Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Other Polar Pesticides

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Overview

Glyphosate and glufosinate are widely used herbicides and, thus, there is an interest in the reliable and sensitive determination of glyphosate in water and food. These pesticides are difficult to extract and analyze because of their high polarity. Here we describe an automated workflow for the FMOC-derivatization, sample cleanup, and LC-MS/MS detection using a GERSTEL Multi Purpose Sampler (MPS) 2XL configured with an online solid phase extraction (SPE^{XOS}) module coupled to an AB SCIEX QTRAP® 4500 system for the identification and quantitation of glyphosate, its major metabolite AMPA, and glufosinate in water and food samples.

Introduction

Glyphosate (N-phosphonomethyl glycine) and glufosinate [ammonium (S) -2-amino-4-[hydroxyl (methyl) phosphinoyl] butyrate] are non-selective post emergence herbicides used for the control of a broad spectrum of grasses and broad-leaf weed species in agricultural and industrial fields. Aminomethyl-phosphonic acid (AMPA) is the major metabolite of glyphosate and also included into the pesticide residue definition.[1, 2]

There is interest in the reliable and sensitive determination of residues of these pesticides in water and food. Due to their high polarity it is difficult to extract these pesticides from samples and to retain them on LC phases. Derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) is a common procedure to improve extraction and separation for the analysis of glyphosate and related compounds. Previously reported methods using derivatization with FMOC-Cl have inherent limitations, such as long derivatization times, long LC run times, and often suffer from lack of repeatability and reproducibility.

Here we present an automated workflow to derivatize and analyze water and food samples for glyphosate, glufosinate and AMPA by LC-MS/MS using a GERSTEL Multi Purpose Sampler (MPS) 2XL with SPE^{XOS} coupled to an AB SCIEX QTRAP® 4500 system (Figure 2).

Water samples were injected directly into the LC-MS/MS system providing sufficient sensitivity to identify and quantify targets at sub 100 µg/L concentrations. Food samples can be injected directly after automatic derivatization followed by extensive dilution or can be cleaned up using online SPE prior LC-MS/MS analysis. Target compounds can be easily identified and quantified at 10 µg/kg levels with excellent repeatability.

Experimental

Derivatization and Sample Preparation

Water samples were analyzed directly and food samples were extracted using the QuPPe (Quick Polar Pesticides) method developed by the EU Reference Laboratories for Residues of Pesticides.[3] QuPPe results were compared to results obtained when using an extraction method reported by Miles et al.[4]

Derivatization and cleanup was performed using the GERSTEL MPS 2XL with SPE^{XOS} system configured for automatic sample handling, derivatization, and online SPE. The Gerstel system is fully controlled using the GERSTEL MAESTRO version 1.4 coupled to Analyst® software version 1.6.

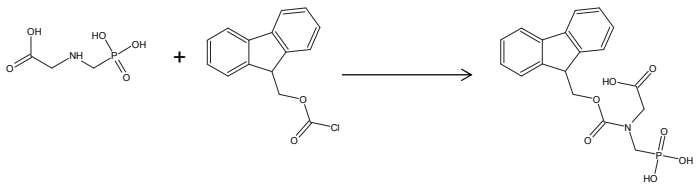

Figure 1. Derivatization of glyphosate using FMOC-Cl

Figure 2. GERSTEL MPS 2XL with SPE^{XOS} coupled to an QTRAP® 4500 system

Automated Derivatization Procedure (Figure 1)

1. Add 100 µL of borate buffer (pH=9) to 1 mL of sample.
2. Add 200 µL of 10mM FMOC-Cl solution.
3. Agitate for 20 min at 50°C.
4. Cool to bring to ambient temperature.
5. Add 130 µL 2% H_3PO_4.

Water samples were derivatized and injected directly (10 µL) into LC-MS/MS.

Figure 3a. Sequence of scheduled events in the Maestro software for online SPE: green - adding buffer and FMOC-Cl, yellow - derivatization, light blue - online SPE, orange LC-MS/MS analysis, dark blue washing of the autosampler, the PrepAhead function increases productivity by simultaneously preparing the following sample while perming LC-MS/MS analysis of the previous sample

Automated Online-SPE Cleanup Procedure

1. Condition GERSTEL SPE^{XOS} C8EC-SE (18.5 mg) cartridge with methanol and water + 100 mM formic acid.
2. Load 1 mL of derivatized sample onto SPE.
3. Wash with water + 100 mM formic acid.
4. Elute with LC pump gradient

Food sample extracts were diluted extensively to minimize possible matrix effects and automatically cleaned up using SPE^{XOS} system. Here we injected 1 mL of the diluted sample extract onto the SPE cartridge. (Figures 3a and 3b)

