thermo scientific

APPLICATION NOTE 666

Routine analysis of polar pesticides in water at low ng/L levels by ion chromatography coupled to triple quadrupole mass spectrometer

Authors

Astrid Kurz,[1] Katerina Bousova,[2] Jonathan Beck,[3] Frans Schoutsen,[2] Cees Bruggink,[2] Milan Kozeluh,[4] Lumir Kule,[4] and Michal Godula[2]

[1]Hochschule für Technik und Wirtschaft Aalen, Aalen, Germany
[2]Thermo Fisher Scientific, Dreieich, Germany
[3]Thermo Fisher Scientific, San Jose, CA, USA
[4]Povodi Vltavy state enterprise, Pilsen, Czech Republic

Keywords

Glyphosate, AMPA, Polar pesticides, Pesticide residues, IC-MS, TSQ Quantiva, Integrion

Goal

To develop an IC-MS/MS based method that can be applied for high-throughput screening and quantitation of polar pesticide residues and their metabolites in water matrices below the current legislative requirements.

Introduction

The analysis of polar ionic pesticides in surface and drinking water as well as food and beverages has become a controversial issue in recent years. The development of genetically modified organism (GMO) crops tolerant to glyphosate (*N*-(phosphonomethyl)glycine) and glufosinate (*(RS)*-2-amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid), for example, promoted the use of these broad spectrum herbicides. In addition, glyphosate is used as a crop desiccant and to suppress weeds in parks and at roadsides. Consequently, polar pesticides are found in foods as residues and in the environment as contaminants of surface waters and soils. There are concerns about their potential adverse effects on human health such as their potential carcinogenicity,[1] although latest toxicological assessments do not predict toxicological risks for humans under normal conditions of human or environmental exposures.[2] Current regulations set maximum levels of

ThermoFisher SCIENTIFIC

glyphosate and its metabolite AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) at 100 ng/L in drinking water. In food and beverage samples, generally higher maximum residue levels (MRL) apply, ranging from 10 µg/kg for food intended for consumption by children up to hundreds of mg/kg in other matrices.[3]

The analysis of glyphosate and other polar compounds presents a difficult analytical challenge. Their polarity does not allow the direct analysis by reversed phase HPLC, so alternative methods need to be applied. Derivatization of glyphosate prior to analysis[4] or the application of specific chromatographic columns, like the porous graphitic carbon (PGC) based Thermo Scientific™ Hypercarb™ column, are the common approaches.[5] With both approaches, varying method robustness and unreliable results are often reported by routine laboratories, especially when the method is applied in high-throughput analysis of samples with rather complex matrix composition.

Recent developments in the hyphenation of ion chromatography (IC) and mass spectrometry (MS) facilitated novel options for the analysis of polar pesticides. IC is the preferred separation technique for polar ionic analytes, such as anions, cations or ionic metabolites as well as sugars. Mass spectrometry, namely in triple quadrupole MS/MS systems, offers very low detection limits and high detection selectivity when operated in selected reaction monitoring (SRM) mode. The system robustness allows the analysis of food and environmental samples. The aim of this work was to develop and validate an IC-MS/MS method for direct analysis of polar ionic pesticides in water samples and to assess its applicability under routine conditions.

Experimental

Samples of drinking and mineral water were analyzed directly; surface water samples were filtered through the membrane filter before injection into the IC-MS system. Standard solutions of glyphosate and other compounds were stored in plastic containers, as it is known to be absorbed to the walls of glassware. Also, the final extract was injected from 2 mL plastic vials.

Instrumentation

- Thermo Scientific™ TSQ Quantiva™ Triple Quadrupole MS, P/N TSQ-50003
- Thermo Scientific™ Dionex™ Integrion™ HPIC™ System, P/N 22153-60208
- Thermo Scientific™ Dionex™ EGC KOH Eluent Generator Cartridge, P/N 075778
- Thermo Scientific™ Dionex™ ASRS™ 300 Anion Electrolytically Regenerated Suppressor 300 – 2 mm, P/N 064555
- Thermo Scientific™ Dionex™ AS-AP Autosampler, P/N 074926
- Thermo Scientific™ Dionex™ CR-ATC 600, P/N 088662
- Thermo Scientific™ Dionex™ AXP-MS Auxiliary Pump (make-up flow), P/N 60684
- Thermo Scientific™ Dionex™ AXP-MS Auxiliary Pump (AERS regeneration), P/N 60684

System control and data evaluation by Thermo Scientific™ Chromeleon™ 7.2 or higher

Consumables

- Thermo Scientific™ Dionex™ IonPac™ AS24 Analytical Column (2 × 250 mm), P/N 064153
- Thermo Scientific Dionex IonPac AG24 Guard Column (2 × 50 mm), P/N 064151
- PES Syringe Filter (0.2 µm), P/N 42213-PS

Instrument and method setup

The instrument system comprised of a metal-free Thermo Scientific™ Dionex™ Integrion™ ion chromatograph and a Dionex AS-AP autosampler coupled to a Thermo Scientific™ TSQ Quantiva™ mass spectrometer (Figure 1). The chromatographic separation was carried out using a polymeric based Thermo Scientific™ IonPac™ AS24 column with guard in the 2-mm format. Additional instrument parameters details are listed in Table 1 and Table 2. The hydroxide eluent was prepared in-situ using eluent generation preventing the use of external chemicals.

