



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

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ABSTRACT

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. In this report, we describe an automated workflow for the FMOC-derivatization, sample cleanup, and LC/MS/MS analysis using a GERSTEL MultiPurpose Sampler (MPS XL) 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. The online SPE-LC/MS/MS method allowed detection and quantitation of all target pesticides in matrix samples at 10 µg/kg concentration levels with excellent reproducibility and values well within the ± 20 % range.

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. Aminomethylphosphonic acid (AMPA) is the major metabolite of glyphosate and also included into the pesticide residue definition [1,2]. According to recent reports, there has been a dramatic increase in the usage of these herbicides which are of risk to both human health and the environment. Therefore, there is a global need for reliable and sensitive methodology to determine trace level residues from these pesticides in water and food [3,4].

Due to their high polarity it is difficult to extract these pesticides from samples and to retain them on common Reverse phase LC columns. Derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) is a common procedure to improve extraction and separation for the analysis of glyphosate and related compounds (Figure 1). 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.

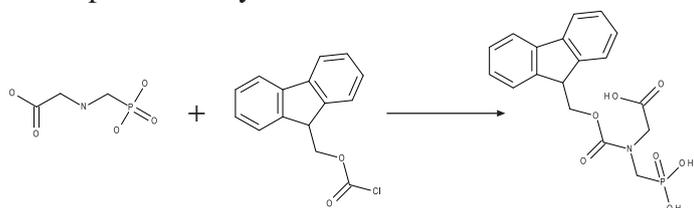


Figure 1. Derivatization of glyphosate using FMOC-Cl.

In this report, we describe an automated workflow to derivatize and determine glyphosate, glufosinate and AMPA residues in water and food samples using a GERSTEL MultiPurpose Sampler (MPS XL) configured with an online solid phase extraction module (SPE^{XOS}) coupled to an AB SCIEX QTRAP[®] 4500 system. The SPE^{XOS} system uses small cartridges packed with 10-50 mg of sorbent, which allows elution to be carried out with small volumes of solvent resulting in high throughput sample cleanup. The total cycle time per sample for the automated sample derivatization and online SPE was approximately 25 minutes, enabling concurrent separation and “just in time” sample preparation using the GERSTEL MAESTRO software.

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 sample extracts were placed on the MPS XL sampler for automated derivatization followed by extensive dilution to minimize matrix effects, online SPE cleanup and concentration with final analysis by LC/MS/MS. Target compounds were easily identified and quantified at 10 µg/kg levels with excellent reproducibility.

EXPERIMENTAL

Materials. Individual dry standards (250 mg) of glyphosate, AMPA and glufosinate were obtained from Sigma Aldrich. GERSTEL SPE^{XOS} C8EC-SE (18.5 mg) cartridges were used for the online SPE cleanup of food matrices. Corn and Soy bean samples were obtained from a local grocery store. All other solvents and reagents used were reagent grade.

Initial Sample Preparation. Water samples were directly spiked and loaded in vials for derivatization and analysis. Different food matrices (corn and soy bean) were spiked with glyphosate, glufosinate, AMPA at 10 and 100 µg/kg and extracted using 2 different methodologies:

A. QuPpe (Quick Polar Pesticides) method developed by the EU Reference Laboratories for Residues of Pesticides [5]:

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. Filter the supernatant, transfer a 1 mL aliquot to a vial and place it in the GERSTEL MPS sample tray.

B. Modified batch equilibrium extraction technique initially reported by Miles C. et al. [6].

1. Add 10 mL water to 2 g of homogenized sample, shake and soak for 15 min.
2. Add 30 mL of 0.1 M HCl and shake vigorously for 30 mins.
3. Centrifuge (3000 rpm) for 10 min.
4. Neutralize a 5 mL aliquot of the supernatant with 5 mL of 0.1 M KOH.
5. Filter and Load 1 mL into a vial and place it in the GERSTEL MPS sample tray.

Automated derivatization and online SPE cleanup. All automated sample preparation Prep Sequences and injections were performed using a dual-head GERSTEL MPS XL equipped with a heated agitator and an online solid phase extraction module (SPE^{XOS}) coupled to an AB SCIEX QTRAP[®] 4500 system as shown in figure 2.



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

The automated sample preparation workflow performed by the MPS is detailed in the flowchart shown in figure 3.

