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Optimization of an Enrichment and LC-MS/MS Method for the Analysis of Glyphosate and Aminomethylphosphonic Acid (AMPA) in Saline Natural Water Samples without Derivatization

Dana Pupke[1]*, Lea Daniel[1] and Daniel Proefrock[2]

[1]*Institute of Coastal Research/Molecular Recognition and Separation, Helmholtz-Zentrum Geesthacht, Max-Planck-Straße 1, D-21502 Geesthacht, Germany*
[2]*Institute of Coastal Research/Marine Bioanalytical Chemistry, Helmholtz-Zentrum Geesthacht, Max-Planck-Straße 1, D-21502 Geesthacht, Germany*

Abstract

Glyphosate represents the main ingredient of commercially available total-herbicides such as Roundup®, which is frequently applied in agricultural areas. Leaching of the herbicide to rivers also results in its transport to the estuarine and coastal environment. The analysis of low concentrations of glyphosate (µg/L range) in sea water samples is a challenge due to the high salinity as well as the overall complex matrix composition of such samples. To overcome these difficulties an optimized two-step method for the enrichment of glyphosate and its main degradation product aminomethylphosphonic acid (AMPA) from saline natural water samples using an ion exchange based procedure is presented in this study. The determination of both analytes was performed using an optimized LC-MS/MS MRM method, which does not require any derivatization of the analytes. The optimized method was further characterized in terms of achievable LODs and LOQs, robustness and repeatability. The achievable enrichment factor dependent on the sample volume was investigated. Finally, the developed method was applied for the analysis of samples from the tidal influenced Elbe region between Hamburg and the Elbe estuary near Cuxhaven. While the glyphosate concentrations determined in the different samples were below the LOQ, AMPA concentrations at almost all sites were above the method´s limit of quantification (6 µg/L) indicating a decreasing gradient from the Hamburg area to the estuary region.

Keywords: Glyphosate; Aminomethylphosphonic acid; Herbicide; Sea water; River Elbe

Abbreviations: AMPA: Aminomethylphosphonic acid; GLY: Glyphosate; LOQ: Limit of quantification; LOD: Limit of detection.

Introduction

The herbicide glyphosate [N-(Phosphonomethyl)glycine] represents the main ingredient of commercially available products such as Roundup® or Vorox®, which are widely applied for weed control in agriculture and on railway systems as well as in private households [1] on a large scale all over the world. The use of glyphosate containing herbicides increased during recent years in particular due to the widespread application of genetically modified soybean and wheat. The global glyphosate application was over 825 kilo tons in the year 2014. The glyphosate use in the United States of America is reported with 125 kilo tons in 2014 [2].

Also in Germany glyphosate is used in agriculture, forestry and by private persons. The amount of glyphosate applied in Germany was estimated by the German Federal Government to be 5 to 6 kilo tons in agriculture and private gardens in the year 2014 [3]. This represents about 25% of all used herbicides [4].

Glyphosate is degraded in various ways in the environment due to biotic / microbial [5,6] and abiotic/chemical [7] processes. Also the photodegradation / photolysis of glyphosate is described in the literature. In particular its binding to solid particle matter and sediments, evacuation by infiltrating water and uptake / sorption by macrophytes (plants) lead to a reduction of the glyphosate concentrations in the water phase [5].

The main metabolite of glyphosate in all degradation processes is aminomethylphosphonic acid (AMPA). Under appropriate conditions glyphosate is metabolized to AMPA in particular by microorganisms, which are present in the soil [8]. In soil glyphosate is degraded faster than in water due to the large number of microbes. In soils half-lives of 56 days [9] to 217 days [10] are reported depending on the temperature and the content of organic matter. The biological degradation can proceed under either aerobic but also anaerobic conditions [8].

As a consequence of its widespread application glyphosate can be detected in natural waters during the whole year, with maximum concentration levels in April and October, correlating with agricultural application events [11]. Even in drinking water glyphosate concentrations in the low µg L^{-1} range have been detected [12]. The degradation of glyphosate and AMPA in natural ground and river waters is described by Mallat et al. with 60 to 100 h [13].

A study of the Bavarian State Institute of Agriculture showed that glyphosate can be detected in surface runoff waters of treated agricultural areas due to the leaching caused by rain events. Concentrations of up to 40 µg/L have been found in the analyzed runoff water samples after the application of 3 L glyphosate per hectare (amount of active ingredient corresponds to 360 g/L). Even after a period of seven months an average glyphosate concentration of 1.84 µg/L has been detected in the runoff

water. For AMPA the mean measured concentration was 0.33 µg/L after the same time period. The correlation with rain events during the investigated time frame showed an un-delayed and fast transport of the contaminant and its metabolite from the soil into the runoff [14] and finally from the treated field into the draining river systems. Run-off modeling [15] for different pesticides e.g., at the Great Barrier Reef already showed the great contribution of such events in terms of matter and pollutant transport from agricultural areas to rivers and the sea. In comparison to soil environments simulation tests in natural sea water of the Australian Great Barrier Reef showed the high persistence of glyphosate in saline water, depending on light conditions and water temperature [16]. At low light conditions and 25°C the degradation of glyphosate proceeded most rapidly and the herbicide could not be detected after 180 days anymore. The slowest degradation was observed at complete darkness and 31°C. In that case glyphosate was still detectable after 330 at a level of 52%. All these reported values are above the reported half lives in soils [17].