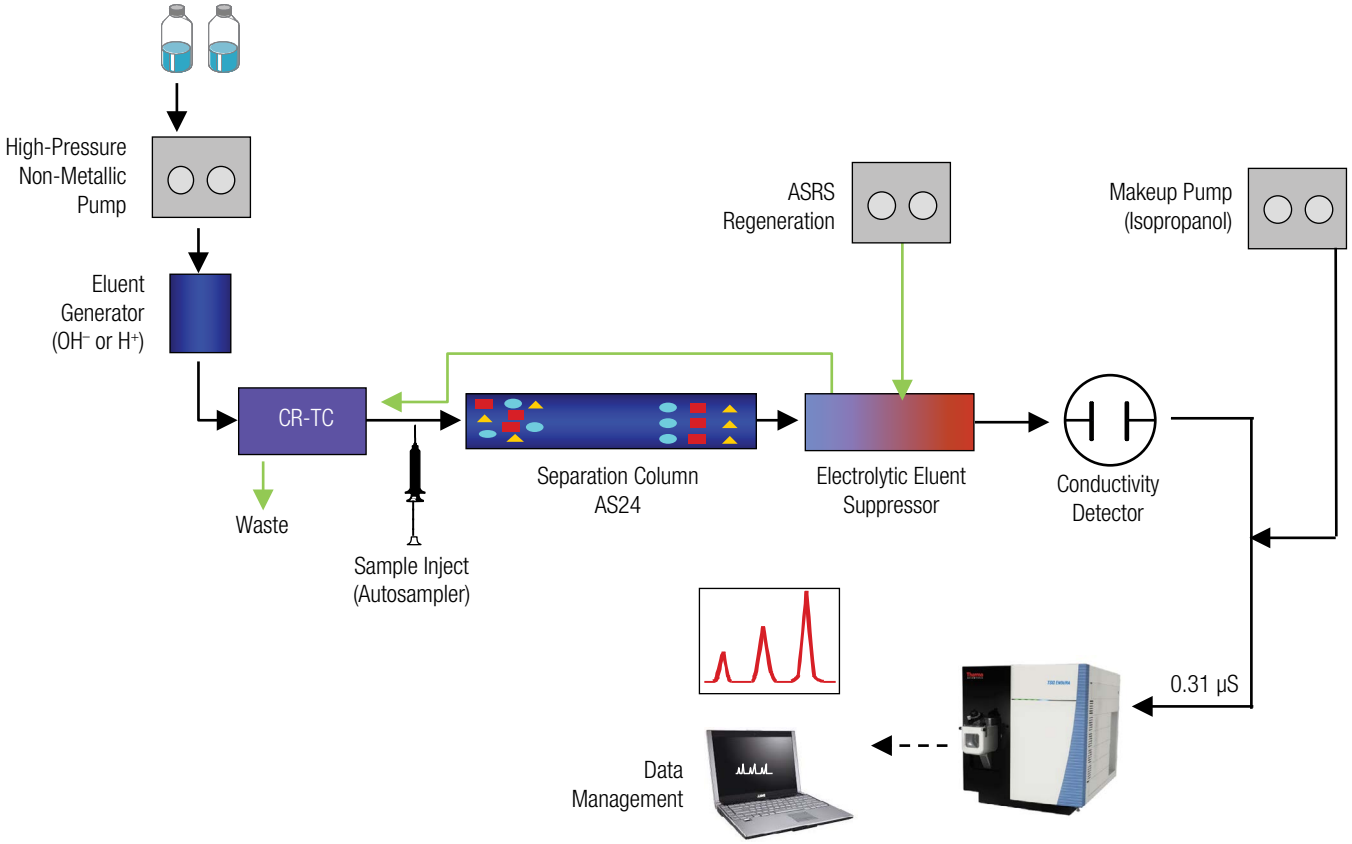

Figure 1. Schematics of the IC-MS/MS system.

Table 1. IC conditions.

Mobile Phase:	KOH (Gradient conditions, Table 2)
Eluent Source:	Eluent Generator
Analytical Column:	Dionex IonPac AS24 (2 × 250 mm) with guard column
Suppressor:	Dionex ASRS 300 – 2mm (External water mode, Table 2)
Flow Pump 1 (AERS regeneration):	1 mL/min
Make-up Solvent:	2-propanol
Flow Pump 2:	0.1 mL/min
Injection Volume:	100 µL
Column Temperature:	30 °C
Flow Rate:	0.3 mL/min

Table 2. IC gradient and suppressor conditions.

Time (min)	Potassium Hydroxide (KOH) (mM)	Suppressor Current (mA)
0.0	22	17
4.1	22	25
7.0	25	25
7.1	40	25
9.5	60	25
12.0	80	60
14.5	80	75
15.0	100	75
17.0	100	75
17.1	22	75
19.9	22	75
20.0	22	17
22.0	22	17

After separation, the eluent passed the electrochemically regenerated AERS suppressor, where the cations from both the eluent and the sample were replaced with hydronium ions, effectively neutralizing the high pH eluent, rendering it compatible with a mass spectrometer. No external chemical regenerants were needed, as an external pump delivered water feeding the electrolytic process to continuously regenerate the suppressor membranes. To improve desolvation, a second external pump added 2-propanol as make-up solvent at a low flow-rate before entering the mass spectrometer.

