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TECHNICAL NOTE

Determination of glyphosate and AMPA in surface and waste water using high-performance ion chromatography coupled to inductively coupled plasma dynamic reaction cell mass spectrometry (HPIC–ICP–DRC–MS)

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Abstract A novel method employing high-performance cation chromatography in combination with inductively coupled plasma dynamic reaction cell mass spectrometry (ICP–DRC–MS) for the simultaneous determination of the herbicide glyphosate (*N*-phosphonomethylglycine) and its main metabolite aminomethyl phosphonic acid (AMPA) is presented. P was measured as $^{31}P^{16}O^{+}$ using oxygen as reaction gas. For monitoring the stringent target value of 0.1 μg L^{-1} for glyphosate, applicable for drinking and surface water within the EU, a two-step enrichment procedure employing Chelex 100 and AG1-X8 resins was applied prior to HPIC–ICP–MS analysis. The presented approach was validated for surface water, revealing concentrations of 0.67 μg L^{-1} glyphosate and 2.8 μg L^{-1} AMPA in selected Austrian river water samples. Moreover, investigations at three waste water-treatment plants showed that elimination of the compounds at the present concentration levels was not straightforward. On the contrary, all investigated plant effluents showed significant amounts of both compounds. Concentration levels ranged from 0.5–2 μg L^{-1} and 4–14 μg L^{-1} for glyphosate and AMPA, respectively.

Keywords Glyphosate · AMPA · Waste water treatment · ICP–MS · Dynamic reaction cell

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Introduction

With the introduction of genetically modified glyphosate-resistant crops in the mid 1990ies glyphosate has become the most widely used herbicide in the world [1]. Moreover, it is used for vegetation control in non-agricultural areas, such as weed control along railway tracks and highways and also in aquatic systems. The main transformation product AMPA [2–4] is formed upon microbial degradation. Recent findings of both substances in groundwater [5–7] and the growing use in agriculture indicate the role of glyphosate and AMPA as emerging contaminants in the environment [8]. For glyphosate, the target value of 0.1 μg L^{-1} in drinking water was issued within the EU, while in the USA based on the safe drinking water act a far less restrictive target value (MCL; maximum contaminant level) of 700 μg L^{-1} is applicable.

Analysis of glyphosate and AMPA at environmental concentration levels is not straightforward, as common matrix separation procedures for pesticides fail due to their high polarity and water-solubility. A detailed overview on

analytical techniques is given elsewhere [9]. Conventionally, HPLC with UV fluorescence detection in combination with either pre-column or post-column derivatization is employed. Recently, LC–MS gained importance in the field [10, 11]. Sadi et al. presented a ICP-MS-based method using octapole collision cell technology and ion-pairing chromatography. [12]. Another ICP-MS-based approach employing anion-exchange chromatography failed for the analysis of AMPA [13].

In the work presented, cation-exchange chromatography was studied for separation of glyphosate, AMPA, and phosphate. P was detected by ICP–DRC-MS using oxygen as reaction gas. Consequently, species-unspecific quantification using phosphoric acid as standard could be carried out. Combination of the HPIC–ICP–MS method with a fit-for-purpose enrichment procedure met the stringent requirements of surface water monitoring.

Materials and methods

Chemicals

Analytical-grade glyphosate (Pestanal grade ≥99.2%) and AMPA (≥99%) were obtained from Sigma–Aldrich (Vienna, Austria). Suprapure phosphoric acid (85%), Suprapure ammonium dihydrogen phosphate (99.99%) and Suprapure potassium chloride (99.999%) were supplied by Merck (Frankfurt, Germany). P.a. grade hydrochloric acid from Merck was distilled in a sub-boiling system (MLS lab systems, Leutkirch, Germany) prior to use. Chelex 100 (100–200 mesh) and AG1-X8 (200–400 mesh, sodium form) resins were obtained from Biorad (Vienna, Austria). $Fe(III)Cl_3$ hexahydrate (99%) from Sigma–Aldrich was used for conditioning of the Chelex resin. For resin-conditioning steps deionized water (Millipore grade) was used. Otherwise, ultrapure water was used: Following reverse-osmosis and ion exchange with an HQ-5 system supplied by REWA (Gladenbach, Germany), the water was finally processed with a duoPUR quartz subboiling-unit.