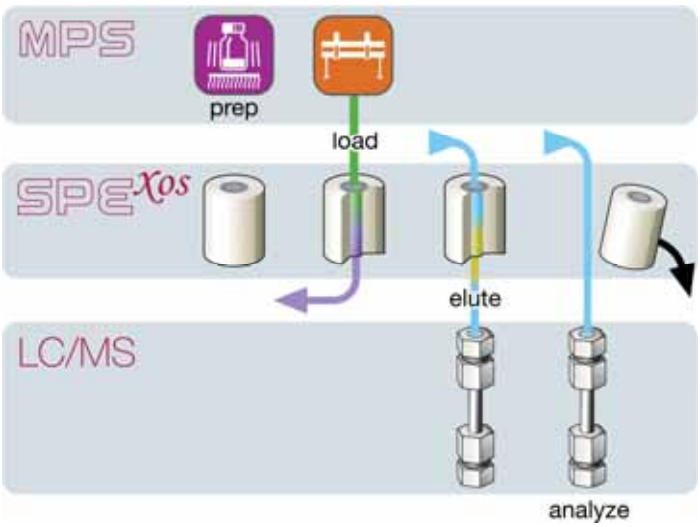

Figure 3b. Sequence of scheduled events when using the automated workflow of FMOC-derivatization, SPE cleanup, and LC-MS/MS detection

LC Separation

The analyses were performed using a Phenomenex Gemini 3µ C18 (150 x 2 mm) column with a gradient of (A) 50 mM ammonium acetate adjusted to pH= 9 and (B) Acetonitrile. The gradient conditions are listed in Table 1.

Table 1. LC gradient used for separation

Time (min)	Flow (mL/min)	A (%)	B (%)
0	0.25	80	20
10	0.25	5	95
15	0.25	5	95
15.1	0.25	80	20
25	0.25	80	20

MS/MS Detection

The analyses were performed on an AB SCIEX QTRAP® 4500 LC/MS/MS system using the Turbo V™ source operated in electrospray ionization and negative polarity with an IS voltage of -4200 V.

The Curtain Gas™ interface (CUR) was set to 30 psi, nebulizer gas (Gas 1) set to 50 psi, drying gas (Gas 2) set to 70 psi, and the source temperature set to 400ºC.

The MRM transitions used for the detection of pesticides are shown in the table below. Each MRM was monitored with a dwell time of 100 ms.

Table 2. MRM transitions used for detection

Compound	Q1	Q3	CE (V)
Glyphosate	390	168, 150	-18, -34
Glufosinate	402	180, 206	-16, -20
AMPA	322	110, 136	-12, -22

Analyst® version 1.6.1 was used for data acquisition and MultiQuant™ version 3.0 software was used for qualitative and quantitative processing.

Results and Discussion

A standard chromatogram after automatic derivatization is shown in Figure 4.

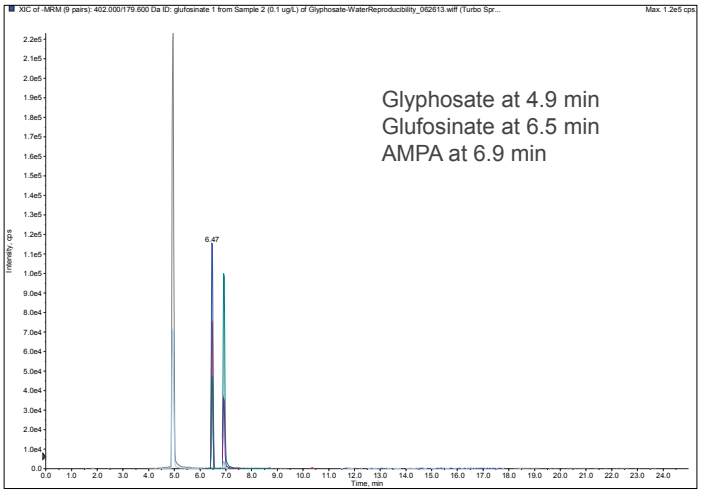

Figure 4. Standard chromatogram at a concentration of 10 ng/mL

A drinking water sample was spiked at 0.1 and 10 µg/L, automatically derivatized, and analyzed in triplicates. The method allowed accurate quantitation of all target compounds well below 0.1 µg/L with excellent repeatability (Figure 5 and Table 3).