Mass spectrometer conditions

SRM was applied for data acquisition. All SRM transitions were individually tuned for each target analyte injecting the corresponding standard solution (1 mg/L). The mass spectrometer conditions are shown in Table 3 and SRM parameters for analyzing targeted analytes are shown in Table 4.

Table 3. Mass spectrometer conditions.

Ionization Mode:	Heated Electrospray (HESI)
Scan Type:	SRM
Polarity:	Negative ion mode
Spray Voltage:	2800 V
Sheath Gas Pressure:	30 arb
Aux Gas Pressure:	12 arb
Ion Sweep Gas Pressure:	1 arb
Capillary Temperature:	340 °C
Vaporizer Temperature:	360 °C
Cycle Time:	0.5 s
Q1/Q3 Resolution (FWHM):	0.7
Collision Gas Pressure (CID) Gas:	1.5 mTorr
Source Fragmentation:	0 V

Table 4. MS/MS parameters for selected reaction monitoring transitions.

Compound	Retention Time (min)	Polarity	Precursor *(m/z)*	Product *(m/z)*	Collision Energy (V)	RF Lens (V)
Fosetyl-Al	4.64	Negative	109.3	80.9	12	45
			109.3	108.7	10	45
Glufosinate	8.20	Negative	180.2	84.9	21	61
			180.2	136.0	18	61
AMPA	8.37	Negative	109.9	62.9	21	53
			109.9	78.8	29	53
Clopyralid	11.22	Negative	191.8	147.8	10	34
			191.8	36.9	20	34
Glyphosate	14.01	Negative	168.3	78.9	40	48
			168.3	149.8	10	48

Note: For each compound quantifier ions are shown in the upper row, and qualifier ions in the lower row.

Analysis of FMOC derivatives

Additional data on accuracy were obtained by analyzing surface water samples provided by laboratory Povodi Vltavy state enterprise, Pilsen, Czech Republic. Surface water samples were collected within the Czech surface waters monitoring program and stored in new bottles at -20 °C. The samples were analyzed by both the conventional LC-MS/MS method after derivatization with fluorenylmethyloxycarbonyl (FMOC)[6] and with the presented IC-MS/MS method. The details of the LC-MS/MS method are shown in Table 5 and Table 6.

Table 5. LC-MS/MS method conditions for the analysis of FMOC derivatives.

Mobile Phase:	A: Methanol B: Water + 0.005% ammonium hydroxide + 5 mM ammonium acetate
Derivatization Agent:	FMOC Chloride (FMOC – Cl)
Ionization Mode:	Electrospray (ESI)
Scan Type:	SRM
Polarity:	Positive ion mode
Instrumentation:	LC-MS/MS
Mass Spectrometer:	Thermo Scientific™ TSQ Vantage™

Table 6. SRM transitions for the analysis of FMOC derivatives.

Compound	Polarity	Precursor (*m/z*)	Product (*m/z*)
Glyphosate	Negative	392.1	88.0
		392.1	179.0
AMPA	Negative	334.1	179.0
		334.1	156.0
Glufosinate	Negative	404.1	179.0
		404.1	182.0

Note: For each compound quantifier ions are shown in the upper row, and qualifier ions in the lower row.

Mass spectrometer calibration - extended mass range (EMR) versus classic (with polytyrosine)

Since the target analytes are small molecules with product ions below 100 Da after fragmentation, it is recommended to calibrate the mass spectrometer with the Thermo Scientific™ Pierce™ Triple Quadrupole, Extended Mass Range (EMR) calibration solution. It consists of 14 components (mass range from 69 *m/z* to 2800 *m/z*) designed for the calibration in both positive and negative ionization mode. This solution has been designed to improve mass accuracy at lower masses compared to conventional calibration solutions containing only three components (polytyrosines) in the narrower mass range (181 *m/z* to 996 *m/z*).

Calculations

Identification of the pesticides was indicated by the presence of two transition ions measured in SRM mode corresponding to the retention times of standards. The quantifier and qualifier ions were selected among the product ions produced by the fragmentation of the selected precursor ion on the basis of the intensity and selectivity. For quantification, a linear calibration was applied. Due to expected matrix induced signal suppression (matrix effects), the quantification for all water matrices (surface, drinking, bottled mineral) was performed by matrix-matched calibration.

Results and discussion

The objective of this study was to evaluate the application of IC-MS/MS for fast routine analysis of polar pesticides and their metabolites in water samples. Various analytical parameters were assessed and the results of these experiments are described.

Suppressed ion chromatography offers the advantage of neutralizing and desalting the mobile phase before the introduction into a detection device, like the MS. To facilitate the ionization efficiency and therefore improve the detection sensitivity of analytes in heated-electrospray source (HESI), organic solvent can be added post column. During method development, we tested acetonitrile, methanol, and 2-propanol. The addition of all solvents significantly improved the ionization efficiency (Figure 2), with the addition of 2-propanol resulting in the best responses and lowest background noise for all analytes.

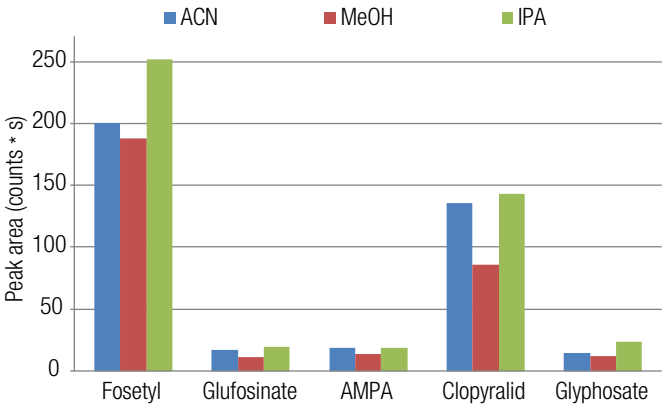

Figure 2. Signal improvement using different make-up solvents, measured at 500 ng/L concentration levels.

