Application Note
Environmental

Glyphosate and AMPA

The finest LC-EC Applications for Environmental analysis

Chloro- and nitrophenols
2,4-dinitrophenol (DNP)
phenol (P)
4-nitrophenol (4-NP)
2-methyl-4,6-dinitrophenol (MDNP)
2-chlorophenol (2-CP)
2-nitrophenol (2-NP)
2,4-dimethylphenol (DMP)
4-chloro-3-methylphenol (CMP)
2,4-dichlorophenol (DCP)
2,4,6-trichlorophenol (TCP)
pentachlorophenol (PCP)
PET Ketones
Glyphosate
Aminomethylphosphonic acid (AMPA)

- **Glyphosate and AMPA in drinking water**
- **Pulsed Amperometric Detection (PAD)**
- **Sensitive method without pre-concentration**
- **Elution and detection within 5 minutes**

Summary

Glyphosate, commonly known by its original trade name Roundup, is the world's most widely used herbicide (weed killer). In March 2015, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic in humans" [1]. Since, the publication of this report the use of glyphosate is under debate worldwide. In addition, in the US, Bayer AG (manufacturer of Roundup) is engaged in billion dollar lawsuits with respect to claims of users that the glyphosate-based weed killer caused cancer.

Due to agricultural use Glyphosate and its metabolite AMPA can make its way into aquatic environments and drinking water, which makes monitoring of these compounds in water and other matrices necessary. In this application note an simple and sensitive analytical method is presented for the analysis of Glyphosate and AMPA in drinking water, using the DECADE Elite electrochemical detector and SenCell. The method is based on separation by High Performance Anion Exchange Chromatography (HPAE) followed by Pulsed Amperometric Detection (HPAE-PAD) on a gold working electrode. The method does not require derivatisation and pre-concentration of the sample.

Introduction

Glyphosate (N-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide and crop desiccant, and is the world's most widely used weed killer in agriculture and gardening. It was discovered in 1970 by Monsanto and brought into the market under the name Roundup in 1974 [2]. Aminomethylphosphonic acid (AMPA) is one of the primary and most stable degradation products of glyphosate. While glyphosate and formulations such as Roundup have been approved by regulatory bodies worldwide, concerns about their effects on humans and the environment persist, and have grown as the global usage of glyphosate increases. In March 2015, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic in humans". However, there is no consensus about this classification. The European Food Safety Authority (ESFA) concluded at the end of 2015 that "the substance is unlikely be genotoxic (i.e. damaging to DNA) or to pose a carcinogenic threat to humans"[3]. In 2017, the European Commission extended the approval for the use of glyphosate for another 5 years until December 2022 [4]. In addition, also the US Environmental Protection Agency (EPA) concluded that glyphosate is unlikely to pose a carcinogenic risk to humans [5].

Due to agricultural use glyphosate and AMPA can make their way into aquatic environments and drinking water, which makes monitoring necessary. Although, glyphosate has a low mobility in soil, it can enter surface and subsurface waters after direct use near aquatic environments or by runoff or leaching. Currently there is no world-wide guideline value established for glyphosate in drinking water [6]. For example, in the US the EPA stated that levels up to 700 µg/L are allowed. In Canada, Australia and the EU member states the Maximum Allowed Concentrations (MAC's) are 280, 10 and 0.1 µg/L, respectively, for glyphosate and AMPA in drinking water. There are several HPLC methods published about the analysis of glyphosate and AMPA, based on Fluorescence detection[7], MS/MS detection[8] and electrochemical detection [9,10]. However, for fluorescence detection derivatisation is necessary and although MS allows direct detection of the herbicides, it requires more expensive analysis equipment. Electrochemical detection is an attractive and cost-effective alternative, it offers quantification at ppb levels in drinking water by direct injection without derivatisation and sample pre-concentration (SPE).

In this application note a sensitive method for the analysis of glyphosate and AMPA in drinking water is presented, based on High Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) using a new type of anion-exchange column with 4µm particle size.

Method

The analysis was performed using an ALEXYS HPAE-PAD analyser with a P6.1L pump and AS 110 autosampler (see figure 1).