In several publications negative effects of glyphosate and AMPA to water organisms are described. It has been figured out that glyphosate and its formulation Roundup® respectively have negative effects onto microbial communities in coastal waters at concentrations of 1 µg/L, reported from microcosm experiments [18]. Another study describes the influence of glyphosate application for weed control onto a nearby river ecosystem. In the case of the water flea *Daphnia magna* a decreased food intake as well as oxidative stress were observed after glyphosate application on adjacent fields. Environmental concentration levels of 20-60 µg/L for glyphosate have been reported in this study [19]. The application of Roundup® to zebrafish e.g., reduced the egg production of female fish and increased the mortality even at low concentration levels of 10 µg/L. The same low glyphosate concentrations inhibit the cholinesterase activity of mussels and fish [20]. Among others the effects [21] due to chronic exposure of glyphosate on three-spined stickleback (*Gasterosteus aculeatus*) have been investigated [22].

In Germany the application and discharge of glyphosate into the environment is still not regulated. Even though environmental authorities in e.g., Hamburg or Schleswig-Holstein detect glyphosate and AMPA in rivers routinely, however to our knowledge the monitoring programs did not include a systematic control of the occurrence of glyphosate and AMPA in coastal waters until now. The lack of information regarding the occurrence of glyphosate and AMPA in the coastal environment might be caused by the challenging detection of both compounds in such complex, matrix rich natural water samples.

Several analytical methods are described for the determination of glyphosate and AMPA. Predominantly HPLC-MS based methods with post-column derivatization with o-phthaldialdehyde (OPA) [23] or pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) [24] have been described in the literature. Also direct LC-MS/MS methods in aqueous samples [25] (instrument detection limits (IDLS) of 1.2 for glyphosate and 0.9 µg/L for AMPA) have been reported. A method for equal complex samples, in this case rat plasma, is applying FMOC-derivatization and ESI-MS [26], resulting in LOQs of 5 (GLY) and 10 µg/L (AMPA) with only fluorescence detection (FLD) and 0.4 and 2 µg/L respectively with ESI-MS detection. Recently the application of an automated detection system based on sequential injection analysis for glyphosate and AMPA with OPA derivatization has been described [27].

The high and sometimes varying salt content of natural water samples represents the main analytical challenge in glyphosate and

AMPA determination. Saline water samples may not be directly injected into the LC-MS/MS system due to destruction of the orifice of the mass spectrometer and corrosion of metal parts in the analytical devices. The salinity is not only problematic for the detection in sea water, but also in estuary samples which show the typical tidal influenced salt gradients. To overcome these issues, an optimized two-step enrichment process will be presented in this study. The enrichment process is coupled to a sensitive direct LC-MS/MS method, which requires no derivatization steps, resulting in a less time-consuming procedure compared to other methods.

Therefore, different anion and cation exchange materials have been used during the sample preparation process allowing the efficient matrix separation of the targeted analytes from the saline water matrix. In order to show the applicability of the optimized method, samples from different locations along the Elbe river and within its estuary, representing a possible transport pathway for glyphosate from agriculturally treated urban areas into the coastal areas, have been analyzed with the optimized method.

Materials and Methods

Chemicals and reagents

All chemicals applied in this study are of analytical grade or higher purity. Glyphosate (N-(Phosphonomethyl)glycine) and AMPA (Aminomethylphosphonic acid) have been obtained as crystalline substances (Sigma-Aldrich, Seelze, Germany). The isotopically labelled standards of Glyphosate (2-13C, 99%; 15N, 98%+, 100 µg/mL) and AMPA (13C, 99%; 15N, 98%, methylene-D2, 100 µg/mL) have been purchased as solution (Cambridge Isotope Laboratories Inc., MA USA).

The mobile phase consists of acetonitrile and water. 500 mL of each solution are supplemented with 2 Vol% (10 mL) formic acid (98-100% suprapur, Merck KGaA, Darmstadt, Germany).