Method performance has been optimized analyzing deionized water spiked at different concentration levels down to 1 ng/L for all analytes. The performance of the method has been evaluated analyzing drinking water, bottled mineral water, and 20 surface water samples provided by laboratory Povodi Vltavy state enterprise, Pilsen, Czech Republic.

As shown in Table 7, the LOQs for most of the compounds were determined to be below 10 ng/L. The LOQ has been determined as the lowest calibration level meeting the 20% RSD criteria.[7] The exception was clopyralid, where higher signal background was observed and the LOQ was determined at 50 ng/L level in all samples.

Representative chromatographic signals at 50 ng/L level are presented in Figure 3 and Figure 4, showing both quantifier transition and qualifier transition used for confirmation. Calibration curves were linear in the range applied (1–1000 ng/L) and correlation coefficients > 0.99 for all analytes.

Table 7. Validation results obtained for drinking water, bottled mineral water, and surface water.

Component	Matrix	LOD (ng/L)	LOQ (ng/L)	Recovery (%)			RSD (n=6) (%)		
c (4 components) (ng/L)	-	-	-	10	20	50	10	100	1000
c (Fosetyl-Al) (ng/L)	-	-	-	5	10	25	5	50	500
Fosetyl-Al	Drinking Water	2.5	5	133	122	132	10	1	1
	Bottled water	1	2.5	107	116	125	2	1	1
	Surface water	2.5	5	121	114	113	4	1	1
Glufosinate	Drinking Water	5	10	139	122	99	12	2	1
	Bottled water	5	10	105	115	94	4	3	1
	Surface water	5	10	105	104	84	4	3	2
AMPA	Drinking Water	5	10	91	95	83	13	2	1
	Bottled water	5	10	105	108	95	9	2	1
	Surface water	5	10	94	111	103	8	5	3
Clopyralid	Drinking Water	10	50	111*	88*	90	14*	1	1
	Bottled water	10	50	103*	87*	85	9*	1	1
	Surface water	10	50	113*	98*	104	7*	2	2
Glyphosate	Drinking Water	10	25	87*	104*	84	8*	3	1
	Bottled water	5	10	79	105	105	14	2	3
	Surface water	5	10	63	102	97	6	4	2

*The level is above LOD, but below LOQ.

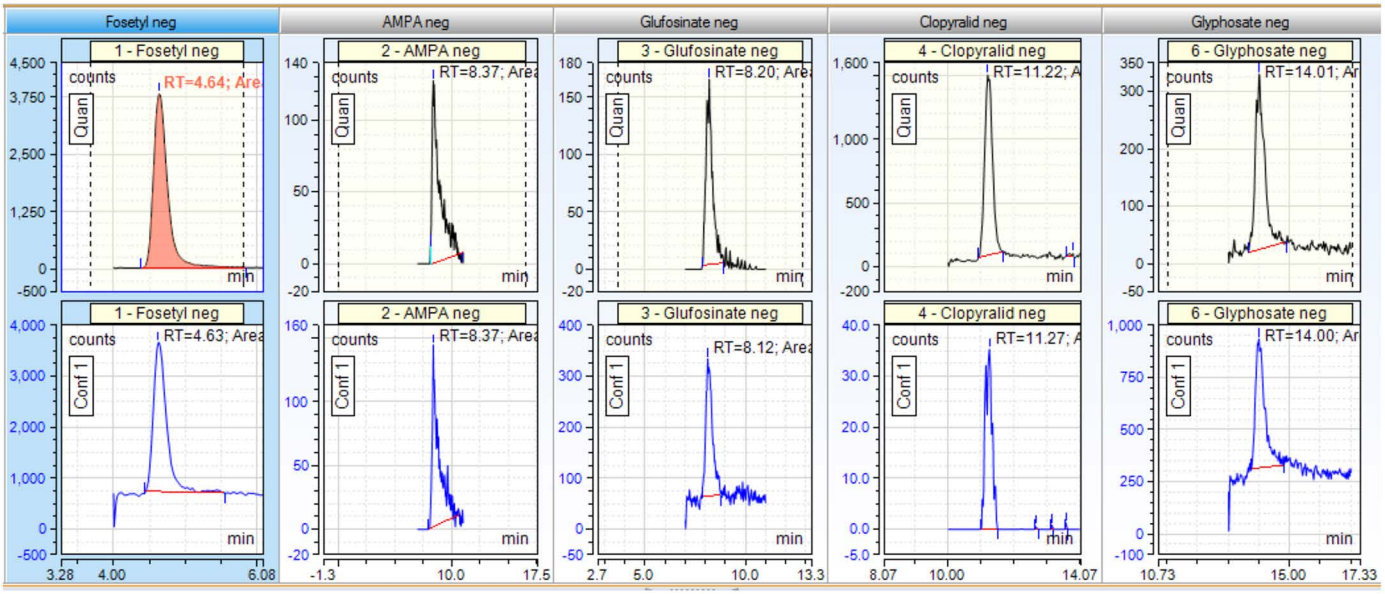

Figure 3. SRM chromatograms of tested analytes in surface water, spiked at 50 ng/L level, quantification and qualification transitions shown.

