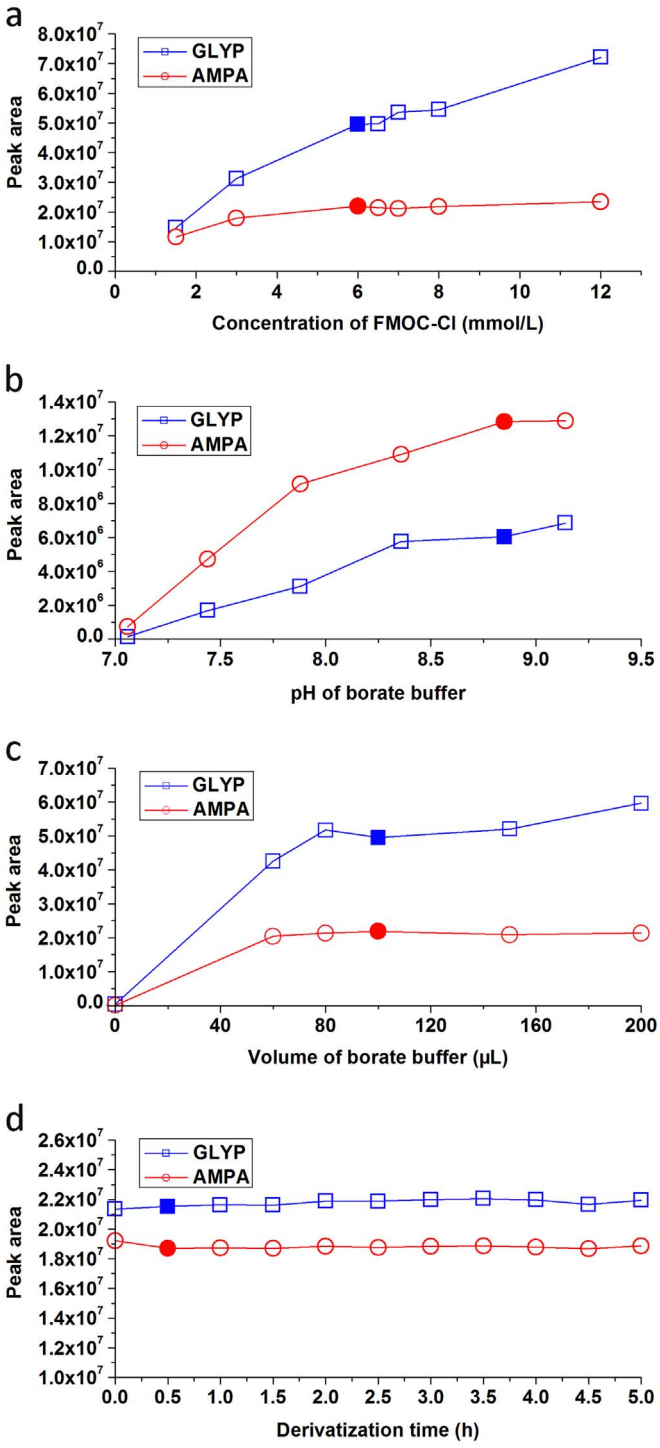

Fig. 1. Effects of (a) FMOC-Cl concentration (1.5–12 mmol/L), (b) borate buffer pH (7.06–9.14), (c) borate buffer volume (0–200 μL), and (d) derivatization time (0–5 h) on peak area. For each (a)–(d) excepting the tested variable, conditions were FMOC-Cl concentration=6.0 mmol/L, pH of borate buffer=8.85, volume of borate buffer=100 μL, mixing time=10–20 s and derivatization time=30 min.

FMOC-Cl was set at different levels for the reaction and the measured values for GLYP and AMPA are shown in Fig. 1a. It can be seen that there is an increase in the response of GLYP when the FMOC-Cl concentration is increased from 1.5 to 12 mmol/L, but this effect was not significant for AMPA. However, the derivatization product of GLYP using more than 6.0 mmol/L FMOC-Cl was not stable and the signal could drop 5% after 1 h. Thus, the optimum FMOC-Cl concentration for good response is 6.0 mmol/L.