Figure 1. ALEXYS HPAE-PAD analyzer based on the DECADE Elite electrochemical detector with SenCell for the analysis of glyphosate and AMPA.

The P6.1L (pn 194.APH31EA) is an isocratic pump with integrated dual channel degasser and solvent switch. The pump has the option to switch between two mobile phases during a run enabling step-gradient profiles. For detection a DECADE Elite electrochemical detector equipped with SenCell flow cell with gold working electrode was used. The system was controlled via a PC using the Thermo Chromeleon Chromatography Data System software (version 7.2.9). The LC-EC conditions are listed in table 1.

Table 1

Conditions	
HPLC	ALEXYS HPAE-PAD analyzer
Detector	DECADE Elite EC detector (FW1.09 or higher)
Column	CarboPac™ PA210-Fast-4µm, 30 x 4 mm guard CarboPac™ PA210-Fast-4µm, 150 x 4 mm analytical
Mobile phase A	200 mM sodium acetate, 10 mM sodium hydroxide (sparged with Helium 5.0)
Mobile phase B	350 mM sodium acetate, 10 mM sodium hydroxide (blanketed with Helium 5.0)
Flow rate	0.8 mL/min
Injection	100 µL (full loop)
Temperature	35°C for separation & detection
Flow cell	SenCell™ with Au WE, HyREF™, AST 2
Potential waveform	See table 3 (Pulse mode -2)
I-cell	about 0.55 - 0.75 µA
ADF	0.1 Hz
Range	5 µA/V

Separation

Both glyphosate and AMPA are weak organic acids having a phosphonic acid group. In addition, glyphosate also has a car-

boxylic acid moiety. Under alkaline conditions (pH around 12) these groups in glyphosate and AMPA are deprotonated and the compounds can be separated based on anion-exchange chromatography. A CarboPac™ PA210-Fast-4µm column (150 x 4 mm ID) Anion-Exchange column with guard (30 x 4 mm ID) was chosen for the separation of glyphosate and AMPA. The temperature for separation was set at 35°C. The analysis is based on a step-gradient elution profile, which is shown in table 2. Both mobile phases A and B contain 10 mM NaOH. Sodium acetate (NaOAc) is used as a modifier. The acetate anion acts as a strong 'pusher' allowing faster elution of strongly retained components.

Table 2

Step-gradient program

Time (min)	*Mobile phase**	*B (%)*	*Description*
0 - 1	200 mM NaOAc	0	Elution of AMPA
1 - 6	350 mM NaOAc	100	Elution of Glyphosate
6 –10	200 mM NaOAc	0	Equilibration, starting conditions

*) Note that both mobile phases also contain 10 mM NaOH, see table 1.

AMPA is eluted with mobile phase A containing 200 mM NaOAc. After one minute the system is switched to mobile phase B with a higher NaOAc concentration (350 mM) to elute glyphosate. To minimize the introduction of carbonate ions in the mobile phase the eluents were carefully prepared manually using a 50% w/w carbonate-free NaOH solution (commercially available). The diluent was DI water (resistivity >18 MΩcm) which was sonicated and sparged with Helium 5.0 prior to use. The mobile phase should be prepared in plastic bottles instead of glass. The mobile phases were blanketed with Helium 5.0 (0.5 bar overpressure) during the analysis, to prevent the introduction of CO_2 over time.

Detection

For the pulsed amperometric detection of glyphosate and AMPA the Antec SenCell™ electrochemical flow cell is used. This flow cell [11] has a confined wall-jet design and consists of a Au working electrode (WE), HyREF (Pd/H_2) reference electrode (RE) and stainless steel auxiliary electrode (AE). A special multi-step potential waveform [10], shown in figure 2, was applied for detection of the compounds. The profile consisted of an upward scan, plateau and downwards scan, during which the current is measured. This particular waveform resulted in a more stabile and reproducible long-term response, compared to the classical 3- or 4-step PAD waveforms with a square wave pattern. The option to program multi-step waveforms up to 30 time points is available for the DECADE Elite from FW version 1.09 and onwards. This new measurement mode,Pulse mode – 2, is only available when the Elite is controlled by software. In the second half of 2019 DECADE Elite control drivers for DataApex Clarity, Thermo Chromeleon and the Agilent OpenLab CDS will become available supporting this new feature.

