A quick and routine analysis of polar pesticides in water by suppressed ion chromatography and mass spectrometry

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ABSTRACT

Polar pesticides analysis in water and foods has become very hot topic in the past couple of years. A famous representative of this group is a broad-spectrum systematic herbicide glyphosate and its metabolite AMPA. Glyphosate was discovered more than 40 years ago and has become popular due to its low toxicity in comparison with other herbicides. It is used to kill weeds competing with crops and in the parks and roadsides. In March 2015 the World Health Organization's (WHO) International Agency for Research on Cancer classified glyphosate as a probable carcinogen[1]. However in November 2015, the European Food Safety Authority (EFSA) concluded glyphosate unlikely to cause cancer[2]. There is a big demand to increase the number of tested water[3] and food samples and monitor the presence of these contaminants carefully.

Because of the chemical properties it is not possible to analyse these compounds with the conventional C18 column. Typically, laboratories use methods that include derivatisation steps or special chromatographic columns, like the porous graphitic-carbon-(PGC)-based Thermo Scientific™ Hypercarb™ columns. With both approaches vary in robustness and reliability. Here, we demonstrate the analysis of polar pesticides with an ion chromatograph coupled to a triple quadrupole mass spectrometer.

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 and glufosinate, for example, promoted the use of these broad spectrum herbicides. Consequently, polar pesticides are found in foods as residues and in the environment as contaminants of surface waters, soils, etc.

There are concerns about their potential adverse effects on human health, such as their potential carcinogenicity,[1] although the latest toxicological assessments do not predict risks for humans under normal conditions or environmental exposures[2]. Current regulations offset maximum residue levels (MRLs) of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) at 100 ng/L in drinking water. In food and beverage samples, higher MRLs typically apply, ranging generally from 10 µg/kg for food intended for consumption by children up to hundreds of mg/kg in other matrices[4] .

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. Derivatisation of glyphosate prior to analysis[5] or application of specific chromatographic columns, such as the Thermo Scientific™ Hypercarb™ column, are the common approaches.[6] With both of these approaches, poor method robustness and questionable results are often reported in laboratories, especially when the method is applied in routine high-throughput analysis of samples with rather complex matrix composition.

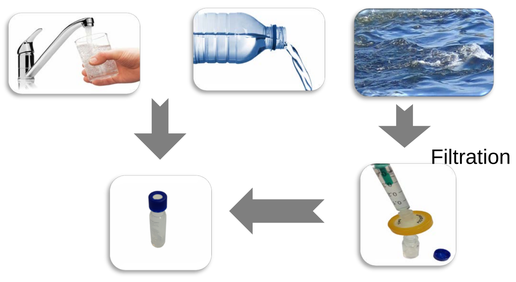
Recent developments in ion chromatography and mass spectrometry offer many advantages for the analysis of very polar substances. Ion chromatography is the preferred separation technique for polar ionic analytes, such as anions, cations, or small polar analytes (metabolites) and sugars. Mass spectrometry, 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 is to develop and validate an IC-MS/MS method for direct analysis of polar ionic pesticides and assess its applicability under routine conditions.

MATERIALS AND METHODS

Sample Preparation

Samples of tap, bottled and surface water were taken and injected directly into the ion chromatograph. The surface water required filtration through a syringe disc filter to remove particulates.

Figure 1. The sample preparation workflow.



INSTRUMENTATION

The instrument system comprised a metal-free Thermo Scientific™ Dionex™ Integrion™ HPIC™ system and a Thermo Scientific™ Dionex™ AS-AP autosampler coupled to a Thermo Scientific™ TSQ Endura™ mass spectrometer (Figure 2). The chromatographic separation was carried out using a polymer-based Thermo Scientific™ Dionex™ IonPac™ AS24 column with guard in the 2 mm format. Instrument parameters and settings are shown in Table 2. The hydroxide eluent was prepared in-situ using an eluent generator, the Thermo Scientific™ Dionex™ EGC-KOH cartridge and a Thermo Scientific™ Dionex™ CR-ATC II continuously regenerated trap column, resulting in a method that does not require external chemicals.

After separation, the eluent passed the electrochemically regenerated Thermo Scientific™ Dionex™ AERS™ suppressor, where the cations from both the eluent and the sample were replaced with hydronium ions, effectively neutralizing the high pH eluent and 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. In order to improve desolvation, a second pump added 2-propanol as a make-up solvent at a low flow rate before entering the mass spectrometer.

Figure 2. Schematic of the ion chromatograph coupled to the mass spectrometer.

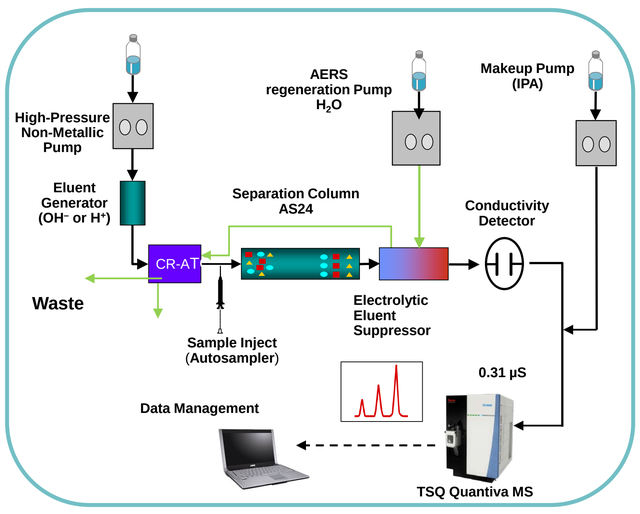


Table 1. Method parameters.