An amount of 10 mg of glyphosate or AMPA respectively is dissolved in 10 mL purified water to prepare a stock solution of 1 g L^{-1}. These solutions were stored at 4°C and have been used for a maximum period of four weeks. Appropriate dilutions are prepared weekly, dissolving the appropriate amount of the stock solution in ultrapure water or artificial/natural sea water respectively. Stability experiments showed that working solutions and calibration solutions are stable for a minimum of 4 weeks when stored at 4°C in the dark.

For the method validation in ultrapure and artificial sea water 10 equidistant concentration levels of glyphosate and AMPA are prepared for both concentration ranges (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µg/L and 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/L respectively)

Hydrochloric acid solutions are prepared by the dilution of 37% HCl solution (Merck KGaA, Darmstadt, Germany) with ultrapure water resulting in 6 M and 0.1 M solutions respectively.

Artificial sea water is prepared after the recipe of Zobell [28]. Therefor the different required salts were dissolved in two thirds of the end volume (1 L) of ultrapure water and then filled up to the end volume. The prepared sea water is stored at 4°C in the dark until usage.

The pH value of this solution is 6. For the examination of the influence of natural pH values, solutions with a pH value of 8 have been also prepared. The pH value is adjusted to 8 using a 0.1 M NaOH solution. All solutions are stored in Nalgene HDPE bottles (Thermo Fisher Scientific, MA, USA) at 4°C in the dark.

Preparation of the sample enrichment columns

An amount of 400 ± 5 mg of Chelex 100 resin is transferred into an empty PolyPrep column (Bio-Rad Laboratories, Inc., CA, USA) and filled up with a volume of 5 mL of pure water. The resin is carefully stirred with a plastic stirrer before the removal of the water with a pipette. Afterwards further 2 mL of pure water are added to the column followed by careful agitation of the column to remove any remaining air bubbles within the resin bed. Then the resin is left to settle and again the supernatant is removed with a pipette without running the resin dry. A slice of clean filter cotton (Fluval, HAGEN Deutschland GmbH and Co. KG, Holm DE) is placed on the top of the resin using a clean tweezer. Such prepared columns are stored in the fridge at 4°C after addition of 10 mL pure water. The water is then drained away directly before use.

In the same way the AG 1-X8 column (resin amount 900 ± 5 mg) is prepared.

Two-step preparation of water samples

Before the application of the matrix separation procedure, the saline water samples are filtrated using hydrophilic syringe filters. Three independent samples with a volume of 10 mL are prepared for each sampling site and each calibration point. The whole sample preparation process is schematically described in Figure 1.

A sample volume of 10 mL is applied to the Chelex column. Then the column is rinsed with 10 mL of ultrapure water. Subsequently 2 mL of 0.1 M hydrochloric acid are applied to the column. Then the sample is eluted with 1.8 mL of 6 M hydrochloric acid. The eluate is collected in a 2 mL Eppendorf tube.

The AG 1-X8 column is preconditioned with 2 mL of 6 M hydrochloric acid. Then the sample is applied and collected in a 2 mL Eppendorf tube again.

The sample preparation with the two-step process is conducted on an EluVac vacuum manifold SPE system (LC-Tech, Dorfen, Germany),

Figure 1: Sample preparation process.

allowing the simultaneous treatment of up to twenty samples.

After preparing the samples with the two-step enrichment process, they are freeze-dried in a SpeedVac vacuum concentrator (45°C, run time 8.5 h, heat time 7.5 h, cooling trap at -100°C) in order to evaporate the hydrochloric acid. Then the residue is filled up to a volume of 1 mL with ultrapure water and stored at 4°C until measurement with LC-MS/MS. 198 µL of this preprocessed sample is applied to a LC vial. A volume of 1 µL of the isotopically labelled standard solutions of glyphosate and AMPA each and 4 µL formic acid are added to the sample directly before measurement, according to RL 2005/6/EG for organic compounds. Then the samples are homogenized for one minute using a lab shaker.

Glyphosate and AMPA analysis by LC/MS-MS method

The detection of glyphosate and AMPA is conducted using a tandem mass spectrometer (API 4000, AB Sciex) coupled to an HPLC system (Agilent type 1100/1200). The chromatographic separation is performed on a Thermo Hypercarb column (100 mm × 3 mm with a particle size of 3 µm). In order to protect the column from pollution due to particles or residues of the sample preparation procedure, a guard cartridge is used (Hypercarb 10 mm × 3 mm). Hypercarb columns consist of 100% porous graphitic carbon and are pH stable from 0 to 14, so they are eminently suitable for acidic samples.

An injection volume of 10 µL of the sample is used during all experiments at a solvent flow rate of 0.2 mL/min. The column temperature is constantly set to 25°C. The separation is conducted under isocratic conditions. The mobile phase consists of acetonitrile (A) and water (B), both supplemented with 2% formic acid. For the removal of analyte residues a gradient is applied at the end of each measurement cycle before re-equilibration of the column (Table 1). All HPLC conditions are summarized in Table 2. The parameters for the MS method are listed in Table 3.