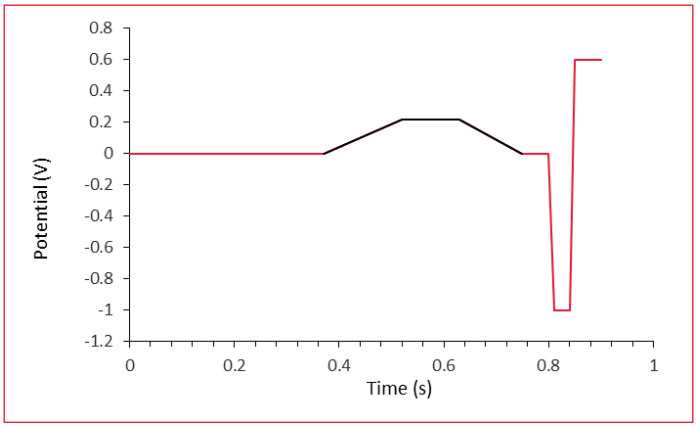

Figure 2. The PAD waveform for the detection of glyphosate and AMPA. The part of the curve shown in black is the time periode in which the current is measured.

Table 3

PAD waveform (Pulse mode -2)

Time (s)	*Potential (V)*	*Data sampling*
0.00	0.00	
0.37	0.00	Begin
0.52	0.22	
0.63	0.22	
0.75	0.00	End
0.80	0.00	
0.81	-1.00	
0.84	-1.00	
0.85	0.60	
0.90	0.60	

The multi-step waveform is programmed by entering time-potential coordinates (t/E), as shown in table 3. The sampling interval is marked by a 'Begin' and 'End' marker. The sampling interval is free programmable in the pulse table.

In the new pulse mode, potential steps as well as slopes can be defined. Allowing the programming of potential scans in the PAD waveform like used in integrated pulsed amperometric detection (IPAD)[12]. So the new pulse mode extends the application areas of the DECADE Elite to IPAD measurements, used for example for the analysis of amino acids [13] and sulfur-containing antibiotics [12]. In fact, glyphosate is an aminophosphonic analogue of the amino acid glycine [2].

Preparation of standards and samples

Standards: 200 mg/L stock standards of AMPA and glyphosate were prepared in 10 mM sodium hydroxide. The addition of 10 mM sodium hydroxide to the standards resulted in a better peak shape (less tailing). Stock standards were kept in the fridge at 4°C and were further diluted in 10 mM NaOH to a concentration range between 0.25 to 10 µg/L.

Sample preparation: A tap water sample was collected and the pH adjusted with the following procedure: 50 µL of a 0.4 M NaOH solution was pipetted into a volume of 1950 µL of the tap water sample, resulting in an end concentration of 10 mM NaOH in the sample. 100 µL was directly injected into the LC system and analyzed.

Results

In figure 3 a chromatogram of a 100 µL injection of a 5 µg/L AMPA and glyphosate standard mixture in 10 mM NaOH is shown.

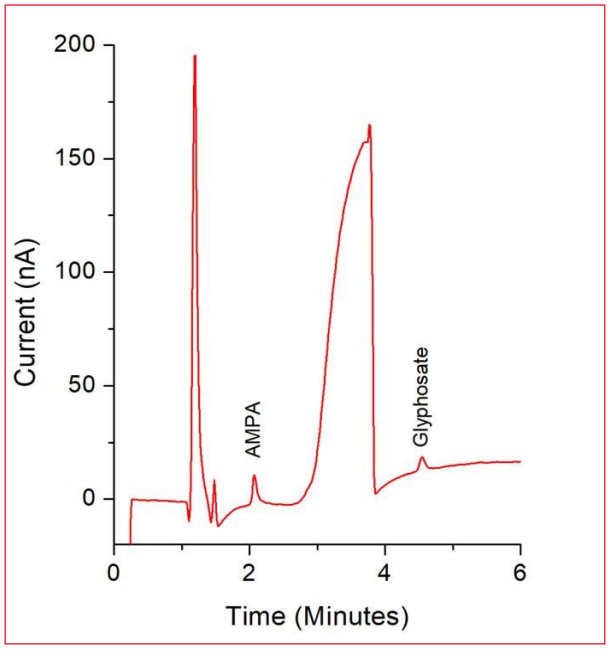

Figure 3. Chromatogram of a 100 µL injection of a 5 µg/L standard mixture of AMPA and glyphosate in 10 mM sodium hydroxide.