3.2.2. Effect of pH of borate buffer

The optimum pH value of borate buffer is also a key factor in this experiment, thus borate buffer solutions with different pH values were prepared (solution proportioning methods are shown in Table S1). Fig. 1b shows that the peak intensities of the derivatized products increased when the pH values of borate buffer increased from 7.06 to 9.14. However, peak tailing problems were observed when the borate buffer was at pH 9.14. The pH of the reaction medium approached pH 10.0 when borate buffer of pH 8.85 was selected, which is consistent with others [24]. Performing derivatizations in reaction media of pH≥10 would lead to several issues such as increased hydrolysis rate of the reagent, need for high FMOC concentration to ensure its excess, and the appearance of a huge FMOC-OH peak in the HPLC chromatogram [24]. Therefore, borate buffer of pH 8.85 was selected in the experiment to keep the reaction medium at a suitable pH value.

3.2.3. Effect of borate buffer volume

It is necessary to add an appropriate volume of borate buffer to maintain the buffer action and to provide a stable basic condition for derivatization. To verify whether the volume of borate buffer in the reaction medium directly interferes with the derivatization reaction, different addition volumes ranging from 0 to 200 μL of 0.20 mol/L borate buffer (pH=8.85) were evaluated. The results are shown in Fig. 1c. It was observed that the derivatization reaction scarcely occurred without borate buffer. The instrument response of GLYP noticeably increased with an increasing volume of borate buffer up to 80 μL. The borate buffer volume was not found to be significant for AMPA when it was more than 50 μL. Increasing the buffer concentration in the medium promotes the reactivity of the amino groups and improves the solubility of the derivatizing reagent, thus favoring the process of derivatization [35]. However, a high content of buffer makes the samples considerably diluted. To get better sensitivity, the volume of borate buffer solution should be kept as low as possible. Furthermore, the presence of more borate in the samples can cause peak tailing problems. Therefore, 100 μL borate buffer solution was selected as an optimal amount for subsequent tests to make the operation and calculations easier.

3.2.4. Effect of derivatization time

Mixing of the sample is important to ensure sufficient interaction of the analyte with the derivatizing reagent. However, prolonged mixing lengthens the analysis time of the procedure. In this study, mixing times of 0, 5, 10, 20 and 30 s were evaluated, and the results showed no significant difference between the mixing time. 10–20 s mixing time was selected to ensure a fast and efficient method.

The derivatization time or reaction time is key to ensure complete acylation reaction in FMOC-Cl by the glyphosate molecule, to achieve complete derivatization of the sample. Data in the literature regarding the derivatization time vary greatly. In some cases, the reaction took 30 min [34,36], whereas in others, the derivatization reaction lasted several hours [29] or even overnight [32,37]. Molnár-Perl [24] studied the relationship between FMOC-Cl concentration and derivatization time for analyzing amino acids with this method, and concluded that 1 min derivatization time was enough when FMOC-Cl concentration was more than 5 mmol/L. In order to obtain a fast and complete reaction, the reaction kinetics of GLYP and AMPA derivatization with FMOC-Cl were analyzed for the first 5 h. Fluorometric analysis was performed immediately after the reaction, as well as 5 h later. The results showed that the derivatized products were more stable after 30 min and did not show any significant variations in the peak areas (Fig. 1d). Therefore, it can be concluded that the derivatization reaction can be stopped after 30 min and the sample can be analyzed immediately afterwards.

3.3. Salinity effect

At this time, to the best of our knowledge, no detailed reports or experimental data could be found in the literature about the effect of salinity on this popular derivatization procedure. Evaluating the effect of salinity on the sample derivatization and detection is imperative because the potential application of this method is seawater culture media analysis. Samples of different salinity values were prepared by diluting seawater or artificial seawater with pure water, and evaluated as described in following sections.

3.3.1. Influence of salinity on the derivatization efficiency

The effect of high levels of salinity on the derivatization efficiency was investigated by measuring five different concentrations of GLYP and AMPA to get calibration curves. The final salinity in the reaction medium was 3.5 (100 μL seawater of salinity 35 was added to 1 mL glass tube). Calibration curves of GLYP and AMPA in pure water and seawater showed that stronger signals for GLYP were obtained when seawater was used as the matrix and this effect was insignificant for AMPA (Fig. S3). Typical HPLC chromatograms of GLYP and AMPA standard solutions, as well as the same concentrations of GLYP and AMPA in seawater are shown in Fig. 2. It can be seen that the retention time of GLYP-FMOC was slowed down to approximately 5.9 min when the sample was detected in seawater.

