The influence of salt matrices on the reversed-phase liquid chromatography behavior and electrospray ionization tandem mass spectrometry detection of glyphosate, glufosinate, aminomethylphosphonic acid and 2-aminoethylphosphonic acid in water

Wael Skeff*, Constantin Recknagel, Detlef E. Schulz-Bull

Leibniz Institute for Baltic Sea Research Warnemuende, Department of Marine Chemistry, Seestrasse 15, 18119 Rostock, Germany

ARTICLE INFO

ABSTRACT

The analysis of highly polar and amphoteric compounds in seawater is a continuing challenge in analytical chemistry due to the possible formation of complexes with the metal cations present in salt-based matrices. Here we provide information for the development of analytical methods for glyphosate, glufosinate, AMPA, and 2-AEP in salt water, based on studies of the effects of salt matrices on reversed-phase liquid chromatography–heated electrospray ionization-tandem mass spectrometry (RP-LC-HESI–MS/MS) after derivatization of the target compounds with FMOC-Cl. The results showed that glyphosate was the only 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 differed by approximately 7.00 min, reflecting their distinct RP-LC behaviors. Divalent cations, but not monovalent (Na^+, K^+) or trivalent (Al^{3+}, Fe^{3+}) cations, contributed to this effect and their influence was concentration-dependent. In addition, Cu^{2+}, Co^{2+}, Zn^{2+}, and Mn^{2+} prevented glyphosate detection whereas Ca^{2+}, Mg^{2+}, and Sr^{2+} altered the retention time. At certain tested concentrations of Ca^{2+} and Sr^{2+} 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. Salt-matrix-induced ion suppression was observed for all analytes, especially under high salt concentrations. For glyphosate and AMPA, the use of isotopically labeled internal standards well-corrected the salt-matrix effects, with better results achieved for glufosinate and 2-AEP with the AMPA internal standard than with the glyphosate internal standard. Thus, our study demonstrated that Ca^{2+}, Mg^{2+}, and Sr^{2+} can be used together with FMOC-Cl to form GMC-FMOC which is suitable for RP-LC-HESI–MS/MS analysis.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Glyphosate (N-phosphonomethyl-glycine) and glufosinate (ammonium dl-homoalanin-4-(methyl) phosphinate) are post-emergence, non-selective, broad-spectrum organophosphorus herbicides intensively used for agricultural and non-agricultural purposes [1]. Their application is growing very rapidly worldwide, mainly due to the development of crops resistant to these herbicides [2]. Aminomethylphosphonic acid (AMPA) is the main metabolite of glyphosate detected in different environmental compartments, such as soil, water, and plants [3]. It is also a key metabolite that can be formed during the degradation of industrial phosphonates used in detergents, laundry agents, and the textile industry, such as ATMP (aminotrimethylene phosphonic acid) and DTPMP (diethylenetriamine pentamethylene-phosphonic acid) [4,5]. The phosphonic acid moiety of these compounds enables their strong adsorption on soils, leading to their low mobility and low potential for contaminating aquatic resources [6–8]. However, despite their high adsorption tendencies, these substances have been detected in different aqueous environments, where they have caused water contamination [1,8,9]. 2-aminoethylphosphonic acid (2-AEP) is a biogenic phosphonate. It has been identified in many types of plankton feeders and marine invertebrates [10]. However,

* Corresponding author.
E-mail addresses: wael.skeff@io-warnemuende.de, drei-stein@hotmail.com
(W. Skeff).

information on the presence of 2-AEP in waters is scarce because of the lack of suitable analytical methods.

The physicochemical characteristics of these analytes, such as their low molecular weight, high polarity, low volatility, thermal liability, high solubility in water, and low solubility in non-polar organic solvents make their analysis using liquid chromatography (LC) more suitable than gas chromatography (GC). Due to the absence of requisite chemical groups (e.g., chromophores and those absorbing UV) their detection using conventional detectors is inadequate [1]. Liquid chromatography coupled with electrospray ionization interface and mass spectrometry (LC-ESI–MS/MS) is a common analytical technique used in environmental samples, due to its high sensitivity, selectivity, and throughput [1,11,12]. Several different LC columns have been employed for the analysis of glyphosate, glufosinate, and AMPA in water, including C18 reversed-phase, anion exchange, mixed mode (C18/anion exchange), cation exchange, –NH2, IonPac, and Hypersil APS [3]. Reversed-phase (RP) in combination with LC-ESI–MS/MS after pre-derivatization of the compound of interest with 9-fluorenylmethyl 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, riverine, and estuarine waters [1,9,11–13]. However, matrix effects caused by the elution of other compounds with the analytes of interest are a major obstacle in the development of analytical LC-ESI–MS/MS methods [14]. The majority of studies of these matrix effects have focused on the interface between LC and MS (e.g., ESI). Their results showed the suppression or enhancement of analyte signals, leading to inaccurate and imprecise quantitative analyses [15]. In addition, Fang et al. [16] showed that the sample matrices can clearly alter the LC behavior of the analytes of interest, such that they are incorrectly identified. These matrix effects are highly diverse and depend on the nature of the matrix, the analytes, the sample preparation methods, and the instruments used in the analysis [17,18]. The amphoteric compounds glyphosate, glufosinate, AMPA, and 2-AEP, with functional groups such as phosphonate, amino, and carboxylate, are expected to be more vulnerable to sample-matrix effects due to the possible formation of pH-dependent complexes with metals ions and mineral surfaces [19–21]. Consequently, their analysis in samples containing metal ions, such as occurs in seawater samples, can be very difficult.

