Glyphosate and AMPA in drinking water

Determination using ion chromatography with pulsed amperometric detection

In March 2015, the International Agency for Research on Cancer (IARC) published a report which stated that glyphosate was «probably carcinogenic to humans». Ever since, the use of this chemical has been highly controversial. In some countries, including the USA, there are already limit values in effect for the weed killer.

Carcinogenic or not?

The broad-spectrum herbicide glyphosate is used all over the world in agriculture. Alongside farming, the chemical is also used for weed-killing in domestic gardens and in public and private spaces kept free from «vegetal invasion», such as railway tracks. Glyphosate has been used since the 1970s in pesticides and was hitherto thought to be harmless at typical levels of exposure. However, since the International Agency for Research on Cancer (IARC) – the specialized cancer-research agency of the WHO – found that glyphosate was «probably carcinogenic to humans» (Group 2A) in a report published in March 2015, the chemical repeatedly made headlines.[1] Experts were then divided over whether glyphosate should be re-approved after the expiry of its EU market approval on June 30, 2016. This is because the European Food Safety Authority (EFSA) only recently arrived at the opposed conclusion that it is unlikely that glyphosate is genotoxic or poses a carcinogenic threat.[2] The approval of glyphosate was initially extended by 18 months, but at the end of 2017, the question of whether glyphosate should remain in use in the EU will resurface.

Limit values for glyphosate in drinking water

Because chemicals used in farming can seep through the ground and into the ground water, limit values are in effect in some countries concerning the concentration of glyphosate in drinking water. For example, the US Environmental Protection Agency (EPA) forbids any concentrations that exceed the limit of 700 µg/L. In Canada, the maximum permissible concentration is 280 µg/L. Australia stipulates much lower limit values at 10 µg/L.

Glyphosate and its metabolite AMPA (aminomethylphosphonic acid) are usually determined by HPLC with post-column derivatization and subsequent fluorescence detection (EPA Method 547), or alternatively by ion chromatography coupled with a mass-selective detector. The following will set out the initial results of the determination of glyphosate and AMPA in drinking water in the low µg/L range using ion chromatography (IC) with pulsed amperometric detection. The detection limits for glyphosate and AMPA previously attained with pulsed amperometric detection were around ≥ 50 µg/L [3]. Given this improvement in terms of sensitivity, the method outlined here represents a promising approach to the screening of water and food samples for glyphosate and AMPA.

Instrumentation

All determinations were performed with an IC system consisting of a 940 Professional IC Vario ONE with an IC Amperometric Detector and an 858 Professional Sample Processor for automatic sample injection (Figure 1). The measuring mode flexIPAD (FLEXible Integrated Pulsed Amperometric Detection) was used on a gold working electrode in the amperometric detector. The flexIPAD mode is characterized by its special, multi-stage potential profile. In the determination of glyphosate and AMPA, this produces a stable signal over a longer period of time than the three-stage potential profile of the regular PAD mode (pulsed amperometric detection). The profile of the potential curve produced in one measuring cycle in flexIPAD mode is presented in Figure 2.

Glyphosate and AMPA were separated on the high-capacity anion separation column Metrosep Carb 2 - 150/4.0. The caustic-soda–acetate eluent used contains 10 mmol/L sodium hydroxide and 300 mmol/L sodium acetate. Under these conditions, AMPA and glyphosate elute after 6.4 and 21.1 minutes, respectively.

Experiment

The goal of this experiment is to investigate the separation of glyphosate and AMPA in the Metrosep Carb 2 separation column, as well as clarifying the detection using pulsed amperometry and its sensitivity. The Metrosep Carb 2 column is used mainly for separating and determining carbohydrates, sugar alcohols, alcohols, etc. Its high column capacity, combined with the high pH value of the eluent (which at approx. 10 is typical for sugar analysis), results in a large difference in retention time for AMPA and glyphosate.