Ionization takes place in the positive mode (MRM). One measurement cycle has the duration of 19.5 min, delay time is set to 0.2 s and the cycle time is set to 1.64 s.

The following MRM settings and transitions are used for the detection of glyphosate and AMPA. In order to verify the presence of the both compounds two mass transitions are utilized each (compare Table 4).

For quantification the mass fragments m/z=88 for glyphosate and m/z=30 for AMPA have been used respectively.

All MRM parameters have been optimized for optimum sensitivity.

Sampling and preparation of the tested natural water samples

The analyzed samples have been collected along a transect from the Elbe estuary upstream to the city of Hamburg during a ship campaign in August 2015. A volume of 100 mL water sample was taken on each of the following sampling sites (compare Figure 2, Table 5). Two samples with a volume of 10 mL were prepared for each sampling site.

Regarding the aim of detecting low concentrations of glyphosate and AMPA, also the influence of the initial sample volume on the enrichment factor of the sample preparation method was investigated. Therefore, sample volumes of 10 mL and 20 mL sampled at the Elbe station Seemannshöft were spiked with 10 µg/L glyphosate and AMPA. Each sample was prepared as triplicate using the two step enrichment method.

Another sample from the same location was prepared with the
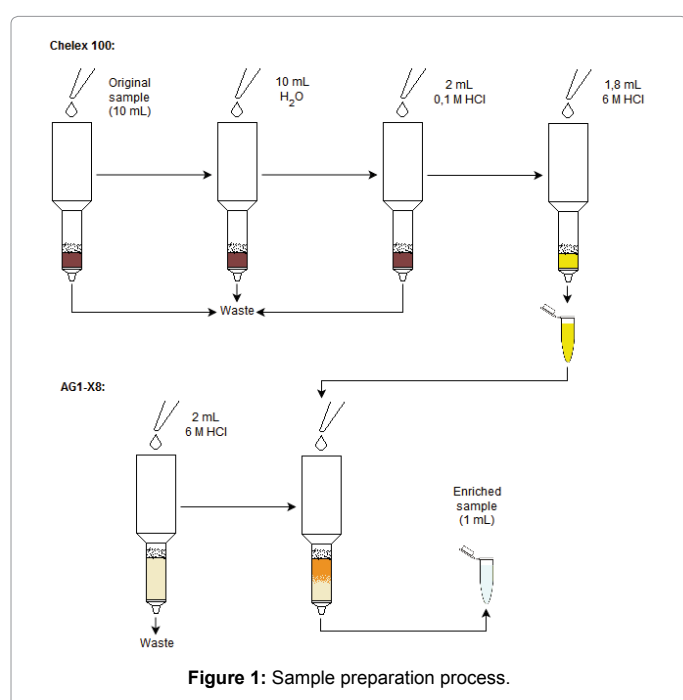
Time t (min)	Phase B (%)	Flow (mL/min)
0.00	100	0.2
3.00	100	0.2
10.00	0	0.2
10.10	100	0.4
15.00	100	0.4
15.10	100	0.2

Table 1: Cleaning gradient scheme.

Separation column	Hypercarb 100 mm × 3 mm, 3 µm
Guard cartridge	Hypercarb 10 mm × 3 mm, 3 µm
Injection volume	10 µL
Flow rate	0.2 mL/min
Temperature	25°C

Table 2: HPLC conditions.

Source/Gas Parameters	
Curtain Gas	40 L/min
Collision Gas	6 L/min
Ion Spray Voltage	4500 V
Temperature	450°C
Ion Source Gas 1	40 L/min
Ion Source Gas 2	35 L/min
Compound Specific Parameters	
Declustering Potential	25 V
Entrance Potential	10 V
Collision Energy	15 V
Collision Cell Exit Potential	12 V

Table 3: Parameters of MS analysis.

	Glyphosate	Glyphosate isotope standard	AMPA	AMPA isotope standard
Quantifier	170.1/88.1	172.1/90.1	112.1/30.1	116.1/34.1
Qualifier	170.1/60.1	172.1/62.1	112.1/83.2	116.1/83.2

Table 4: Mass transitions glyphosate and AMPA (m/z).