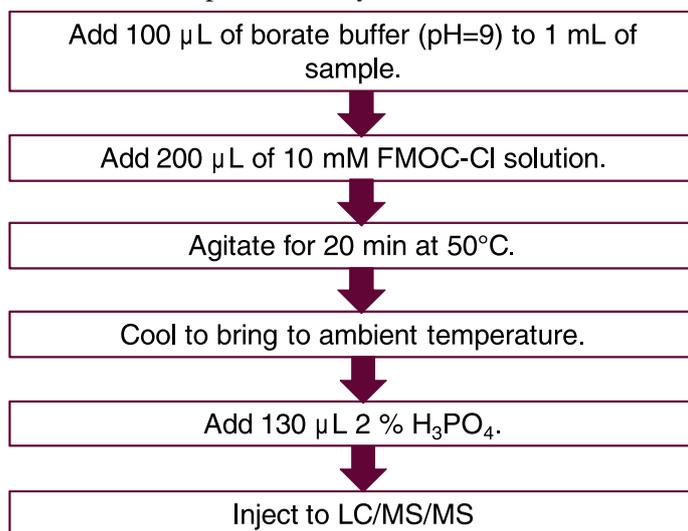


Figure 3. Automated derivatization procedure.

The automated Online SPE cleanup performed by the SPE^{XOS} module is described in figure 4.

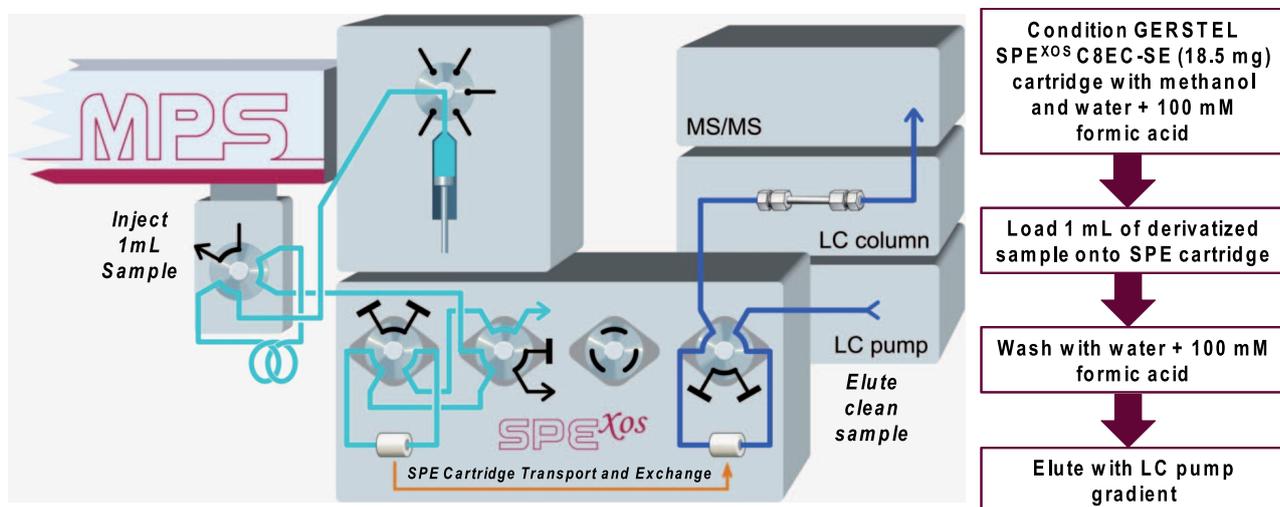


Figure 4. Automated online SPE sample cleanup workflow.

Using the GERSTEL MAESTRO software, it is possible to enhance the productivity of the proposed sample preparation workflow by using the PrepAhead feature, which allows staggering the derivatization and online-SPE cleanup process with the analysis of the previous sample. Graphic representations of this feature and its benefits are shown in figure 5.