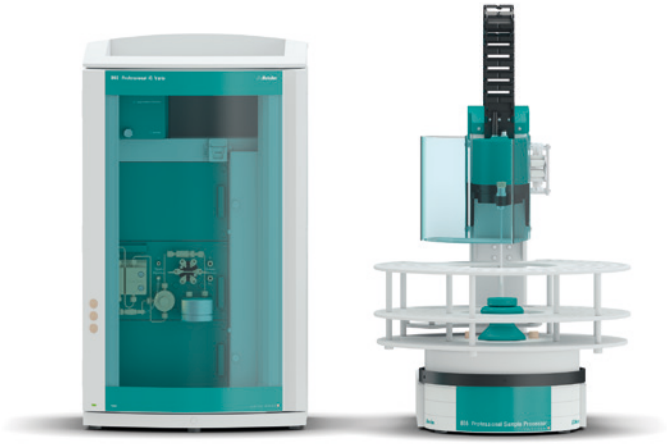

Figure 1. Glyphosate and AMPA were determined with the ProfIC IC Vario ONE Amperometry IC system.

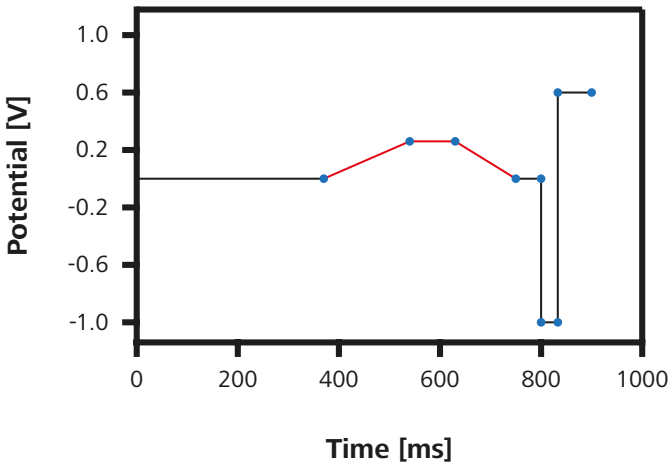

Figure 2. Pulse profile of the flexIPAD method: A measuring cycle lasts 0.9 s; measurement of the current is performed during the phase shown in red.

This is because, at a pH value of 10, all three acid groups are deprotonated in part of the glyphosate, meaning that it is partially present as a trivalent anion. Meanwhile, the metabolite AMPA, which is missing the carboxyl group, is present as a divalent anion.

In order to accelerate the elution of glyphosate, a flow gradient is used: after AMPA elution at 6.4 minutes, the flow rate is doubled from 0.4 mL/min to 0.8 mL/min. This results in a retention time of 21 minutes for glyphosate. The chromatographic conditions are summarized in Table 1.

Results

Figure 3 shows the chromatogram of the determination of AMPA and glyphosate under the conditions listed in Table 1. An aqueous standard solution containing 10 µg/L each of both components was injected. In order to investigate the suitability of the process for drinking water, tap water from Herisau (Switzerland) was analyzed and spiked with different amounts of AMPA and glyphosate. The concentrations and peak areas found are shown in Table 2.

The detection limits for both components were determined using the signal-to-noise (S/N) ratio, i.e., the ratio of the peak height to the baseline noise. At the detection limit, the S/N ratio is 3; with smaller values, secured detection is not possible. The detection limit found for AMPA was considerably lower than 1 µg/L, while the limit for glyphosate was approx. 1 µg/L. Figure 4 shows the chromatogram of the drinking water spiked with 2 µg/L glyphosate and AMPA.