No	Place	Elbe (km)
1	Lt. Vogelsand	746.3
2	Cuxhaven, Kugelbake	727.0
3	Neufeld	721.6
4	Glameyer	716.1
5	Otterndorf	710.0
6	Brunsbüttel Elbhafen	693.0
7	Glückstadt	675.5
8	Pinnau	660.3
9	Blankenese	636.1
10	Seemannshöft	628.8
11	Köhlbrandbrücke	622.6
12	Zollenspieker	598.7

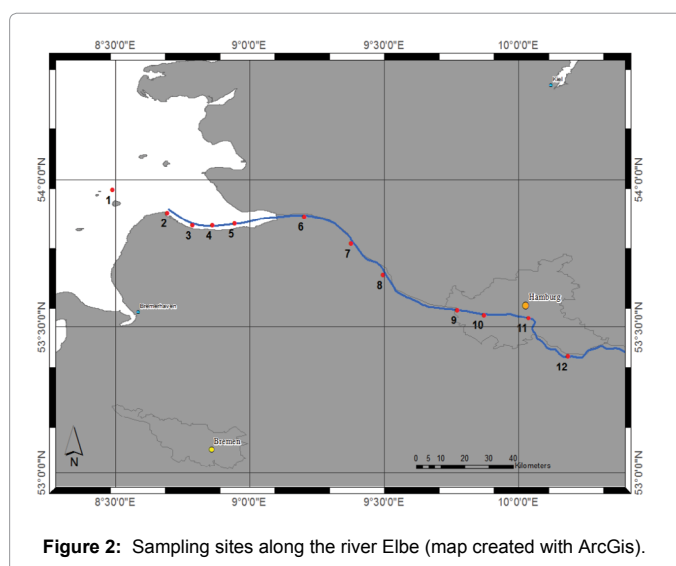**Table 5:** Sampling sites along the river Elbe (map created with ArcGis).

Figure 2: Sampling sites along the river Elbe (map created with ArcGis).

described method to compare the effects when processing either filtered or unfiltered samples. In this case the samples have been spiked with 5 and 10 µg/L glyphosate and AMPA respectively.

Results and Discussion

Method validation

Linearity: In order to investigate the linearity of the method, the calibration curves of the method including the enrichment process of glyphosate and AMPA in ultrapure water and artificial sea water samples were calculated. These curves were determined for ten different concentration levels in the range of 10 to 100 µg/L, as shown in Figure 3. The ratio of the analyte (glyphosate/AMPA) to its isotope standard is plotted across the adjusted concentration of the analyte. The ratio of the analyte to the analyte standard is directly proportional to the concentration, so the method is linear in the examined range.

Sensitivity: As to learn from the calibration curves, the sensitivity of our optimized method is better for glyphosate than for AMPA and expectably better in ultrapure water than in artificial sea water for both analytes, in both examined concentration ranges. The regression lines show good coefficients of determination for glyphosate and for AMPA. The increases of the regression lines for glyphosate are 2 to 4 times higher than for AMPA, ensuing the presented method being more sensitive for glyphosate than for AMPA. It has to be taken into consideration that there are still some matrix effects. The worse sensitivity for AMPA may be contributed to the lower m/z ratio.

Robustness: In order to validate the optimized method for natural water samples, additional samples from the Elbe have been collected at the measuring station "Seemannshoft" in June 2015. Filtered and unfiltered samples were compared with spiked samples of the same sampling site in the Elbe. In both cases a sample volume of 10 mL was tested.

As shown in Figure 4 we interestingly observed AMPA findings at this sampling site. In the filtered samples an AMPA concentration of 18 ± 2 µg/L was found, while in the unfiltered samples 18 ± 6 µg/L AMPA has been detected respectiveley. The slight difference between filtered and unfiltered samples is within the standard deviation of the method. Due to this fact, all further samples have been filtered before the final enrichment process. In both samples the glyphosate levels were below the LOD.

In the spiked samples (5 µg/L) glyphosate is detected at a concentration level of 36 ± 14 µg/L and AMPA at 48 ± 13 µg/L after the enrichment process. For the spike level of 10 µg/L the mean glyphosate concentration after enrichment was 202 ± 81 µg/L while an AMPA concentration of 225 ± 35 µg/L has been quantified. The slightly higher relative standard deviations in the higher spiked samples may point out a non-linear adsorption behavior of the Chelex 100 resin for glyphosate and AMPA. This corresponds to the different sensitivities for different concentration ranges and our batch experiments concerning multilayer adsorption in another study (not published yet).

Accuracy and repeatability: Three independently preprocessed samples for each sample volume were analyzed and compared to the nominal values in order to examine the accuracy and repeatability of the described method.

The good accuracy and repeatability of the method can be seen from Figure 5. The mean values for all three samples are within the standard deviation of the expected concentrations (dashed and dotted lines in the Figure 5). For glyphosate the accuracy and repeatability are better than for AMPA due to a lower standard deviation for glyphosate.

The accuracy is moreover defined as a combination of the selectivity and the linearity of a method, and the calibration curve must meet the origin point of that curve. All of these three requirements are fulfilled, showing the method presented here being accurate.