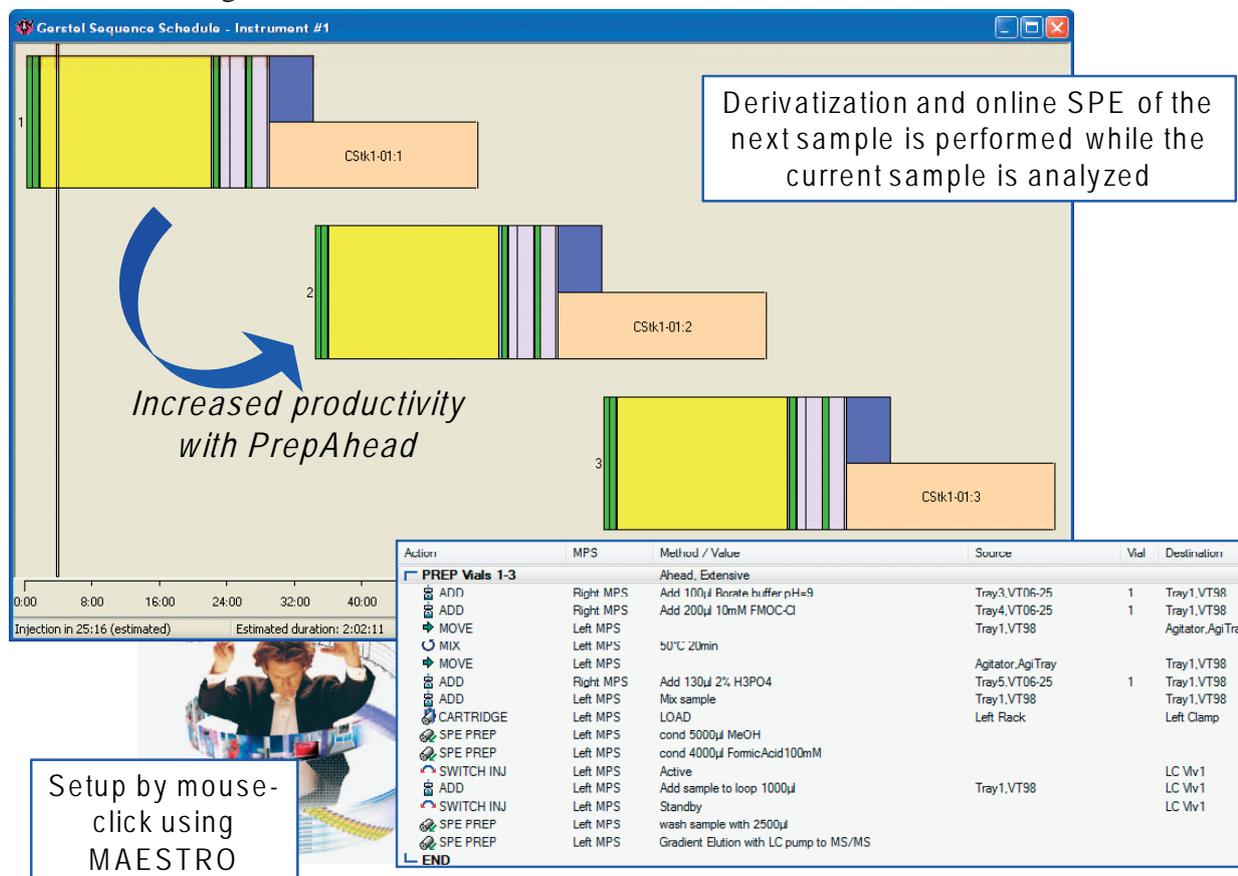


Figure 5. Sequence of scheduled events in the MAESTRO software for online SPE: green - adding buffer and FMOC-Cl, yellow - derivatization, light blue - online SPE, orange LC/MS/MS analysis, dark blue – rinsing the injection syringe.

LC/MS/MS Parameters. The LC/MS/MS analyses were performed using an Agilent 1200 Series LC pump configured with a Phenomenex Gemini 3 µm (150 x 2.0 mm) column, an AB SCIEX QTRAP® 4500 System and GERSTEL MPS XL Dual Head autosampler equipped with a Modular Washstation configured for Active Wash. Sample injections were made using a 6 port (0.25 mm) Cheminert C2V injection valve.

Mobile Phase: A – 50 mM ammonium acetate (adjusted to pH = 9)
B – Acetonitrile

LC Gradient:

| Time (min) | Flow (µL/min) | % B |
|------------|---------------|-----|
| 0 | 250 | 20 |
| 10 | 250 | 95 |
| 15 | 250 | 95 |
| 15.1 | 250 | 20 |
| 25 | 250 | 20 |

Run time: 25 minutes.

Column Temperature: 40°C

Water samples were injected directly (10 µL) into the LC/MS/MS. Food sample extracts were extensively diluted to minimize possible matrix effects and automatically cleaned up and concentrated using the SPE^{XOS} system. In this case, a 1 mL injection of the diluted sample extract onto the SPE cartridge was performed.

Mass Spectrometer Parameters:

Source/Gas Parameters

| | |
|------|----------------|
| CUR: | 30 psi |
| IS: | -4200 V (ESI-) |
| TEM: | 400°C |
| GS1: | 50 psi |
| GS2: | 70 psi |
| CAD: | Medium |

The MRM transitions used for the compounds are shown in table 1; each MRM was monitored with a dwell time of 100 ms.

