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НОВЫЕ МЕТОДЫ И РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЙ ЛАНДШАФТОВ В ЕВРОПЕ, ЦЕНТРАЛЬНОЙ АЗИИ И СИБИРИ

Монография в 5 томах

Том II Изучение и мониторинг процессов в почвах и водных объектах

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This monograph shall inform you about up to date methodologies and recent results in landscape research. It is intended as a guide for researchers, teachers, students, decision makers, stakeholders interested in the topic of landscape science and related disciplines. It provides information basis for decision makers at various levels, from local up to international decision bodies, representing the top level of landscape science in a very short form.

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Chapter II/78: AN LC-HESI-MS/MS METHOD FOR THE ANALYSIS OF GLYPHOSATE AND AMINOMETHYPHOSPHONIC ACID IN SALTWATER MATRICES

Глава II/78: Метод ЖХ-МС/МС с высокотемпературной электрораспылительной ионизацией для определения глифосата и аминометилфосфоновой кислоты в водно-солевых матрицах

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ABSTRACT. The analysis of amphoteric compounds, as glyphosate and AMPA, in saltwater matrices is a continuing challenge in analytical chemistry due to (i) their possible formation of complexes with the metal cations present in salt-based matrices and (ii) problems related to their extraction from the salt matrices. For the method development, the influence of salt matrices on the reversed-phase liquid chromatography behavior and heated electrospray ionization tandem mass spectrometry detection (RP-LC-HESI-MS/MS) was investigated [1]. Furthermore, a fast and sensitive LC-MS/MS method was developed and validated, that allows the analysis of glyphosate and AMPA in saltwater matrices at trace level concentrations (LOD\textsubscript{glyphosate}: 27 ng/L, LOD\textsubscript{AMPA}: 31 ng/L) [2]. The method was effectively applied to study the occurrence of both contaminants in the estuaries of the Baltic Sea and their potential transport into the Baltic Sea.

Резюме. Определение таких амфотерных соединений, как глифосат и аминометилфосфоновая кислота, в водно-солевых матрицах остается сложной задачей в аналитической химии вследствие 1) возможного образования их комплексов с катионами металлов, присутствующими в солевых матрицах, и 2) проблем, связанных с их выделением из солевых матриц. Для разработки метода было исследовано влияние солевых матриц на разделение соединений в жидкостной хроматографии с обращенной фазой и тандемное масс-спектрометрическое детектирование с использованием высокотемпературной электрораспылительной ионизации [1]. Кроме того, была выполнена разработка и валидация быстрого и чувствительного метода ЖХ-МС/МС, который позволяет определять глифосат и аминометилфосфоновую кислоту в водно-солевых матрицах в следовых концентрациях (предел обнаружения соответственно 27 и 31 нг/л) [2]. Метод был эффективно применен для изучения присутствия обоих загрязнителей в эстуариях Балтийского моря и их потенциального переноса в Балтийское море.
INTRODUCTION
Glyphosate (N-phosphonomethyl-glycine) is a post-emergence, non-selective, broad-spectrum organophosphorus herbicides intensively used for agricultural and non-agricultural purposes [3]. AMPA is the main break-down product of glyphosate detected in different environmental compartments, such as soil, water, and plants [4]. Reversed-phase (RP) in combination with LC-ESI–MS/MS after pre-derivatization of glyphosate and AMPA with 9-fluorenymethyl chloroformate (FMOC-Cl) is the predominant analytical method for these highly polar and amphoteric compounds in different water matrices, including ground water, drinking water, rain and riverine waters [3,5,6]. Glyphosate and AMPA are not regularly monitored in the marine environment, among other reasons owing to analytical challenges due to the compounds’ chemical properties. Their amphoteric characterization with functional groups such as phosphonate, amino, and carboxylate, are expected to be more vulnerable to saltwater matrices due to the possible formation of pH-dependent complexes with metals ions and mineral surfaces [7–8]. Consequently, their analysis in samples containing metal ions, such as occurs in seawater samples, can be very difficult.