To verify whether seawater improved the efficiency of the derivatization reaction or enhanced the sensitivity of FLD, GLYP-FMOC solutions of same concentration were prepared in pure water and seawater, and were analyzed by HPLC immediately. The results showed that retention time of GLYP-FMOC in seawater was again delayed, but the FLD signals were the same for both pure water and seawater samples (Table S2). To investigate the effect of salinity on the derivatization efficiency, GLYP and AMPA were derivatized and detected in different salinity matrices (the final salinities in the reaction medium were 1.4–7). The results show that there was little difference observed between the different saline samples (Fig. S4). Moreover, no interference was observed from the seawater when the signals of samples of the same concentration in natural seawater and artificial seawater at the same salinity level were compared (Fig. S5). However,

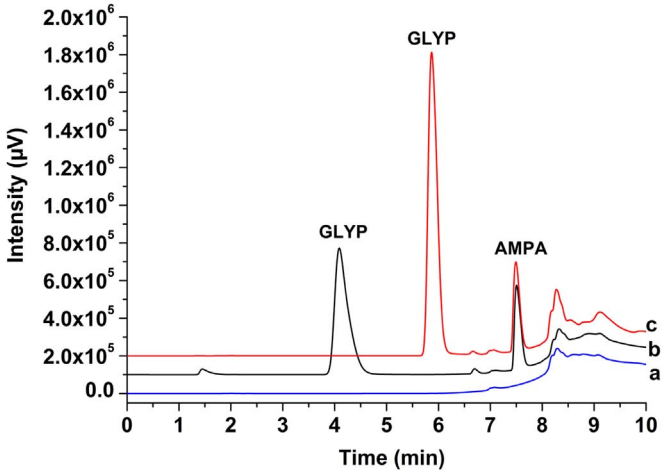

Fig. 2. Chromatograms of (a) seawater sample, (b) 400 μg/L GLYP and 20 μg/L AMPA in pure water, (c) 400 μg/L GLYP and 20 μg/L in seawater.

salinity was found to affect both derivatization efficiency and peak shapes when the samples had low concentration of salts (Fig. S6).

In order to better understand the effect of salinity on the derivatization reaction of GLYP and AMPA, salt was removed with a desalting step before detection. An Oasis HLB SPE cartridge (60 mg, 3 mL) was used to concentrate the derivatized product of GLYP and AMPA. After pre-concentration, the cartridges were rinsed with 5.0 mL pure water to remove the salt, and then analytes were eluted with 5.0 mL of MeOH and injected to HPLC-FLD system for detection. Fig. S7 shows HPLC chromatograms of same concentrations of GLYP and AMPA in pure water and seawater after being subjected to the same derivatization and desalting procedures. After desalting, the retention time of GLYP-FMOC reverted to 4.0 min. Moreover, higher signals were obtained for GLYP when seawater was used as matrix. One possible explanation could be that chloride can act as efficient base to trap the liberated protons in derivatization in acetonitrile/methanol based solvents where chloride is not solvated [38]. Although further study is required to provide a detailed explanation of the effect of salinity, it can definitely be concluded in this study that seawater promotes the derivatization efficiency of GLYP but does not change the actual products.

3.3.2. Method for quantification of GLYP and AMPA in seawater matrix

Since the salinity was found to improve the efficiency of the derivatization reaction, pure water couldn't be used for preparing calibration curves. Therefore, five different concentrations of GLYP and AMPA were prepared in seawater and artificial seawater (the final salinity in the reaction medium was 3.5), and analyzed to get the respective calibration curves. No significant difference was observed between the calibration curve obtained in artificial seawater and natural seawater (P=0.95) (Fig. S5), indicating that there was no interference from the buffering ability of natural seawater. Therefore, artificial seawater could be used as a high salinity matrix for preparing calibration curves without having to collect GLYP-free seawater. HPLC chromatograms of the GLYP and AMPA standard solutions as well as the same concentrations of GLYP and AMPA in seawater and artificial seawater are shown in Fig. 3.