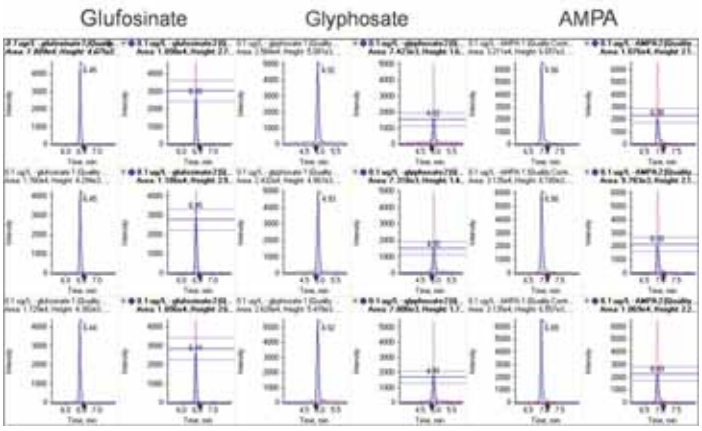

Figure 5. Triplicate analysis of polar pesticides in a spiked water sample at 0.1 µg/L (injection volume of 10 µL), ion ratios for compound identification were calculated automatically in MultiQuant™ software version 3.0

Table 3. Triplicate analysis of polar pesticides in a spiked water sample at 0.1 µg/L (injection volume of 10 µL)

Compound	Concentration (µg/L)	%CV of MRM 1	%CV of MRM 2
Glyphosate	0.1	4.0	3.9
	10	7.7	8.9
Glufosinate	0.1	2.3	4.5
	10	4.6	5.4
AMPA	0.1	1.4	5.3
	10	5.1	5.4

Different food matrices (corn and soy bean) where spiked with glyphosate, glufosinate, AMPA at 10 and 100 µg/kg and extracted using the QuPPe (Quick Polar Pesticides) method:

1. Add 10 mL water to 5 g of homogenized sample, shake and soak for 10 min.
2. Add 10 mL of acidified methanol (1% formic acid).
3. Shake vigorously for 1 min and centrifuge (at 3000 rpm) for 10 min.
4. Load 1 mL onto the Gerstel MPS 2XL system for automated dilution, derivatization, and SPE cleanup followed by LC-MS/MS analysis.

Corn and soy samples were spiked at 10 and 100 µg/kg and analyzed in triplicates using the automated derivatization and cleanup procedure. The method allowed accurate quantitation of all target compounds well below the target concentration of 100 µg/kg with excellent repeatability (Table 4, Figures 6 and 7).

Table 4. Triplicate analysis of polar pesticides spiked into corn and soy samples 100 µg/kg

Compound	Concentration (µg/kg)	%CV of MRM 1	%CV of MRM 2	Ion ratio (%RSD)
Glyphosate	100 (in corn)	3.6	6.0	0.36 (1.9%)
	100 (in soy)	5.1	5.9	0.31 (1.9%)
Glufosinate	100 (in corn)	1.6	12.5	0.71 (8.9%)
	100 (in soy)	5.2	7.7	0.67 (3.9)%
AMPA	100 (in corn)	5.7	4.8	0.43 (0.9%)
	100 (in soy)	5.3	6.2	0.38 (2.2%)

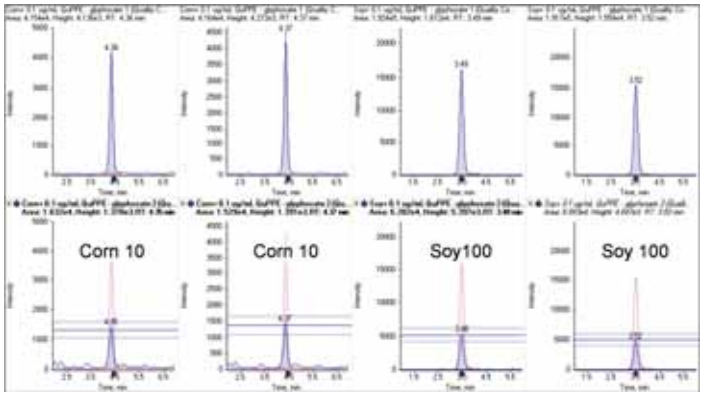

Figure 6. 10 and 100 µg/kg of glyphosate spiked into corn and soy and analyzed using automatic derivatization, dilution, and cleanup followed by LC-MS/MS, ion ratios for compound identification were calculated automatically in MultiQuant™ software version 3.0