Detection and quantification limits: The limits of detection and quantification for the sample preparation process coupled to the optimized LC-MS/MS method were determined using the software tool DIN Test, which calculates both parameters according to DIN 32645 [29-31]. Three samples for each of ten equidistant concentration levels in the range of 1 … 10 µg/L glyphosate and AMPA in a common mixture were prepared with the presented two step enrichment process and analyzed with LC-MS/MS. The detection limits of the entire preparation and detection process are 4 µg/L for glyphosate and 0.8 µg/L for AMPA, the limits of quantification are 18 µg/L for glyphosate and 6 µg/L for AMPA. Equal LODs for glyphosate and AMPA are reported in literature, but in that study the water samples were prepared with a much more time-consuming procedure, followed by a derivatization step using trifluoroacetic anhydride and trifluoroethanol before the detection by GC-MS. MDLs of 6 µg/L for glyphosate [19] in reagent water and 9 µg/L for ground water are reported in the EPA method for the determination of glyphosate with post-column derivatization and LC-MS/MS. Other authors describe MDLs of 0.8 µg/L for AMPA and 0.5 µg/L for glyphosate with IC-ESI-MS. But in that method the separation time with about 30 minutes was longer than that of our presented method [32]. The earlier reported limit of detection for glyphosate attained with an equal LC-MS method on a Hypercarb column is obviously worse providing a LOD of 40 µg/L in drinking water [33]. In fishpond water a LOD of 2 µg/L was achieved with an LC-MS method on a HILIC column. To our knowledge for saline water matrices no comparable [34] studies do exist. After the much more time consuming FMOC derivatization LODs in the ng/L range were achieved in saline water samples [35]. Further method optimization would be necessary to detect concentrations in the ng/L range with this method as well. Higher sample volumes would either result in a longer sample preparation time or would require other dimensions of the sample preparation columns.

Additionally, the hydrochloric acid leads to massive corrosion of metal parts, probably resulting in metal complex forming with glyphosate. Glyphosate that has formed a metal complex can no longer be detected with LC/MS-MS. This may explain our higher
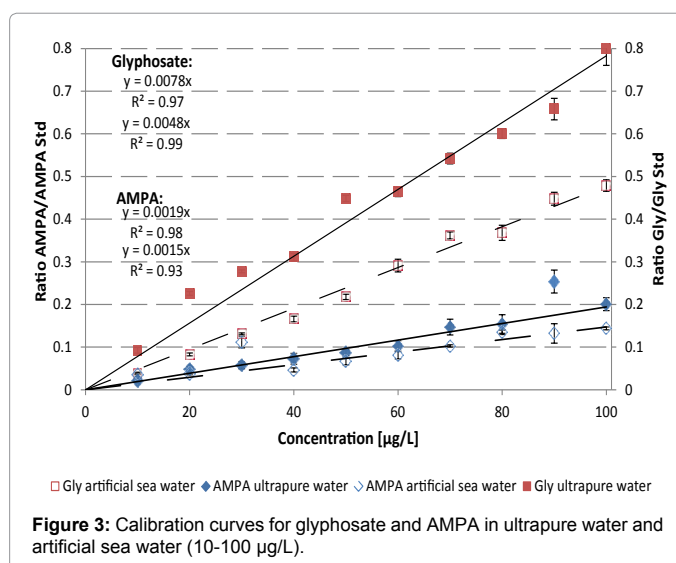

Figure 3: Calibration curves for glyphosate and AMPA in ultrapure water and artificial sea water (10-100 µg/L).
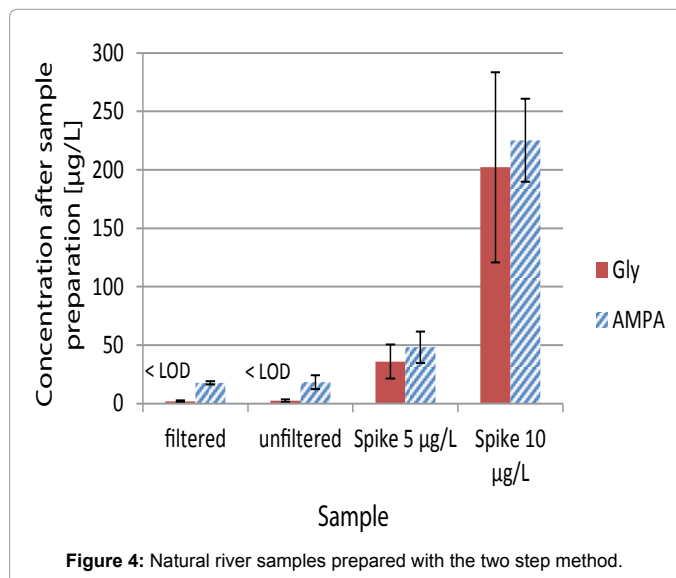