3.4. Analytical figures of merit

To quantify GLYP and AMPA in actual environmental samples and seawater culture media samples, the linear range for HPLC analysis should be extended to higher concentrations. Therefore, linearity was established over several calibration ranges in both pure water and artificial seawater. Regression analyses of serially diluted standards showed good linear relationships (correlation coefficient, $R^2 > 0.99$) over different concentration ranges for GLYP and AMPA in pure water and seawater matrix (Table 1).

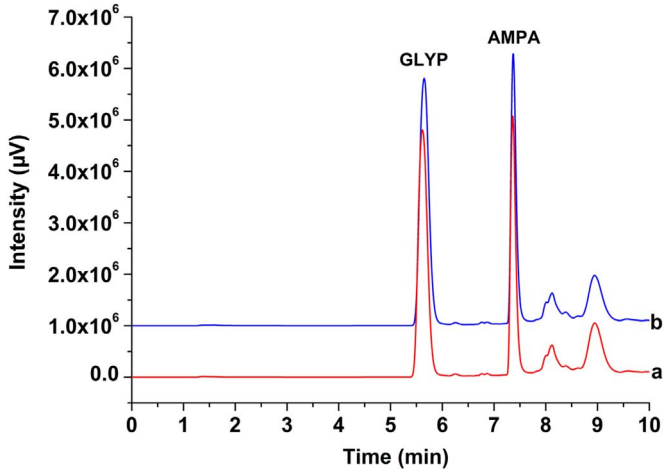

Fig. 3. Chromatograms of 1000 μg/L GLYP and 200 μg/L AMPA in (a) artificial seawater and (b) seawater at the same salinity.

The relative standard deviations for repetitive derivatization followed by HPLC analysis of 1.00 mg/L GLYP and 100 μg/L AMPA in artificial seawater (the final salinity in the reaction medium was 3.5) were 14.0% and 3.1% (n=24). The retention times were 6.370 ± 0.021 min for GLYP and 7.584 ± 0.008 min for AMPA. This experiment was conducted by three operators with basic analytical chemistry knowledge and lab skills on the same day. It should be noted that two of them had no previous experience with HPLC determination of organic compounds and they watched the experimental procedure only once before performing the experiment themselves and obtaining the data shown in Fig. 4. The reproducible results illustrate the ease of operation, stability of derivatized products, and the potentially wide applicability of this method.

LOD and LOQ values of the method were estimated on the basis of signal/noise ratios (S/N) of 3 and 10, respectively. The LOD and LOQ values for GLYP and AMPA in different matrices are shown in Table 1. Among the other LC-FLD-based methods, a similar LOD was achieved by Báez et al. [34] and Corbera et al. [39], although these studies used SPE procedure or other complex steps.

3.5. Application and recovery

To evaluate the degree of interference from the different matrices, recovery tests were conducted using several different fresh water samples. The recovery was found to vary from 80.6–134.6% with RSD≤10.1% for GLYP and from 68.9–90.5% with RSD≤4.1% for AMPA (Table S3). The results showed good accuracy, precision and reproducibility for the determination of GLYP and AMPA using LC-FLD method, without any complicated sample pretreatment steps.

Seawater culture media samples (n=32) collected on different days were analyzed before and after spiking them with standard solutions. Spiked GLYP was recovered 80–120% in more than 90% seawater culture media samples (Table S4). Excellent recoveries were observed for both high and low level spiking, indicating that this method is not affected by the matrix or salinity effect during determination of seawater culture media. Moreover, the change in the concentration of GLYP and AMPA with culture time and biological growth showed good agreement with other physiological parameters and gene expression data (data not shown), which will be discussed in detail elsewhere. Furthermore, as this method is based on LC-FLD without any requirement for enrichment or sample pretreatment steps, it provides a simple derivatization method for the simultaneous determination of GLYP and AMPA in seawater culture media. Thus, it is potentially a powerful tool to investigate the bioavailability of GLYP and AMPA as well as to facilitate research works where GLYP and AMPA have to be determined in seawater or surface water at low concentration levels.