Eluent	KOH with gradient
Eluent source	EGC KOH
KOH flow rate	0.3 ml/min
IC column	IonPac AG24 (2 x 50 mm) + AS24 (2 x 250 mm)
Suppressor	ASRS 300 – 2 mm external water mode
AXP-MS Pump 1 deionised water flow rate	1 ml/min
Make up solvent	2-propanol
AXP-MS Pump 2 2-propanol flow rate	0.1 ml/min
Injection volume	100 µl
Column temperature	30°C
MS source	Heated Electrospray (H-ESI)
MS detection mode	t-SRM

Mass spectrometer conditions

Data acquisition was performed in selected reaction monitoring mode (SRM). All SRM traces (parent, quantifier, and qualifier ions) were individually tuned for each target analyte injecting the corresponding standard solution (10 mg/L). Data was acquired and processed using Thermo Scientific™ TraceFinder™ 4.0 software allowing easy building of the acquisition and processing methods for high-throughput quantitative analysis with improved data reviewing and reporting.

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

Since the target analytes are small molecules with product ions after fragmentation < 100 Daltons, it is recommended to calibrate the mass spectrometer with the Thermo Scientific™ Pierce™ triple quadrupole, EMRS, calibration solution. It consists of 14 components (mass range from 69 m/z to 2800 m/z) for the calibration in both positive and negative ionization modes. This solution improves mass accuracy and transmission compared to conventional polytyrosine tune solutions, especially in the low m/z range where many of the polar pesticides are found.

Figure 3. Photograph showing IC coupled to MS plus additional pumps.

METHOD OPTIMISATION

During method optimisation, various analytical parameters including the influence of make-up solvent were assessed. The performance of the method has been evaluated by analyzing fortified drinking water, bottled mineral water and surface water samples. Additional data on accuracy were obtained by analyzing surface water samples provided by Water Laboratory Pilsen, Czech Republic. The method results are shown in Table 2.

Table 2. Method results obtained for drinking, bottled mineral and surface water at three spiking levels.

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

RESULTS

Samples from a survey conducted on the Vltava river in the Czech Republic (CR) were analyzed using the FMOC LC-MS/MS method by the Water Laboratory, Pilsen for conc-entrations of glyphosate and AMPA. The samples were then run on the newly developed IC-MS/MS for comparison of the two techniques and to determine accuracy of the method. Results are shown in Figure 4 and an example of the SRM chromatogram is shown in Figure 5.

Figure 4. Comparison of the concentrations of glyphosate and by FMOC LC-MS/MS and IC-MS/MS methods.

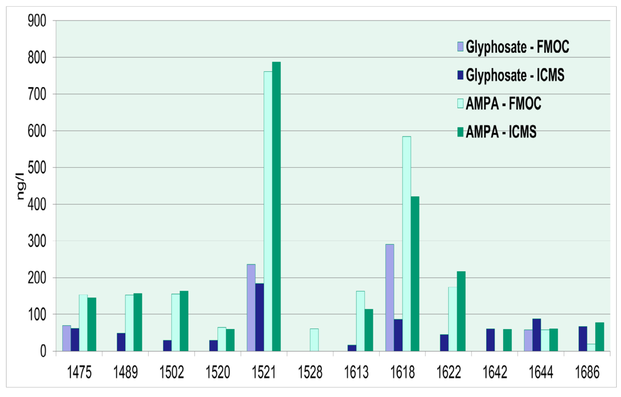
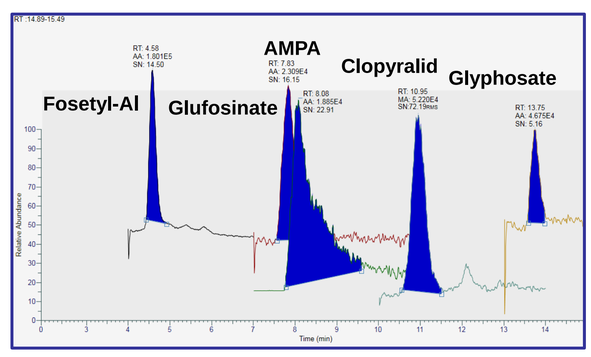


Figure 5. SRM chromatogram of target analytes in blank surface water, spiked at 50 ng/l level.



CONCLUSIONS

- The reported IC-MS/MS method enables the quantitative analysis of five polar ionic pesticides with respect to the actual MRL levels.
- The method showed good repeatability, recovery, and limit of detection and quantitiation for drinking, bottled mineral and surface water.
- The developed method has many benefits in comparison with traditionally used LC-MS/MS methods utilising FMOC derivatization. Thanks to the direct injection without a long and laborious sample preparation the method is more sensitive, very fast and avoids sample manipulation errors.

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ACKNOWLEDGEMENTS

We would like to thank Milan Kozeluh from the water laboratory Povodi Vltavy a.s., Pilsen, Czech Republic for supplying the survey surface water samples.

TRADEMARKS/LICENSING

PO72392-EN 0517S