AIM OF THE METHOD
The objectives of the present work are (i) to obtain information on the influence of salt matrices in analyses of glyphosate and AMPA using RP-LC-HESI–MS/MS after derivatization of the analytes with FMOC-Cl in water (ii) to develop a fast and easy LC-MS/MS method that enables their identification and quantification in marine water samples at a trace concentration level.

PRINCIPLE AND PROCEDURE

Principle: The detailed description of the used materials, sample preparation, derivatization process, instrumentations and analytical conditions can be found in Skeff et al. [1, 2]. In brief, glyphosate and AMPA were derivatized with FMOC-Cl at pH 9 for 2 hours. Samples were directed to reverse phase chromatographic separation on a Gemini-NX C18 column coupled to a Gemini-NX Security Guard cartridge. Samples were eluted gradually from the column with a 2 mM ammonium hydrogen carbonate buffer and ammonia solution (32% (v/v)) with a pH of 9 and B acetonitrile. Glyphosate and AMPA analysis was conducted on an LC triple quadrupole mass analyzer. The compounds were ionized through the HESI interface and detected in the Selected Reaction Monitoring (SRM) mode.

Procedure: The effect of a salt matrix on the RP-LC behavior of glyphosate, and AMPA in water and, consequently, on the identification of these compounds was investigated. The results showed that glyphosate, not AMPA, was the analyte with a strong tendency to form glyphosate-metal complexes (GMC), which clearly influenced the analysis. The retention times (RTs) of GMC and free glyphosate (FG) differed by approximately 7.00 min (Figure 1), reflecting their distinct RP-LC behaviors. Due to the different molecular masses of GMC and free glyphosate, diverse parent ions should be formed in the ionization source. The formation of same parent ion (m/z 390) by the two analytes may have been caused due to the thermal liability of GMC derivative in the HESI interface during the ionization process (vaporizer temperature 200 °C). This may have induced derivatized free glyphosate formation [FG-FMOC-H] and, consequently, the production of the same product ions (m/z 150 and 168) in the second quadrupole (Q2). Divalent cations, but not monovalent (Na+, K+) or trivalent (Al3+, Fe3+) cations, contributed to this effect and their influence was concentration-dependent. In addition, Cu2+, Co2+, Zn2+, and Mn2+ prevented glyphosate detection whereas Ca2+, Mg2+, and Sr2+ altered the retention time. At certain tested concentrations of Ca2+ (Figure 1) and Sr2+, glyphosate yielded two peaks, which violated the fundamental rule of LC, that under the same analytical conditions a single substance yields only one LC-peak with a specific RT. Our research finding demonstrated that Ca2+, Mg2+, and Sr2+ can be used together with FMOC-Cl to form GMC-FMOC which is suitable for RP-LC-HESI–MS/MS analysis. The impact of salt matrices on the quantitative analysis of both compounds was also carried out in this work. Salt-matrix-induced ion suppression was observed for both analytes, especially under high salt concentrations [1]. The use of stable, isotopically labeled glyphosate and AMPA internal standards prepared in the same sample matrix can well-correct the problems related to sample preparation, complexes formation, and ion suppression.

KEYWORDS: Glyphosate, AMPA, LC-MS/MS, reversed phase, metal complexes, method development, Salt matrices, Baltic Sea estuaries

Ключевые слова: Глифосат, AMPA, LC-MS / MS, обратная фаза, комплексы металлов, разработка методов, Солевые матрицы, эстуарии Балтийского моря
Due to the ionic property of glyphosate and AMPA, their extraction from saltwater matrices can be an analytical problem. Therefore, our strategy for the method development was based on the optimization of the chromatographic and detector specific parameters to yield the best resolution of the compounds as well as the highest detector sensitivity possible. LC conditions were tested with respect to eluent compositions, different buffer concentrations in the eluent A and B, various variants gradient elution and different mobile phase flow rates. Mass detector parameters such as spray voltage, auxiliary gas flow rates, sheath gas pressure, vaporizer temperature and capillary temperature were also optimized for both compounds. The optimized parameters greatly improved the sensitivity of the method that allows the measurement of glyphosate and AMPA until concentration limits (LODs) of 27 ng/L and 31 ng/L, respectively. Satisfactory control data including linearity ($R^2 > 0.999$), precision (relative standard deviation RSD < 12%), accuracy (relative error RE < 16%), analytes and system stability were obtained. A sensitive and straightforward method in hand might allow monitoring programs to be expanded by glyphosate and AMPA measurements in the marine environment.