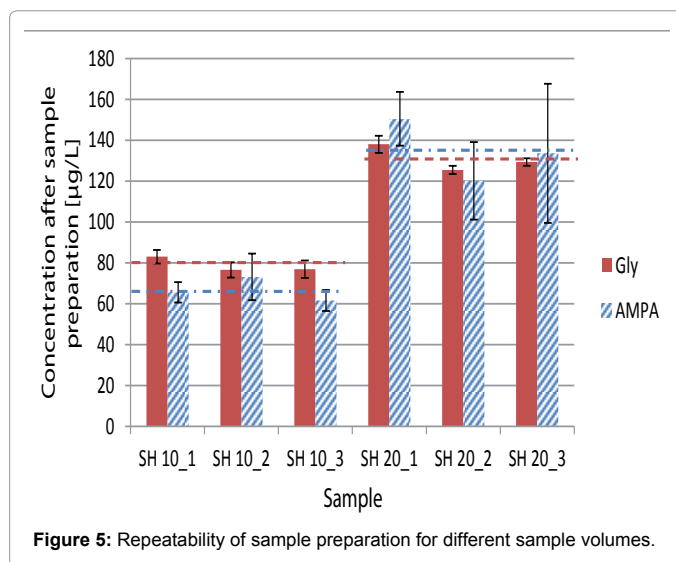**Figure 4:** Natural river samples prepared with the two step method.

Figure 5: Repeatability of sample preparation for different sample volumes.

limits of detection and quantification compared to reported levels. Nevertheless, the obtained limits of detection and quantification enable the measurement of environmentally relevant concentration levels.

Enrichment factors: Due to the low expected concentration levels of both analytes in natural water samples, a pre concentration of glyphosate and AMPA is essential. Figure 6 shows the influence of the initial sample volume on the enrichment factor. The enrichment factor is defined as the ratio of the concentration after the two-step sample preparation process towards the adjusted concentration before preparation. It was determined in three different samples for 10 mL and 20 mL each. For glyphosate enrichment factors of approximately 8 with sample volumes of 10 mL and 13 with sample volumes of 20 mL were attained, while for AMPA factors of about 7 and 14 have been achieved depending on the sample volume.

So with the sample preparation process not only matrix reduction in complex natural water samples is possible, but also a satisfying detection improvement for both analytes becomes possible. This enables the detection of environmentally relevant concentration levels.

With larger sample volumes even higher enrichment factors and lower concentration levels could probably be attained in the future. However, for the application of larger sample volumes the column dimensions may need to be adjusted to ensure a sufficient binding capacity of the ion exchange resins, as well as to maintain a good passage of the higher sample volume through the columns.

Application of the optimized method to natural water samples from the River Elbe

Having proven the applicability of the method for natural water samples, a sample transect along the river Elbe between the Elbe estuary in Cuxhaven and the city of Hamburg has been taken. As one can see from Figure 7, AMPA could be detected at all sampling sites.

A trend of decreasing concentrations between the sampling sites 7 (Gluckstadt, industry site) with a value of 23 ± 6 µg/L and sampling site 1 (Cuxhaven, North Sea) with a concentration of 1 ± 1 µg/L has been observed. This is mainly a result of the increasing dilution effects along the river Elbe in the direction of the North Sea.

At the Hamburg sampling sites 8 to 12 AMPA concentrations between 7 ± 5 and 14 ± 3 µg/L have been found. At Seemannshöft an AMPA concentration of 12 ± 3 µg/L has been found. This value is comparable to data obtained during an earlier sampling campaign in June 2015.

The high AMPA concentrations that have been found may not only be contributed to the degradation of glyphosate. As described by other authors, AMPA is also formed from other nitrogen containing organic phosphonates. Certain methylenephosphonic acids as ATMP, EDTMP, DTPMP, HDTMP or 1-Hydroxy-(ethandiphosphonic acid) are used in detergents, as corrosion inhibitors and in textile and paper industry in large amounts [36-38]. Therefore, an identification of the particular source for our AMPA findings is not obvious. The high findings at the sampling sites 6 and 7 perhaps may result from the paper and color industry located in Glückstadt [3]. The concentrations of glyphosate are under the LOD of the whole method on all sampling sites except 6 and 7. The mean concentration levels at these sites are even above the limit of quantification with mean values of 73 and 30 µg/L. Due to the high standard deviation within those sample sites we have to consider these values as outliers (results not shown).