Instrumentation

A metal-free DX-500 ion chromatography system (Dionex, Sunnyvale, CA, USA) was used. The chromatographic separation was conducted on a 4.0×20 mm Pickering cation-exchange pre-column (potassium form) and a 4.0×150 mm analytical cation-exchange column (Pickering Laboratories, Mountain View, CA, USA). The eluent was 10 mmol L^{-1} KCl and 10 mmol L^{-1} HCl (isocratic elution). A flow rate of 0.5 mL min^{-1} and an injection volume of 50 μL were used. The column was flushed with 50 mmol L^{-1} KCl-50 mmol L^{-1} HCl for regeneration.

The Elan 6100 DRC II plus (Perkin–Elmer–Sciex, Ontario, Canada) was operated in combination with a PFA microflow nebulizer (ESI, Omaha, NE, USA) and a cyclonic spray chamber (PE Sciex). A quartz injector (2.0 mm i.d.), a quartz torch, and nickel sampler and skimmer cones (all AHF, Tuebingen, Germany) were used. Table 1 lists the operational conditions.

All working steps for the preparation of standards, samples, and eluents were conducted under clean-room conditions (class 100,000). ICP–DRC-MS measurements were conducted under clean-room conditions (class 10,000).

Offline enrichment of glyphosate and AMPA using ion exchange resins

A two-step solid-phase enrichment procedure was applied [14, 15]. First, the Chelex resin was treated with acidic 0.033 mol L^{-1} $Fe(III)Cl_3$ solution and subsequently washed with dilute HCl (0.02 mol L^{-1}). The AG1-X8 resin was washed with water and the preconditioned with 10 mol L^{-1} HCl. PE cartridges (15 mL) were filled with Chelex 100 or AG1-X8 resins (4 mL). At the bottom of the cartridge and on top of the resin bed 20-μm polyethylene (PE) frits were placed.

Following filtration through 0.45 μm cellulose membrane filters (Schueller, Germany), the pH value of the samples was set to 2.0±0.1 by addition of 6 mol L^{-1} HCl. The sample (each 220 mL) was then loaded on to the Chelex 100 resin via a 150-mL PE reservoir at a maximum flow rate of 6 mL min^{-1}. The flow was regulated by use of a Vacmaster 10 vacuum manifold (Separtis, Grenzach-Wyhlen, Germany) and stopcocks at the bottom of the cartridges. A washing step followed (20 mL water and 40 mL 0.02 mol L^{-1} HCl). Next, the Chelex cartridge was mounted via adapters on the cartridge with AG1-X8. The analytes were then slowly eluted through the two resins with 10 mol L^{-1} HCl. The eluate was collected in 50-mL PE-Flasks. In a final step the HCl was evaporated completely at 55°C and a pressure of 50 mbar. The solid residue was dissolved in 500 μL IC-Eluent (10 mmol L^{-1}

Table 1 ICP–DRC-MS operating conditions

Rf power	1250 W
Plasma gas flow	16.0 L min^{-1}
Auxiliary gas flow	1.25 L min^{-1}
Nebulizer gas flow	0.83 L min^{-1}
Cell gas flow	0.45 mL min^{-1}
Monitored Ion	$^{31}P^{16}O^{+}$ (m/z=47)
Dwell time	50 ms
Rpq	0.35