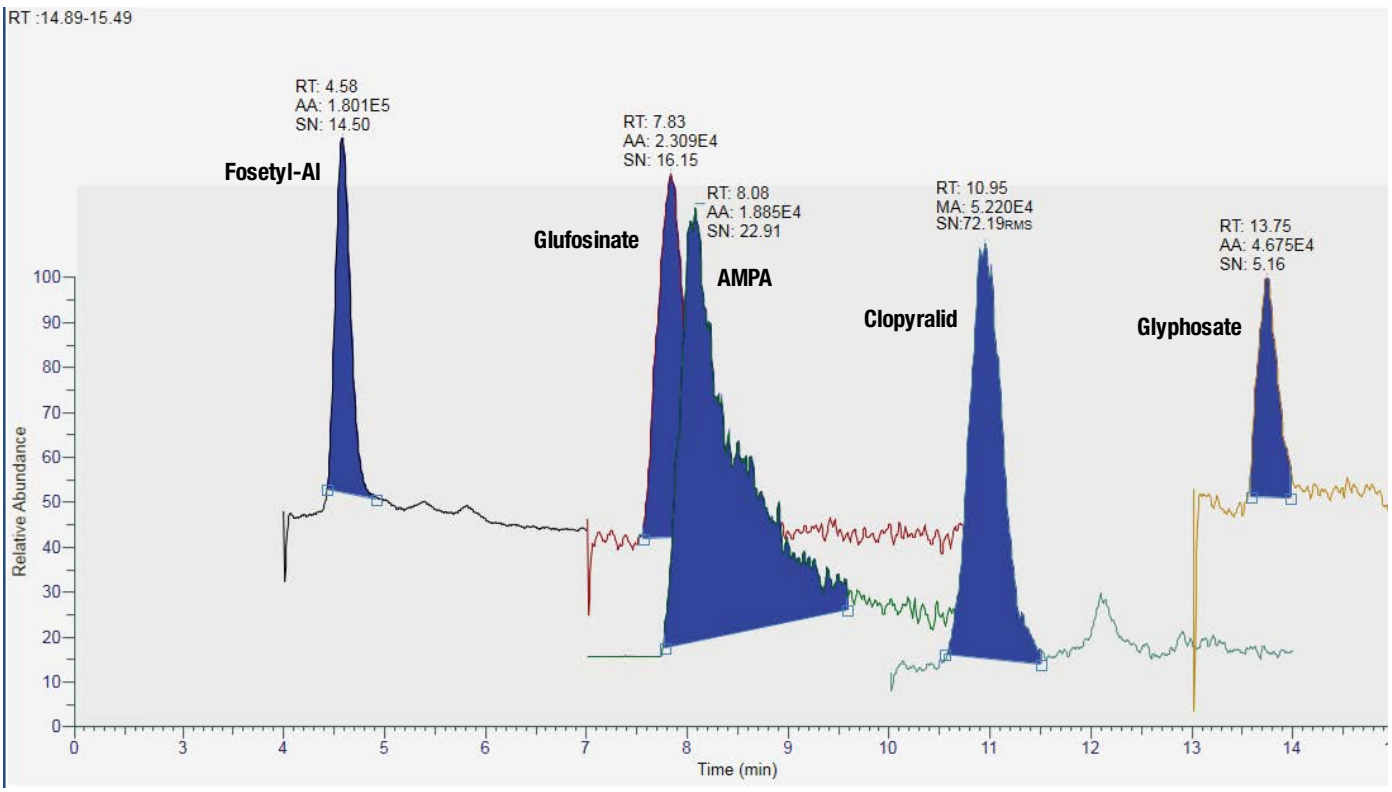

Figure 4. SRM chromatogram of tested analytes in surface water, spiked at 50 ng/L level.

Matrix effects

When considering the calibration of the system, the possibility of matrix-effect-like signal suppression in the HESI probe must be taken into account. The most severe signal suppression was observed during the analysis of drinking water and artificial matrix water containing high salts concentrations.

To examine the influence of high ion matrix concentrations on the measurements, an artificial water was prepared. This sample consisted of 250 mg/L Cl^- and SO_4^{2-}, each, 150 mg/L HCO_3^-, and 20 mg/L NO_3^- in deionized water. The multi-standard solution with a concentration of 1 mg/L (except fosetyl-Al 500 µg/L) was diluted using this water matrix. Multi-level calibrations

were prepared in both the artificial matrix as well as in deionized water, drinking water, surface water, and bottled water. Added high salt amounts into the deionized water disturbed the detection of FOS, AMPA, as well as CLO. High background noise was observed in these cases. For this reason, it was possible to obtain the linear calibration curves only at higher concentration ranges. Table 8 shows the levels of LOD and LOQ that are, as expected, significantly higher in comparison to other tested water matrices.

Table 8. LODs/LOQs for target analytes in artificial water matrix and surface water.