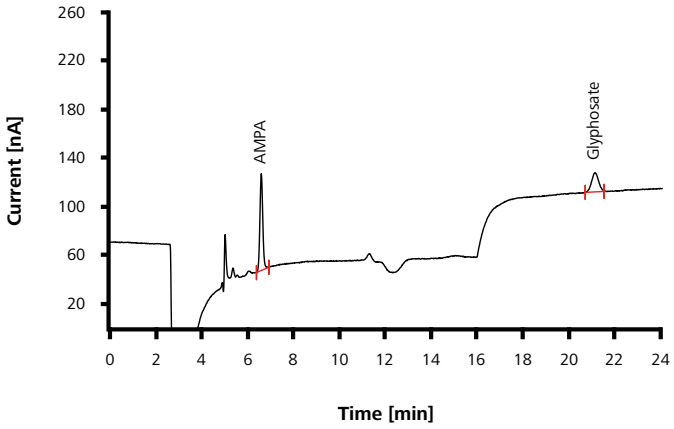

Figure 3. Separation of AMPA and glyphosate: A standard solution containing 10 µg/L of each component in ultrapure water was analyzed. For conditions see Table 1.

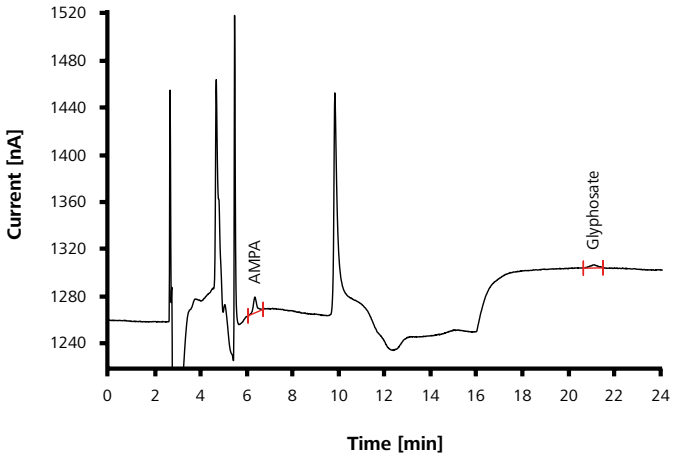

Figure 4. Determination of AMPA and glyphosate in drinking water which was spiked with 2 µg/L of each component. For conditions and results, see Tables 1 and 2.

Table 2. Investigated drinking water samples and the peak areas found

Sample	Peak area of AMPA [nA · min]	Peak area of glyphosate [nA · min]
Tap water	Not detectable	Not detectable
Tap water spiked with 2 µg/L	2.47	1.13
Tap water spiked with 5 µg/L	4.96	2.73
Tap water spiked with 10 µg/L	8.97	5.20

Table 1. Chromatographic conditions

Chromatography	
Column	Metrosep Carb 2 - 150/4.0
Eluent	10 mmol/L sodium hydroxide 300 mmol/L sodium acetate
Flow rate	0.4 mL/min (0–16 min) 0.8 mL/min (16–25 min)
Injection volume	250 µL
Chromatography duration	25 min
Column temperature	30 °C
Amperometric detection	
Cell	Wall-Jet cell
Working electrode	Gold
Reference electrode	Palladium
Spacer	50 µm
Temperature	35 °C
Measuring mode	flexIPAD
Measured quantity	Current

Summary

For the first time, glyphosate and its primary metabolite AMPA were determined in drinking water in the low µg/L range using ion chromatography with pulsed amperometric detection (flexIPAD). This puts at our disposal a reliable and – compared with HPLC with a mass-selective detector – very inexpensive method for determining the glyphosate and AMPA content in water and foodstuffs. With a detection limit of approx. 1 µg/L, the adherence to limit values for glyphosate can be verified in the USA, Canada, and Australia, among others.

References

[1] IARC Monographs Volume 112 (2015). Retrieved from http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-09.pdf on June 27, 2016.
[2] EFSA press news, 151112 (2015). Retrieved from http://www.efsa.europa.eu/en/topics/factsheets/glyphosate151112 on June 27, 2016
[3] F. Sanchez-Bayo, R. V. Hyne, and K. L. Desseille (2010) Anal. Chim. Acta, 675 125–131