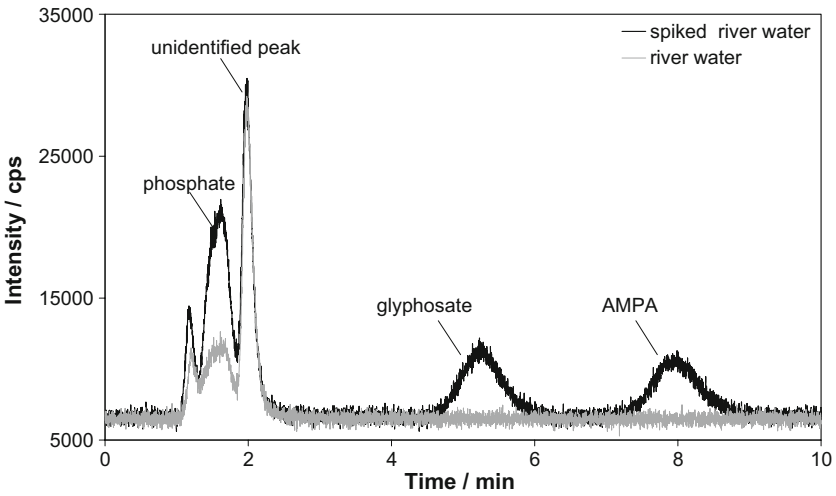

Fig. 1 Overlaid chromatograms of HPIC–ICP–DRC-MS runs of a spiked (*dark grey*) and a non-spiked (*light grey*) river water sample (River Danube). The water sample was filtered (0.45 μm) and spiked with phosphate (156 μg L^{-1}), glyphosate (280 μg L^{-1}), and AMPA (183 μg L^{-1})

KCl–10 mmol L^{-1} HCl) and filtered through a 0.45-μm ion-disc filter (Supelco, Vienna, Austria).

Measurement campaign

In summer 2005 and spring 2006 samples were taken at three waste water-treatment plants (WWTP) and one industrial site (IS) in Vorarlberg. For the WWTP1 80% of the inflowing waste water originated from the metal, food, and textile industries. For WWTPs 2 and 3 the industrial character was less pronounced with 50% and 30% of the influent derived from industrial sites, mainly textile production. Industrial site IS 1 was a regional metal processing company (electroplating and galvanic processing) with daily waste water discharge of 500 m^3. Additionally, the River Dornbirner Ache, one of the region's main water bodies, was investigated close to the village Lauterach.

Results and discussion

HPIC–ICP–DRC-MS

Glyphosate, AMPA, and phosphate were separated using cation-exchange chromatography applying isocratic elution with 10 mmol L^{-1} KCl–10 mmol L^{-1} HCl. P was detected as $^{31}P^{16}O^{+}$ using oxygen as reaction gas with ICP–DRC-MS. Figure 1 Shows the overlaid chromatograms of an unspiked aliquot of a surface water sample and an aliquot of the same sample fortified with phosphate (156 μg L^{-1}), glyphosate (280 μg L^{-1}), and AMPA (183 μg L^{-1}). Phosphate eluted nearly in the dead volume. For glyphosate and AMPA retention times of 5.2 min and 7.9 min, respectively, were obtained. At the pH of the eluent both target analytes contain a protonated amino-functionality which is the active site for cation exchange. Due to the strong acidity of the phosphonic acid sites these function-

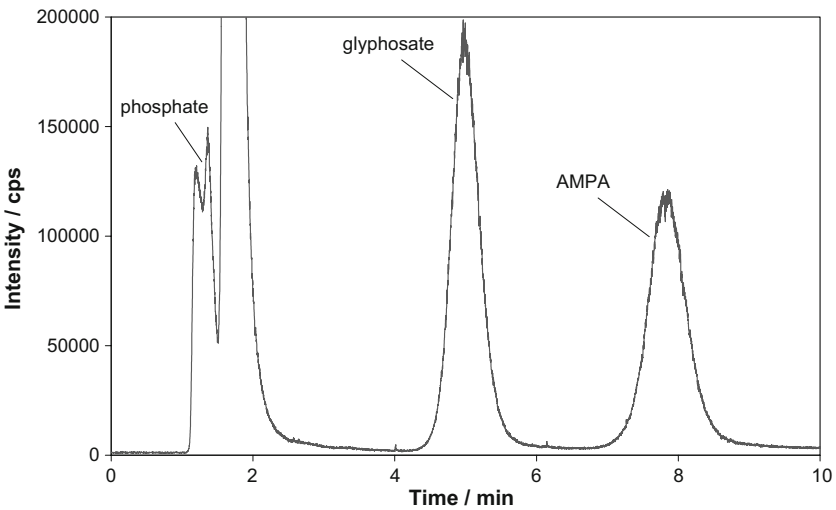

Fig. 2 Spiked surface water sample (River Danube) after enrichment by two-step SPE procedure on Chelex 100 and AG1-X-8 ion-exchange resins. Spiked concentrations: 58 μg L^{-1} glyphosate and 22 μg L^{-1} AMPA (enrichment factor 400)