4. Conclusions

The purpose of this study was to establish a simple and rapid determination method for GLYP and AMPA in seawater matrix, which can be easily used by marine biologists in the biological incubation experiment. Therefore, only a simple derivatization procedure and LC-FLD were utilized, reducing the cost of materials and instruments, as well as the labor budget for well-trained operators. Without preconcentration or desalting steps, derivatization and chromatographic analysis were significantly improved by merely optimizing the experimental parameters such as pH, buffer volume, mixing time, derivatization time, etc.. The LOD for the entire method was found to be 0.60 μg/L for GLYP and 0.30 μg/L for AMPA in seawater matrix, with a linear response over a large concentration range, thus allowing the determination of GLYP and AMPA dissolved in seawater culture media at different concentration levels. This method is one of few that can be

Table 1
Linear relationships, LOD (S/N=3) and LOQ (S/N=10) of GLYP and AMPA.

Matrix	Analyte	Linear range (μg/L)	Regression equation	Correlation coefficient	LOD (μg/L)	LOQ (μg/L)
Pure water	GLYP	0.8–10	y=5.047E+04 x–1.173E+04	0.9986	0.24	0.80
		10–160	y=5.479E+04 x–1.224E+05	0.9980		
		160–6000	y=6.410E+04 x–1.726E+06	0.9960		
	AMPA	0.2–1.0	y=3.004E+05 x+7.288E+03	0.9974	0.06	0.20
		1.0–32	y=2.432E+05 x+1.822E+04	0.9998		
		32–160	y=4115E+05 x–2.782E+06	0.9932		
Artificial seawater	GLYP	2.0–160	y=1.120E+05 x–1.531E+05	0.9988	0.60	2.00
		160–480	y=1.804E+05 x–5.653E+06	0.9984		
		480–2400	y=1.506E+05 x–1.960E+06	0.9964		
	AMPA	1.0–16	y=2.562E+05 x–1.582E+03	1.0000	0.30	1.00
		10–200	y=2.940E+05 x+2.200E+05	0.9998		

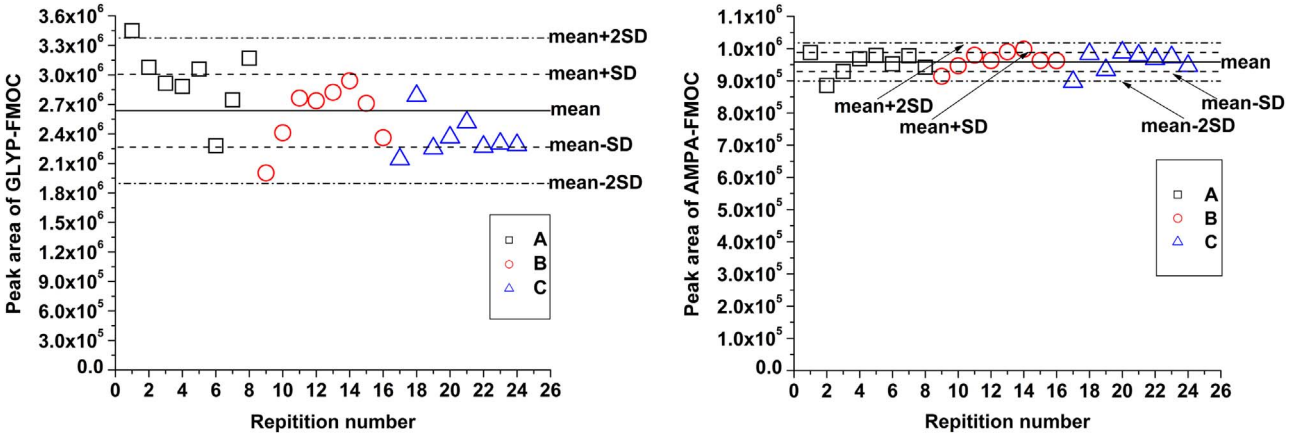

Fig. 4. Reproducibility of this method. The letters (A, B, C) present different individuals.

Table 2
Summary of the published papers for the determination of GLYP and AMPA using LC and FMOC-Cl derivatization.