Studies of the presence of glufosinate and 2-AEP in seawater have yet to be published and only a very few have investigated glyphosate and AMPA. In the study of German estuaries that feed into the Baltic Sea, Skeff et al. [12] reported the detection of glyphosate and AMPA at concentrations up to μg/L level, sufficient to contaminate estuarine waters. However, that method was not sensitive enough to determine the levels of these compounds in open seawater samples (i.e. lower concentrations and higher salt contents). The objective of the present work was to obtain information on the influence of salt matrices in analyses of glyphosate, glufosinate, AMPA, and 2-AEP using RP-LC-HESI–MS/MS after derivatization of the analytes with FMOC-Cl in water. Matrix effects on the identification and quantification of these compounds were investigated. The results enabled the formulation of methodological approaches that resolve the problems posed by salt-matrix effects in LC–MS/MS studies.

2. Experimental

2.1. Chemicals and reagents

Stock solutions of glyphosate, a glyphosate internal standard (1-2-$^{13}C_2$ ^{15}N glyphosate), AMPA, and an AMPA internal standard (^{13}C ^{15}N AMPA), each at a concentration of 100 ng/μL in

water, were supplied together with glufosinate ammonium, at a concentration of 100 ng/μL in methanol, by Dr. Ehrenstorfer GmbH (Augsburg, Germany). 2-AEP was purchased from Sigma-Aldrich (Taufkirchen, Germany). A stock solution of 2-AEP, at a concentration of 100 ng/μL, was prepared by dissolving 10 mg in 100 mL LC–MS-grade water (VWR international GmbH, Darmstadt, Germany). All standards were stored at 5 °C in the dark. Borate buffer (pH 9) was prepared by dissolving 1 g of sodium tetraborate decahydrate (Sigma-Aldrich) in 50 mL of Milli-Q water (Merck KGaA, Darmstadt, Germany). A stock solution of FMOC-Cl (purity 99.0%, Sigma-Aldrich) was prepared by dissolving 1 g in 58 mL of acetonitrile (Walter-CMP GmbH, Kiel, Germany), yielding a final concentration of 66.6 mM. The FMOC-Cl solution was stored in a brown glass flask in the dark at 5 °C. A working solution was prepared daily by diluting the appropriate amount of the stock solution in acetonitrile using a glass syringe. Eluent A (2 mM NH_4HCO_3) was prepared by first dissolving 158 mg of ammonium bicarbonate (Sigma-Aldrich) in 1 L of LC–MS-grade water and then adding 100 μL of ammonia solution (32% v/v; Sigma-Aldrich) to adjust the pH to 9. Artificial sea salt was purchased from Tropic Marin®, Germany. The following salts were dissolved in LC–MS-grade water based on the required concentrations: $CaCl_2$ (AppliChem; Darmstadt, Germany), $AlCl_3$ (Alfa Aesar; Karlsruhe, Germany), and $ZnSO_4$*H_2O (Sigma-Aldrich). $CuSO_4$*$5H_2O$, $MgCl_2$, NaCl, and $SrCl_2$ were obtained from Merck, and $CoSO_4$*$7H_2O$, $MnSO_4$*H_2O, $FeCl_3$ and KCl from VWR.

2.2. Sample preparation and derivatization

Working solutions of the target compounds, including the internal standards, were prepared in LC–MS-grade water at a concentration of 100 μg/L. An appropriate volume of the working solution was diluted with LC–MS-grade water, either alone or with the different dissolved salts, to the required concentration. 800 μL of each sample was then transferred to a 2 mL Eppendorf vial, adjusted to pH 9 by adding 100 μL of borate buffer, and treated with 100 μL of 3.33 mM FMOC-Cl in acetonitrile to allow their derivatization. After 2 h of incubation at the lab temperature of 21 °C, the derivatized samples were filtered through a 0.45 μm Phenex-RC 15 mm syringe filter (Phenomenex, Germany) and subjected to LC–MS/MS.

2.3. Instrumentation

The target substances were analyzed on a LC–MS/MS system (Thermo Fisher Scientific; Dreieich, Germany) comprising an Accela autosampler (series: 750477), an Accela pump (series: 700862), and a Maylab MistraSwitch column oven (model 886, series: 100027). The TSQ Vantage triple-quadrupole mass analyzer (series: TQU 02725) was equipped with a heated electrospray ionization source interface (HESI). Instrument operation as well as data processing and evaluation were managed using XCalibur® version 2.1 (Thermo Fisher). Chromatographic separation was carried out at 20 °C on a Gemini-NX C18 column (150 × 2.0 mm, 3 μm) coupled to a 4 × 2.0 mm Gemini-NX Security Guard cartridge (Phenomenex, Germany). The LC and HESI–MS/MS parameters were developed in previous work, in which glyphosate and AMPA in estuarine water were analyzed [12]. All analytes were ionized in negative ionization mode. The collision energy and S-lens values were optimized for each compound. Data were acquired in selected reaction monitoring (SRM) mode. Two transitions per substance, including the internal standards, were chosen. The most abundant transition was used for quantification and the other transition for confirmation. Details of the parent ions, product ions, quantifier ions (Qn), qual-