Table 2 Validation data for two-step solid-phase extraction of glyphosate and AMPA on Chelex 100 and AG1-X8 ion-exchange resins

Sample	Determined concentration		Spiked concentration (μg L^{-1})	Recovery
	Mean (μg L^{-1})	RSD		
Glyphosate Level 1	1.2	33%	1.03	114%
Glyphosate Level 2	5.8	5%	5.18	112%
Glyphosate Level 3	9.6	18%	10.25	94%
AMPA Level 1	1.1	35.7%	1.01	112%
AMPA Level 2	5.6	5.7%	5.08	110%
AMPA Level 3	9.3	19.6%	10.02	93%

Determination of target analytes was by HPIC–ICP–DRC-MS

alities are at least partially deprotonated. In addition, glyphosate contains a carboxylic acid group that will also partially be deprotonated. The concentrations of the analytes were determined by species-unspecific external linear calibration using FI and phosphoric acid standards. Excellent recoveries of 103% and 104% for glyphosate and AMPA were found. Due to the partial co-elution with an unidentified peak close to the dead-time volume, for phosphate only a recovery of 82% was achieved. Methodological LODs of the HPIC–ICP–DRC-MS were 42 μg L^{-1} and 33 μg L^{-1} for glyphosate and AMPA (calculated from the peak height and the threefold standard deviation of the baseline; Fig. 2). These values correspond to P LODs of 8.4 and 10.1 μg L^{-1}.

However, monitoring the legislative value of 0.1 μg L^{-1}, set by the EU for pesticides, demanded for an enrichment procedure. In preliminary experiments ion exchange on Bakerbond SPE quaternary amine columns (500 mg) (J.T. Baker, Deventer, The Netherlands) was evaluated. However, only for glyphosate could efficient enrichment be achieved while recoveries were very poor for AMPA. Therefore, a different two-step approach was validated. At pH 2 the iron form of the Chelex 100 resin acted as a chelator for both glyphosate and AMPA. This strategy allowed efficient and selective pre-concentration of glyphosate and AMPA even from matrix-rich samples. In a second step the target analytes were eluted by HCl. Fe(III) ions co-eluting in the form of a $[FeCl_4]^-$ complex were

Table 3 Analysis of surface water and waste water samples

Sampling Site	July 2005		April 2006	
	Glyphosate (μg L^{-1})	AMPA (μg L^{-1})	Glyphosate (μg L^{-1})	AMPA (μg L^{-1})
Dornbirner Ache – Lauterach	0.67	2.81	n.a.	n.a.
IS 1 – Waste water	< LOD	< LOD	< LOD	< LOD
Receiving water upstream IS 1	< LOD	< LOD	< LOD	< LOD
Receiving water downstream IS 1	< LOD	< LOD	< LOD	< LOD
WWTP 1 – Influent	< LOD	< LOD	n.a.	n.a.
WWTP 1 – Effluent	< LOD	1.77	n.a.	n.a.
Receiving water upstream WWTP 1	< LOD	< LOD	n.a.	n.a.
Receiving water downstream WWTP 1	< LOD	< LOD	n.a.	n.a.
WWTP 2- Influent	< LOD	14.3	< LOD	2.63
WWTP 2- Effluent	1.49	12.6	0.72	4.89
Receiving water upstream WWTP 2	0.33	< LOD	< LOD	< LOD
Receiving water downstream WWTP 2	1.39	4.3	< LOD	< LOD
WWTP 3 – Influent	1.08	4.5	< LOD	< LOD
WWTP 3 – Effluent	1.93	8.1	< LOD	2.42
Receiving water upstream WWTP 3	< LOD	< LOD	< LOD	< LOD
Receiving water downstream WWTP 3	0.63	1.5	[0.26]	0.50

< LOD denotes below the limit of detection; values in brackets are below the limit of quantification
n.a. denotes samples were not available
Results from two sampling campaigns at three waste water-treatment plants (WWTP), one industrial site (IS), and the river Dornbirner Ache in Lauterach. All sites are situated in western Austria in the Province of Vorarlberg

separated from both compounds by subsequent anion-exchange clean-up.