Analyte	Artificial Water		Surface Water	
	LOD (ng/L)	LOQ (ng/L)	LOD (ng/L)	LOQ (ng/L)
Fosetyl-Al	25	50	2.5	5
Glufosinate	5	10	5	10
AMPA	25	50	5	10
Clopyralid	250	500	10	50
Glyphosate	10	25	5	10

Figure 5 and Figure 6 present the differences in calibration curves obtained for glyphosate and clopyralid in different matrices and their effect on the MS signals. For glyphosate, almost no matrix dependency was observed. However, clopyralid sensitivity and recovery was strongly affected by the high ionic strength of the artificial matrix, as sulfate, one of the major anionic components, elutes in the vicinity of the pesticide apparently causing ion suppression effects in the MS (Figure 7). As shown in Table 8, the presence of high concentrations of anions in artificial water lead to higher LOQs, impacting clopyralid the strongest with an estimated LOQ of 500 ng/L. For the other compounds, LOQs of 25–50 ng/L were obtained. It should be noted, that the artificial water matrix was selected to closely match the maximum concentration levels and parametric values described in COUNCIL DIRECTIVE 98/83/EC.[8] In most of real life samples, the concentrations of the major anionic components can be expected to be lower.

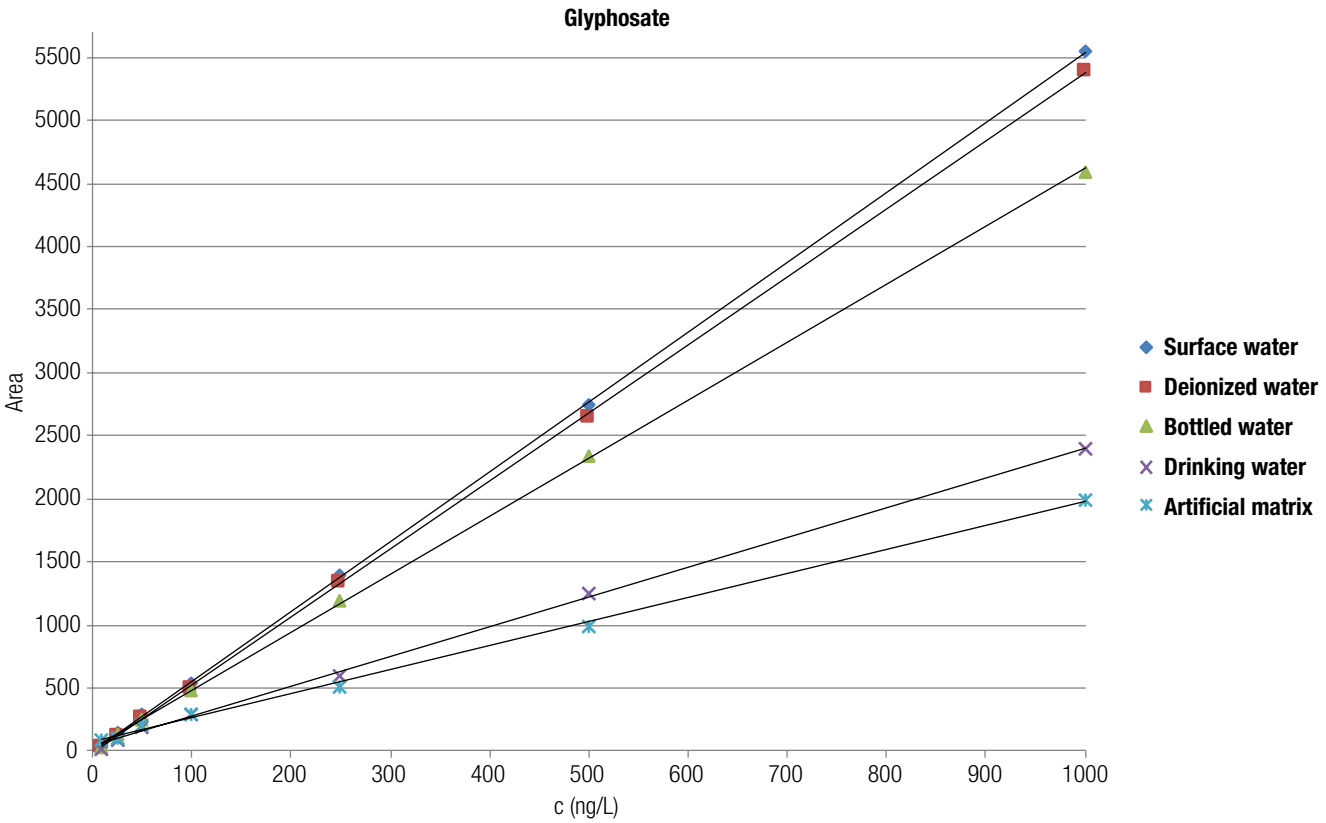

Figure 5. Calibration curves of glyphosate obtained in different matrices (surface water, deionized water, bottled water, drinking water, artificial matrix).