Sample type	Preconcentration method	Detection	LOD (μg/L, μg/1000 g)		Analyte	Reference
			GLYP	AMPA		
Seawater	–	LC-MS/MS	0.027	0.031	GLYP, AMPA	[27]
Seawater	SPE	LC-MS/MS	< 0.1	< 0.1	GLYP, AMPA	[28]
Ground, surface, river waters	SPE	LC-MS/MS	< 0.0005	< 0.0004	GLYP, AMPA, Glufosinate	[29]
Water, soil	Online SPE	LC-MS/MS	0.005, 50	0.05, 500	GLYP, AMPA, Glufosinate	[32]
Soil	–	LC-FLD	0.6	0.4	GLYP, AMPA	[34]
Natural waters	Online SPE	LC-FLD	0.03	0.03	GLYP, AMPA	[37]
Natural waters	Anion-exchange resin	LC-FLD	0.1	0.3	GLYP, AMPA	[39]
Natural waters	–	LC-FLD	0.04	0.01	GLYP, AMPA	[40]
Drinking and ground waters	Anion-exchange resin	LC-LC-FLD	0.007	0.03	GLYP, AMPA	[41]
Different fresh waters	–	LC-FLD	0.24	0.06	GLYP, AMPA	This study
Seawater	–	LC-FLD	0.60	0.30	GLYP, AMPA	

used efficiently in seawater matrix [27,28].

Among the other reviewed LC methods (Table 2), the sensitivity of this method is comparable or even better than those provided by the similar methods [34,38,40]. However, it is lower than that of advanced methods such as coupled-column LC system using fluorescence detection (LC-LC-FLD) [41] or SPE procedure and liquid chromatography-tandem mass spectrometry (LC-MS/MS) determination [27–29,32]. Hence, the sensitivity can be further improved with SPE procedure and LC-MS/MS determination, if necessary (e.g. determination of GLYP and AMPA in natural waters and open ocean waters).

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (41306090) and MEL Internal Research Funding Program (MELRI1601). We thank Dr. Xin Lin for discussing the results, Mr. Lemeng Zhang and Miss Cong Wang for providing samples, Miss Minjuan Ma and Mr. Qipei Shangguan for participating the reproducibility work. We gratefully acknowledge the constructive and thoughtful comments from two anonymous reviewers.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2016.09.023.

References

[1] C. Benitez-Nelson, Science 348 (2015) 759–760.
[2] S.T. Dyhrman, J.W. Ammerman, B.A.S. Van Mooy, Oceanography 20 (2007) 110–116.
[3] D.M. Karl, K.M. Björkman, Dynamics of DOP, in: D.A. Hansell, C.A. Carlson (Eds.), Biogeochemistry of Marine Dissolved Organic Matter2nd ed., Elsevier, 2014.