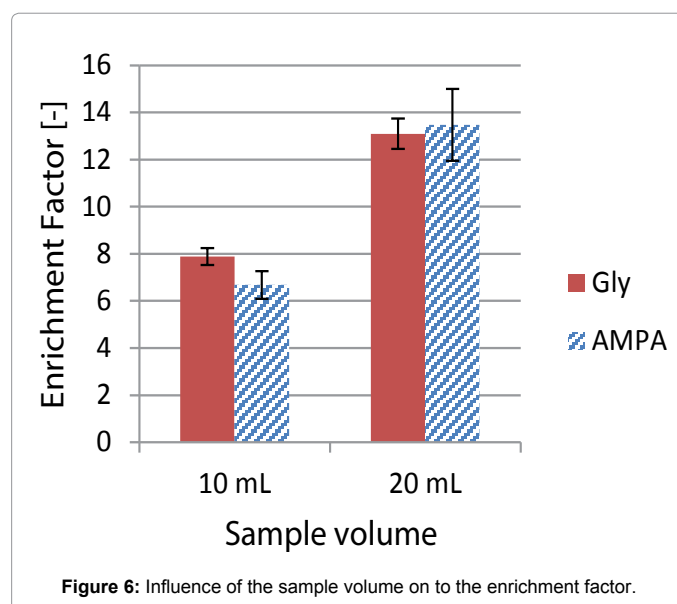To our knowledge no transect along the river Elbe between

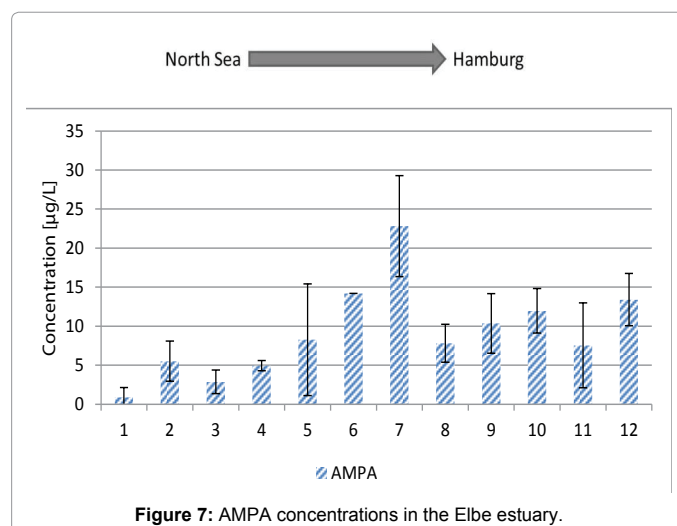**Figure 6:** Influence of the sample volume on to the enrichment factor.

Figure 7: AMPA concentrations in the Elbe estuary.

Hamburg and Cuxhaven has been investigated regarding glyphosate and AMPA concentration levels before.

However, the detected AMPA concentrations in the estuary near Cuxhaven in this study correspond good with reported findings in the estuaries of the Baltic sea. The highest concentration levels of 2 µg/L for glyphosate and 4 µg/L for AMPA have been found in the Muehlenfliess estuary near Rostock. In that paper no transects along rivers were investigated [35].

Another study shows high glyphosate and AMPA concentration levels in extracted sediment samples along the same Elbe transect in May 2014. In the Elbe estuary near Cuxhaven the AMPA concentration level in the sediment was below the LOD, increasing to a level of about 35 µg/kg in the direction of Hamburg city. Glyphosate concentrations between approximately 5 and 12 µg/kg have been reported for all sampling sites. Water samples have been investigated only for the city of Hamburg during the time period of November 2012 to November 2013 in that study. The highest concentration level of glyphosate has been about 3 µg/L in June 2013. The AMPA concentration levels in any of the water samples in the city of Hamburg have been below the LOD [39].

Conclusion

A two-step enrichment process without time-consuming derivatization steps was developed and validated in this study. The determination of glyphosate and AMPA with the described LC/MS-MS method in these preprocessed samples is possible in different complex water matrices. Overall the developed two step enrichment process with subsequent LC-MS/MS detection shows a good accuracy and repeatability with a satisfying sensitivity depending on the water matrix.

Enrichment factors between 7 and 14 for sample volumes of 10 and 20 mL respectively have been achieved for both analytes in natural water samples from the river Elbe.

The applicability of the method for the detection of glyphosate and AMPA in natural water samples was shown. Therefore, during a ship campaign in August 2015 the Elbe estuary between Hamburg and Cuxhaven has been sampled. The detected glyphosate concentrations were below the calculated limit of detection for almost all sampling sites. However, at all stations AMPA could be detected at decreasing concentration levels with increasing distance to Hamburg. This may be contributed to dilution effects of the water body. Our findings may be contributed to a high discharge of glyphosate and AMPA in the industry region Hamburg.

As described above, even concentration levels of glyphosate in the low and middle μg/L range may have negative effects to water organisms. The concentration levels detected in this study along a transect of the river Elbe may consequently affect the local aquatic and estuarine fauna as well. Further sampling campaigns and the regular monitoring of glyphosate and AMPA concentrations in the Elbe estuary by the responsible authorities should be taken into consideration.

Acknowledgements

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