Five blanks and triplicates of standards at three concentration levels (1.0 μg L^{-1}, 5.0 μg L^{-1}, and 10 μg L^{-1}) were processed and analysed by HPIC–ICP–MS. Again, quantification was carried out via species-unspecific calibration. As can be readily observed in Table 2, complete recovery of the target analytes over a working range of two orders of magnitude was obtained. Moreover, blank contributions were found to be negligible. Enrichment factors in the range of 400 were gravimetrically assessed (ratio of applied sample to final extract).

River water and waste water samples

Knowledge on the fate of glyphosate and AMPA in wastewater plants is rather fragmentary. Table 3 lists data from samples collected at three WWTPs, one industrial site, and a river. The influent and the effluent (both 24-h mixed samples) and the on-site receiving water up and downstream of the WWTPs were investigated.

In the case of the river Dornbirner Ache for both, glyphosate and AMPA, minor concentrations of 0.67 μg L^{-1} and 2.81 μg L^{-1}, respectively, were determined during the first campaign in 2005. Generally, elevated concentrations were found in the effluent compared to the influent. This is in contradiction to the general opinion that glyphosate and AMPA are effectively removed in WWTP through adsorption on suspended solids and sewage sludge. One possible explanation for this finding could be release from suspended particles during the waste water processing. All concentrations of glyphosate and AMPA are comparable with those found in previous studied [7].The highest concentrations of AMPA of up to 14 μg L^{-1} were found in the influent of WWTP 2. At the same time glyphosate concentrations below the LOD were present in the influent and only comparatively low concentrations of the herbicide in the effluent. Phosphonates, are besides glyphosate other possible sources of AMPA [16].

Conclusions

IC–ICP–DRC-MS in combination with suitable enrichment procedures allowed monitoring of glyphosate and AMPA at environmental concentration levels. Both substances were found in river water in western Austria. The investigated WWTPs did not efficiently eliminate glyphosate and AMPA from the water cycle.

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References

1. Woodburn A (2000) Pestic Manag Sci 56:309–312
2. Rueppel ML, Brightwell BB, Schaefer J, Marvel JT (1977) J Agric Food Chem 25:517–528
3. Strange-Hansen R, Holm PE, Jacobsen OS, Jacobsen CS (2004) Pestic Manag Sci 60:570–578
4. Forlani G, Mangiacalli A, Nielsen E, Suardi CM (1999) Soil Biol Biochem 31:991–997
5. Vereecken H (2005) Pestic Manag Sci 61:1139–1151
6. GEUS (2004) Grundvandsovervagning 1998-2003.Geological survey of Denmark and Greenland. <http://www.geus.dk>
7. Pfeffer M, Kress K, Busch D (2002) Erste Ergebnisse des Glyphosate Monitorings im Rahmen der WGEV. Bericht ALVA - Jahrestagung 2002
8. Richardson SD (2006) Anal Chem 78:4021–4045
9. Stalikas CD, Konidari CN (2001) J Chromatogr A 907:1–19
10. Bauer KH, Knepper TP, Maes A, Schatz V, Voihsel M (1999) J Chromatogr A 837:117–128
11. Vreeken RJ, Speksnijder P, Bobeldijk-Pastorova I, Noij ThHM (1998) J Chromatogr A 794:187–199
12. Sadi BBM, Vonderheide AP, Caruso JA (2004) J Chromatogr A 1100:95–101
13. Guo ZX, Cai Q, Yang Z (2005) J Chromatogr A 1100:160–167
14. Björjesson E, Torstensson L (1998) J Chromatogr A 886:207–216
15. Standardarbeitsanweisung SAA33R6502, Bestimmung von Glyphosate und AMPA in Wasser und Böden mittels HPLC-FLD, 2001, ICS-Nummer 67.060, Bundesamt und Forschungszentrum für Landwirtschaft, Vienna
16. Skark C, Zullei-Seibert N, Schottler U, Schlett C (1998) Int J Environ Anal Chem 70:93–104