Within 5 minutes both AMPA and glyphosate are eluted. The large baseline disturbance, starting at 2.7 min is caused by the introduction of mobile phase B which has a much higher concentration of NaOAc. The change from 200 to 350 mM NaOAc results in an increase of approximately 170 nA of the back ground current. At t = 3.7 min an autozero is executed automatically, using a timed-event programmed in the method.

Linearity, Repeatability and LOD

The linearity of the response of glyphosate and AMPA was investigated in the concentration range of 1 — 63 µg/L, which corresponds with a molar concentration range of 6 nmol/L - 373 nmol/L and 9 nmol/L - 567 nmol/L for glyphosate and AMPA respectively. For both compounds the linearity was excellent with correlation coefficients better than 0.999 (see figure 4).

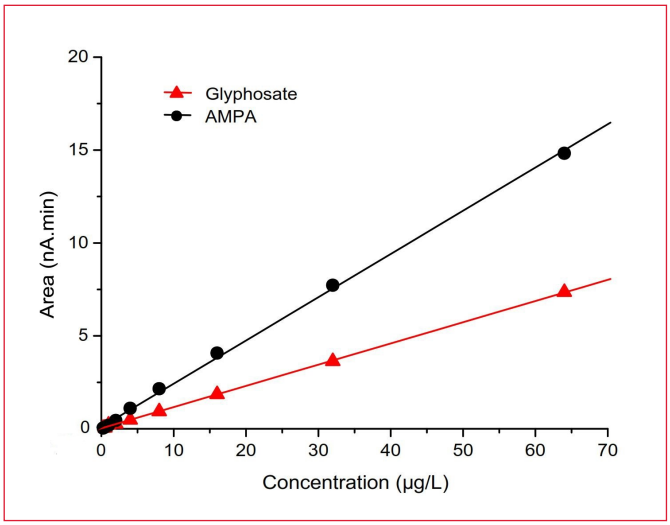

Figure 4. Calibration curve of glyphosate and AMPA in the concentration range of 1 - 63 µg/L. (R > 0.999).

The repeatability of the method was evaluated by ten repetitive injections of a 1, 5 and 50 µg/L standard mix of glyphosate and AMPA. The relative standard deviations (RSD) for retention times and peak area are shown in table 4.

Repeatability of 100 µL injections of a 1 and 10 µg/L AMPA and glyphosate standard mix in 10 mM NaOH (n=10)

	RSD's (%) 1 µg/L		*RSD's (%) 5 µg/L*		*RSD's (%) 50 µg/L*	
Compound	t_R	*Area*	t_R	*Area*	t_R	*Area*
AMPA	0.00	4.4	0.38	1.7	0.30	0.7
Glyphosate	0.32	4.7	0.36	0.9	0.31	0.5

Table 4

RSD's for retention time were < 0.4% for all repro data sets. A good repeatability of the response (peak area) for both AMPA and glyphosate was obtained for the 5 and 50 µg/L standards. It is evident from table 4 that the RSD's of the peak area of the 1 µg/L standard were higher (<5%). This is caused by the fact that this concentration is close to the detection limit of both compounds.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) was determined based on the response of the 1µg/L standard mix. The LOD was calculated as the analyte response corresponding to 3x the ASTM noise. The LOQ was calculated as the analyte response corresponding to 10x the ASTM noise. The noise was calculated based on a blank injection with a 5 minute section of the baseline close to the compounds of interest (10 segments of 0.5 minutes). The results are shown in table 5.