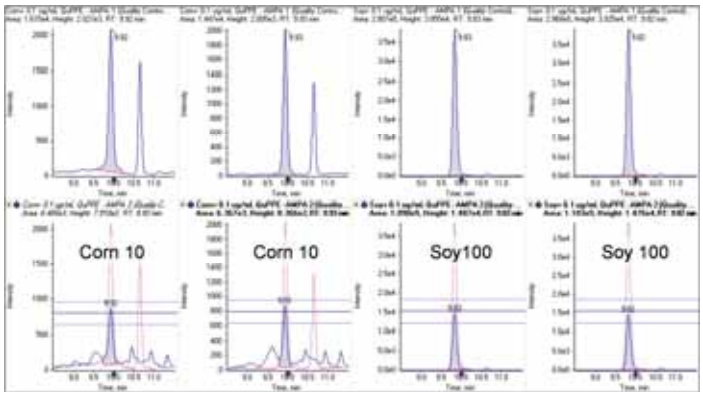

Figure 7. 10 and 100 µg/kg of AMPA spiked into corn and soy and analyzed using automatic derivatization, dilution, and cleanup followed by LC-MS/MS, ion ratios for compound identification were calculated automatically in MultiQuant™ software version 3.0

Ion ratios for compound identification where automatically calculated in the result table in MultiQuant™ software version 3.0. The quantifier and qualified ratio was found to be a valuable tool to identify all target pesticides in matrix samples with excellent reproducibility and values well in between ± 20% (Table 4).

The slightly higher %RSD of the ion ratio of glufosinate in corn can be explained by interfering matrix signals (Figure 8). Stable LC separation was essential for confident identification and accurate quantitation of glufosinate.

The results using the QuPPe extraction where compared to results obtained when using the none QuPPe procedure based on extraction with 0.1 M HCl.[8] In general, recoveries were between 70-120% for both matrices when using the QuPPe protocol with slightly better recoveries in corn due to the lower protein content.

Recoveries using the none QuPPe extraction where found to be lower in all cases. However, in the case of corn this extraction resulted in cleaner MRM chromatograms for glufosinate (Figure 8).

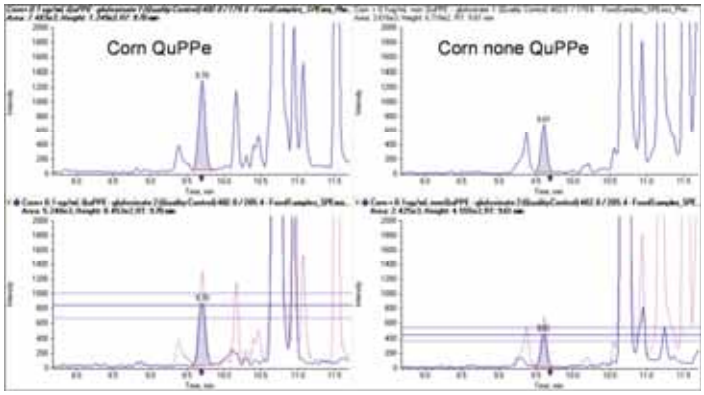

Figure 8. Corn analyzed for glufosinate using the QuPPe and a none QuPPe extraction procedure with higher recoveries but more matrix interferences when using the QuPPe protocol

The total cycle time per sample for the automated sample derivatization and online SPE was approximately 25 minutes, enabling "just in time" sample preparation using the GERSTEL MAESTRO software PrepAhead function. Using this automated procedure for derivatization, extraction and analysis over 55 samples can be processed per day.

Future studies will include the use of isotopically labeled standards to compensate for possible matrix effects. Also retention time shifts were observed when analyzing glyphosate in matrix samples with high protein content. The use of an internal standard will increase confidence in identification using relative retention times.

Summary

As a result of this study, we were able to show:

- Glyphosate, glufosinate, and AMPA can be detected after automatic derivatization using FMOC-Cl at relevant concentration in drinking water and food samples[5-7]
- The described workflow using the GERSTEL MPS 2XL with SPE^{XOS} coupled to an AB SCIEX QTRAP® 4500 system enabled automated derivatization, dilution, and SPE cleanup and analysis of water and QuPPe extracts of food for LC-MS/MS of polar pesticides.
- The method is highly repeatable with %CV well below 10% due to the automation of sample handling and derivatization.
- Sensitivity was sufficient to inject water samples directly and detect all target compounds below 0.1 µg/L. Food samples can be diluted prior SPE cleanup using the online SPE to monitor at 10 µg/kg.

References

1 M. Singh and D. P. H. Tucker: Proc. Fla. State Hort. Soc. 100 (1987) 58-61

2 http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1_glyphosate_en.pdf

3 http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf

4 C. Miles and A. Moye: 'Extraction of glyphosate herbicide from soil and clay minerals and determination of residues in soils' J. Agric. Food Chem. (1988) 486-491

5 Regulation (EC) 'concerning the placing of plant protection products on the market' No 1107/2009

6 Commission Regulation (EU) 'regards maximum residue levels' No 441/2012

7 Council Directive 'concerning the quality of water intended for human consumption' 98/83/EC

8 O. Cabrices et al.: 'Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Other Polar Pesticides' Application Note Gerstel (2013) #AN9/2013

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