**Figure 1.** SRM chromatograms obtained from the analysis of 5 µg/L glyphosate in solutions supplemented with different Ca2+ concentrations: A. 4 mg/L, B. 30 mg/L and C. 40 mg/L. The first peaks are shown according to the quantified ions (Qn) and the second peaks according to the qualified ions (Qi).

**TEST RESULT**

The influence of sample matrices on glyphosate and AMPA analysis was examined in enormous environmental aqueous samples of rain, ground, drainage, riverine, estuarine, lagoon, Baltic Sea, and North Sea waters. The samples were collected at different sampling sites from Germany in 2016 and included different matrix and salt contents. Each of the aqueous samples was spiked with glyphosate and AMPA, and analyzed using the LC-MS/MS method. The result indicated the negligible influence of the sample matrices on the RP-LC behaviors of AMPA and therefore on their identification. In the case of glyphosate, it was eluted as GMC at RT $\approx 13.00$ min in all analyzed samples with a maximum RTs variation of 0.3 min. The exception was the rainwater sample (RT $\approx 6.00$ min), which because of the low ionic content of rainwater, glyphosate was eluted as free glyphosate. None of the environmental samples yielded two glyphosate elution peaks in the same chromatogram. Instead, the results showed the strong tendency of glyphosate in these samples to form complexes in different types of water, with the subsequent detection of those newly formed GMC. SRM chromatograms obtained from glyphosate and AMPA analysis in Baltic Sea estuarine water samples (salinity 0.4) are shown in figure 2.
**Figure 2.** SRM chromatograms obtained from analysis A. glyphosate and B. AMPA in a natural water sample collected from Muehlen estuary (Station 7 figure 3). Two peaks of each compound are represented in the chromatograms. First peaks are according to the quantifier ions (Qn) and the second to qualifier ions (Ql).

**Figure 3.** The Baltic Sea estuary sampling sites 1 to 10 (1: Uecker, 2: Peene, 3: Ryck, 4: Barthe, 5: Recknitz, 6: Warnow, 7: Muehlenfließ, 8: Hellbach, 9: Wallensteigraben and 10: Stepenitz) are distributed along the coast of Mecklenburg-Vorpommern. Glyphosate (red) and AMPA (green) concentrations are represented in ng/L.
APPLICATION
Water samples were collected during the application period of pesticides between May and September from ten different estuarine stations distributed along the Baltic Sea coastline of Mecklenburg-West Pomerania, Germany (Figure 3) at different time intervals. The samples were analyzed using the developed method in order to study the presence of glyphosate, AMPA and their potential transport into the Baltic Sea. As it can be seen from Figure 3, all estuaries were contaminated with AMPA and nine of them with glyphosate. Glyphosate concentrations ranged from 28 to 1690 ng/l, while AMPA was found at higher concentrations (from 45 to 4156 ng/l). The detection frequencies of glyphosate and AMPA in the water samples were 73% and 93%, respectively. Our method was effectively applicable for glyphosate and AMPA analysis in saltwater matrices. This allowed gaining the first information on the presence of both contaminants in the marine environment.

CONCLUSIONS
1. We studied the influence of salt matrices on the RP-LC behavior and HESI-MS/MS detection of glyphosate and AMPA.
2. We were able to analysis glyphosate as metal complexes.
3. A straightforward LC-MS/MS method was developed for glyphosate and AMPA analysis in saltwater matrices.
4. The method was effectively applicable for the analysis of glyphosate and AMPA in estuarine water.
5. We conducted the first study on transport glyphosate and AMPA from land to sea.

REFERENCES