[4] A. Paytan, K. McLaughlin, Chem. Rev. 107 (2007) 563–576.
[5] A. White, S. Dyhrman, Front. Microbiol. 4 (2013), Article 105.
[6] S.A. Sañudo-Wilhelmy, Nature 439 (2006) 25–26.
[7] S.T. Dyhrman, P.D. Chappell, S.T. Haley, J.W. Moffett, E.D. Orchard, J. B. Waterbury, E.A. Webb, Nature 439 (2006) 68–71.
[8] R. Feingersch, A. Philosof, T. Mejuch, F. Glaser, O. Alalouf, Y. Shoham, O. Beja, ISME J. 6 (2012) 827–834.
[9] J.A. Gilbert, S. Thomas, N.A. Cooley, A. Kulakova, D. Field, T. Booth, J. W. McGrath, J.P. Quinn, I. Joint, Environ. Microbiol. 11 (2009) 111–125.
[10] J.W. McGrath, J.P. Chin, J.P. Quinn, Nat. Rev. Microbiol. 11 (2013) 412–419.
[11] S. Thomas, H. Burdett, B. Temperton, R. Wick, D. Snelling, J.W. McGrath, J. P. Quinn, C. Munn, J.A. Gilbert, ISME J. 4 (2010) 459–461.
[12] J.F. Villarreal-Chiu, J.P. Quinn, J.W. McGrath, Front. Microbiol. 3 (2012), Article 19.
[13] X. Yu, J.R. Doroghazi, S.C. Janga, J.K. Zhang, B. Circello, B.M. Griffin, D.P. Labeda, W.W. Metcalf, Proc. Natl. Acad. Sci. USA 110 (2013) 20759–20764.
[14] M.J. Lovdahl, D.J. Pietrzyk, J. Chromatogr. 602 (1992) 197–204.
[15] A.D. Baylis, Pest Manag. Sci. 56 (2000) 299–308.
[16] R. Annett, H.R. Habibi, A. Hontela, J. Appl. Toxicol. 34 (2014) 458–479.
[17] J.W. McGrath, N.G. Ternan, J.P. Quinn, Lett. Appl. Microbiol. 24 (1997) 69–73.
[18] C. Wang, X. Lin, L. Li, S. Lin, PLoS One 11 (2016) e0151633.
[19] C.D. Stalikas, C.N. Konidari, J. Chromatogr. A 907 (2001) 1–19.
[20] W.C. Koskinen, L.J. Marek, K.E. Hall, Pest Manag. Sci. 72 (2016) 423–432.
[21] T. Arkan, I. Molnár-Perl, Microchem. J. 121 (2015) 99–106.
[22] T.C.P.G. Catrinck, A. Dias, M.C.S. Aguiar, F.O. Silvério, P.H. Fidêncio, G.P. Pinho, J. Braz. Chem. Soc. 25 (2014) 1194–1199.
[23] A. Jámbor, I. Molnar-Perl, J. Chromatogr. A 1216 (2009) 6218–6223.
[24] I. Moláar-Perl, J. Chromatogr. B 879 (2011) 1241–1269.
[25] R. Hanczko, A. Kőrös, F. Toth, I. Molnár-Perl, J. Chromatogr. A 1087 (2005) 210–222.
[26] H.A. Moye, A.J. Boning, Anal. Lett. 12 (1979) 25–35.
[27] P. Mercurio, F. Flores, J.F. Mueller, S. Carter, A.P. Negri, Mar. Pollut. Bull. 85 (2014) 385–390.
[28] W. Skeff, C. Neumann, D.E. Schulz-Bull, Mar. Pollut. Bull. 100 (2015) 577–585.
[29] I. Hanke, H. Singer, J. Hollender, Anal. Bioanal. Chem. 391 (2008) 2265–2276.
[30] M. Popp, S. Hann, A. Mentler, M. Fuerhacker, G. Stingeder, G. Koellensperger, Anal. Bioanal. Chem. 391 (2008) 695–699.
[31] M. Ibáñez, Ó.J. Pozo, J.V. Sancho, F.J. López, F. Hernandez, J. Chromatogr. A 1134 (2006) 51–55.
[32] M. Ibáñez, Ó.J. Pozo, J.V. Sancho, F.J. López, F. Hernández, J. Chromatogr. A 1081 (2005) 145–155.
[33] C.E. Ramirez, S. Bellmund, P.R. Gardinali, Sci. Total Environ. 496 (2014) 389–401.
[34] M.E. Báez, E. Fuentes, M.J. Espina, J. Espinoza, J. Sep. Sci. 37 (2014) 3125–3132.
[35] J. Patsias, A. Papadopoulou, E. Papadopoulou-Mourkidou, J. Chromatogr. A 932 (2001) 83–90.
[36] J.V. Sancho, F. Hernández, F.J. Lopez, E.A. Hogendoorn, E. Dijkman, P. vanZoonen, J. Chromatogr. A 737 (1996) 75–83.
[37] R.J. Vreeken, P. Speksnijder, I. Bobeldijk-Pastorova, T.H.M. Noij, J. Chromatogr. A 794 (1998) 187–199.
[38] J. Clayden, S. Warren, Solutions Manual to Accompany Organic Chemistry, 2nd ed., Oxford University Press, Oxford, 2013, pp. 464–465.
[39] M. Corbera, M. Hidalgo, V. Salvadó, Microchim. Acta 153 (2006) 203–209.
[40] E. Le Fur, R. Colin, C. Charrêteur, C. Dufau, J.J. Péron, Analusis 28 (2000) 813–818.
[41] C. Hidalgo, C. Rios, M. Hidalgo, V. Salvado, J.V. Sancho, F. Hernández, J. Chromatogr. A 1035 (2004) 153–157.