Table 5

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

	Limit of detection (LOD)		*Limit of Quantification (LOQ)*
Compound	*µg/L (ppb)*	*nmol/L*	*µg/L (ppb)*
AMPA	0.4	3	1.2
Glyphosate	0.9	5	2.8

The Detection limits found for AMPA and glyphosate are 0.4 µg/L and 0.9 µg/L, respectively. These LOD's are sufficient to reliably quantify and monitor the maximum allowed concentrations (MAC's) of Glyphosate and AMPA in drinking water in the US, Canada and Australia and other countries in the world*. For application in the EU member states some additional sample preparation step (SPE) to pre-concentrate the sample might be required to monitor concentrations around the EU guideline value of 0.1 µg/L.

Sample analysis

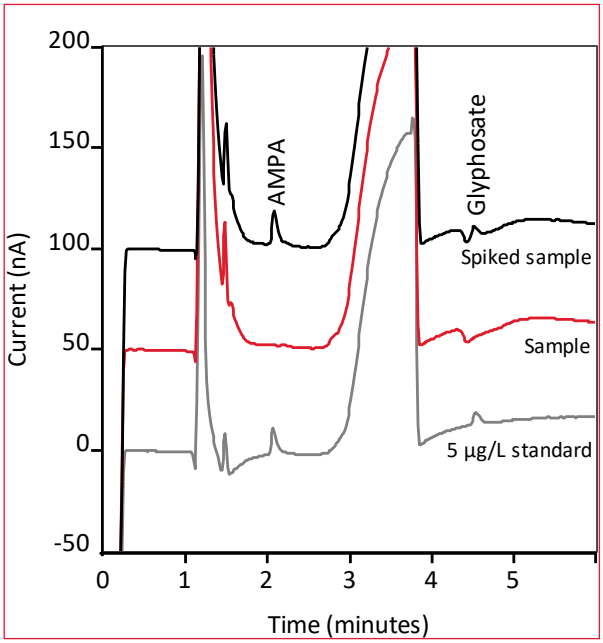

Figure 5. Stacked overlay of a chromatogram of a tap water sample (red), tap water sample spiked with 5 µg/L AMPA and glyphosate (black) and a 5 µg/L AMPA and glyphosate standard (grey).

A tap water sample was analyzed using the described method. The sample preparation of the tap water sample is simple and described in the previous section. The chromatogram of the tap water sample is shown in figure 5 together with a chromatogram of a 5µg/L standard and spiked tap water sample (5 µg/L std) for reference and identification. Note that only the relevant time window (first 6 minutes) is shown where the analytes of interest are eluting. The total run duration was 10 minutes taking into account the equilibration step. In the shown tap water sample no quantifiable amount of glyphosate and AMPA was present.

*) Preliminary experiments with a column with a smaller bore-size (pn 250.1174 CarboPac™ PA210-Fast-4µm, 150 x 2 mm analytical column) have shown that the detection sensitivity can be further improved. The smaller i.d. column allows more sensitive quantification with LOD's of 0.3 and 0.4 µg/L for AMPA and glyphosate respectively. In addition, such 2 mm I.D. column has to be operated at a much lower flow rate of 250 µL/min., resulting in a lower mobile phase consumption.

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Conclusion

The ALEXYS HPAEC-PAD system based on the DECADE Elite detector, SenCell flow cell and 'fast-4µm' HPAE column, offers a simple and cost-effective analysis solution for the quantification of Glyphosate and AMPA in drinking water. The presented HPAEC-PAD method allows fast separation, within 5 min, of both herbicides followed by sensitive PAD detection without the need for derivatization. LOD's of 0.4 µg/L and 0.9 µg/L for AMPA and glyphosate were achieved, without preconcentration of the sample. The new PAD mode in the DECADE Elite offers the possibility to program multi-step potential waveforms, commonly used in ion chromatography applications based on Integrated Pulsed Amperometric Detection.

Ordering information

180.0055W	ALEXYS HPAEC-PAD Analyzer (isocratic. step-gradient applications) including DECADE Elite SCC and SenCell 2 mm Au HyREF
250.1173A*	CarboPac PA210-4µm guard column, 30 x 4.0mm ID (088955)
250.1174A*	CarboPac PA210-4µm anal. column, 150 x 4.0mm ID (088953)

*) Columns are products of Thermo Fisher Scientific Inc. (USA), between parenthesis the Thermo pn's are shown for reference.

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