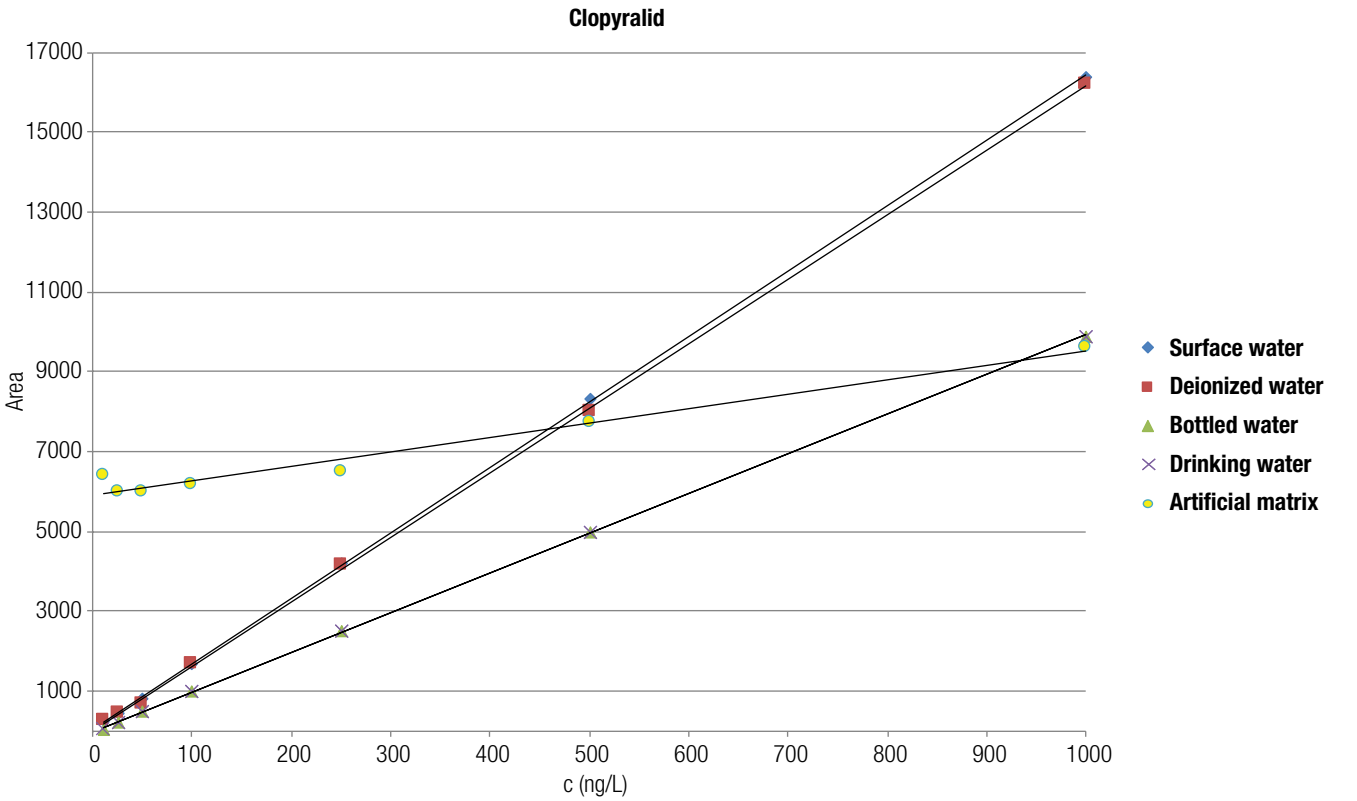

Figure 6. Calibration curves of clopyralid obtained in different matrices (surface water, deionized water, bottled water, drinking water, artificial matrix).

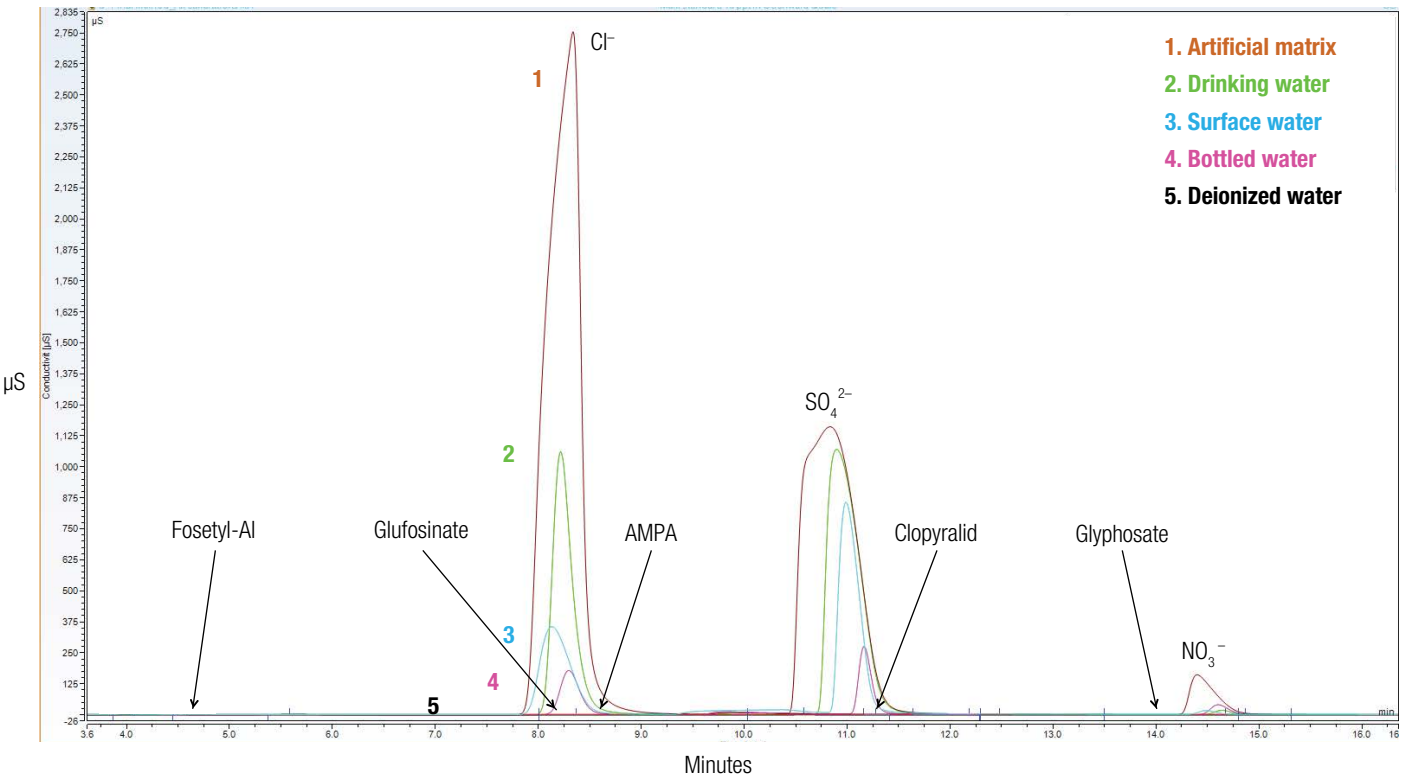

Figure 7. Conductivity traces of anions present in different matrices together with elution times of target analytes.

Precision and accuracy

The method's precision and accuracy were determined by analyzing blank water samples fortified with the working solution. Six replicates at three different concentration levels were analyzed. Very good results were reached and are shown in Table 7.

Additional data on accuracy were obtained by analyzing surface water samples collected within the Czech surface waters monitoring program. The samples were analyzed by both the conventional LC-MS/MS method after derivatization with fluorenylmethyloxycarbonyl (FMOC)[6] and with the presented IC-MS/MS method.

Results shown in Table 9 demonstrate good agreement between both techniques used. The IC-MS/MS approach does not require derivatization, which simplifies the method and translates into significant time savings improving the laboratory efficiency and sample throughput. Furthermore, the number of analytically accessible compounds is larger with IC-MS/MS (like clopyralid, fosetyl-Al) as those compounds cannot be derivatized with FMOC. The analytical workflow of the laboratory using IC-MS/MS is simplified and the cost of analysis is reduced.

Table 9. Results for analytes obtained with LC-MS/MS derivatization based method (FMOC) and IC-MS/MS method.

Sample Name	FMOC LC-MS/MS Method (ng/L)			IC-MS/MS Method (ng/L)		
	Glyphosate	AMPA	Glufosinate	Glyphosate	AMPA	Glufosinate
1475	69	154	n.d.	63	145	n.d.
1489	n.d.	152	n.d.	48	157	n.d.
1502	n.d.	156	n.d.	29	164	n.d.
1520	n.d.	65	n.d.	29	60	9
1521	235	761	n.d.	183	787	n.d.
1524	n.d.	1880	n.d.	18	1801	n.d.
1528	n.d.	61	n.d.	n.d.	n.d.	n.d.
1613	n.d.	163	n.d.	15	114	n.d.
1618	291	585	n.d.	86	421	n.d.
1622	n.d.	174	n.d.	45	217	n.d.
1624	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1642	n.d.	n.d.	n.d.	61	59	n.d.
1644	58	58	n.d.	87	61	33
1686	n.d.	67	n.d.	20	77	n.d.
1701	n.d.	471	n.d.	46	537	n.d.
1740	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
1829	n.d.	n.d.	n.d.	n.d.	59	n.d.
1837	n.d.	n.d.	n.d.	n.d.	16	n.d.
1840	n.d.	173	n.d.	48	175	n.d.
1843	n.d.	152	n.d.	38	128	n.d.

n.d.– below method LOQ

Conclusion

The reported IC-MS/MS method enables the quantitative analysis of five polar ionic pesticides including closely watched glyphosate and AMPA in different water matrices. The method was in-house validated with very good method performance results for drinking, bottled mineral, and surface water. The reliability was proven by measurement surface water samples from Czech monitoring program supplied by laboratory Povodi Vltavy state enterprise, Pilsen, Czech Republic.

The developed method has many benefits in comparison with traditionally used LC-MS/MS methods utilizing FMOC derivatization. Thanks to direct injection without a long and laborious sample preparation, the method is more sensitive, very fast, and avoids errors during the manipulation with the samples. Adopting this method gives the routine laboratories the potential to increase cost savings, provide more reliable results, and increase the sample throughput.

References

1. https://www.iarc.fr/en/media-centre/iarcnews/pdf/Q&A_Glyphosate.pdf.
2. https://www.efsa.europa.eu/sites/default/files/4302_glyphosate_complementary.pdf.
3. Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC Text with EEA relevance., http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN.
4. Hanke, I.; Singer, H.; Hollender, J. Ultratrace-level determination of glyphosate, aminomethylphosphonic acid and glufosinate in natural waters by solid-phase extraction followed by liquid chromatography-tandem mass spectrometry: performance tuning of derivatization, enrichment and detection. *Anal Bioanal Chem.* **2008,** *391*(6),2265-76. doi: 10.1007/s00216-008-2134-5.
5. http://quppe.eu/
6. LC-MS/MS in house validated method for water analysis, laboratory Povodi Vltavy state enterprise, Pilsen, Czech Republic.
7. B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org.
8. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, http://data.europa.eu/eli/dir/1998/83/oj.

Find out more at **thermofisher.com/chromatography**

ThermoFisher SCIENTIFIC