Table 1. 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 |

The AB SCIEX QTRAP® 4500 LC/MS/MS System was operated with Turbo V™ source and Electrospray Ionization (ESI) probe. 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

Environmental testing. A standard chromatogram obtained after automated derivatization is shown in figure 6.

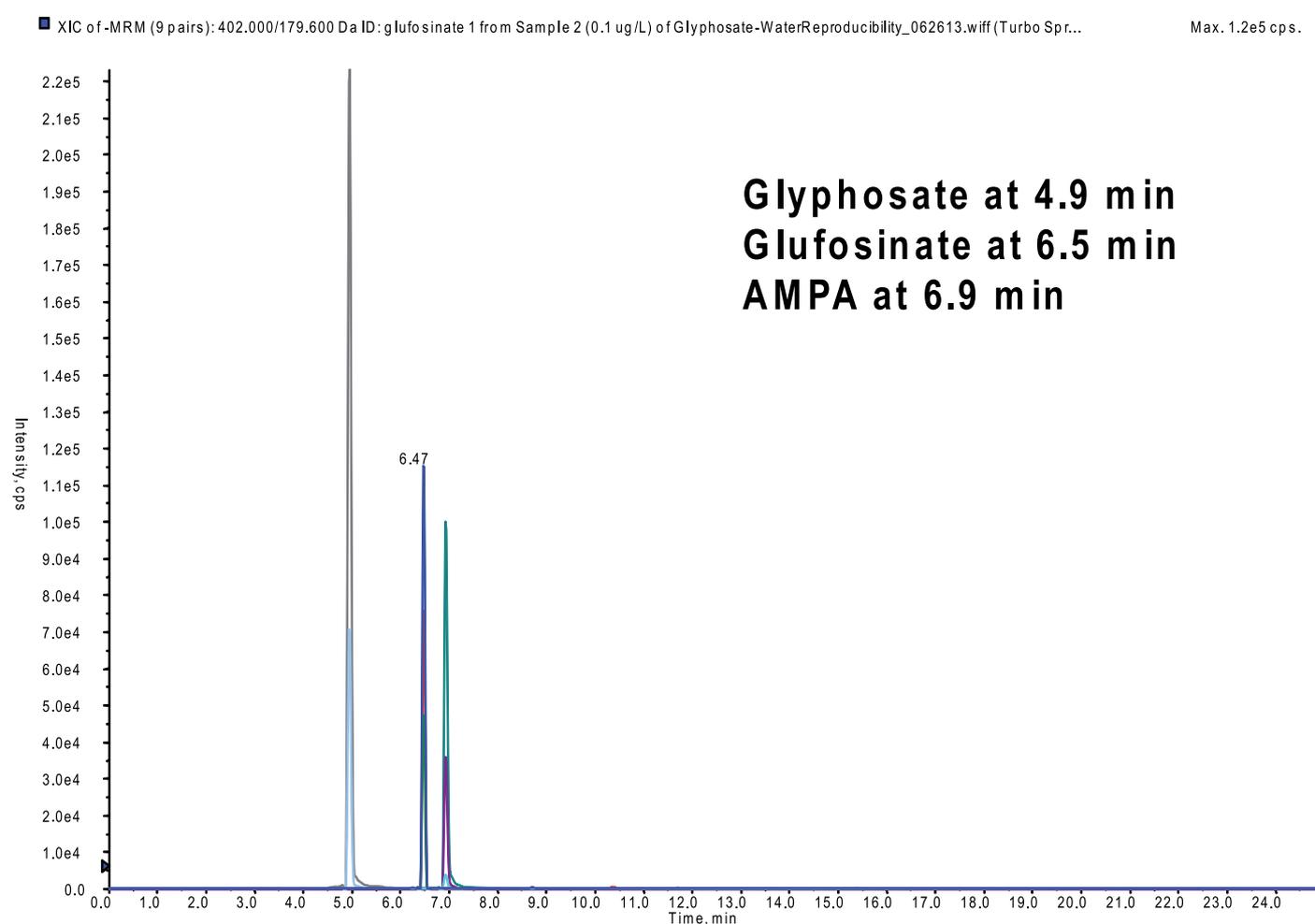


Figure 6. Standard chromatogram at a concentration of 10 ng/mL.

A drinking water sample was spiked at 0.1 and 10 $\mu\text{g/L}$, automatically derivatized, and analyzed in triplicate. The method allowed accurate quantitation of all target compounds well below 0.1 $\mu\text{g/L}$ with excellent repeatability (Figure 7 and Table 2).

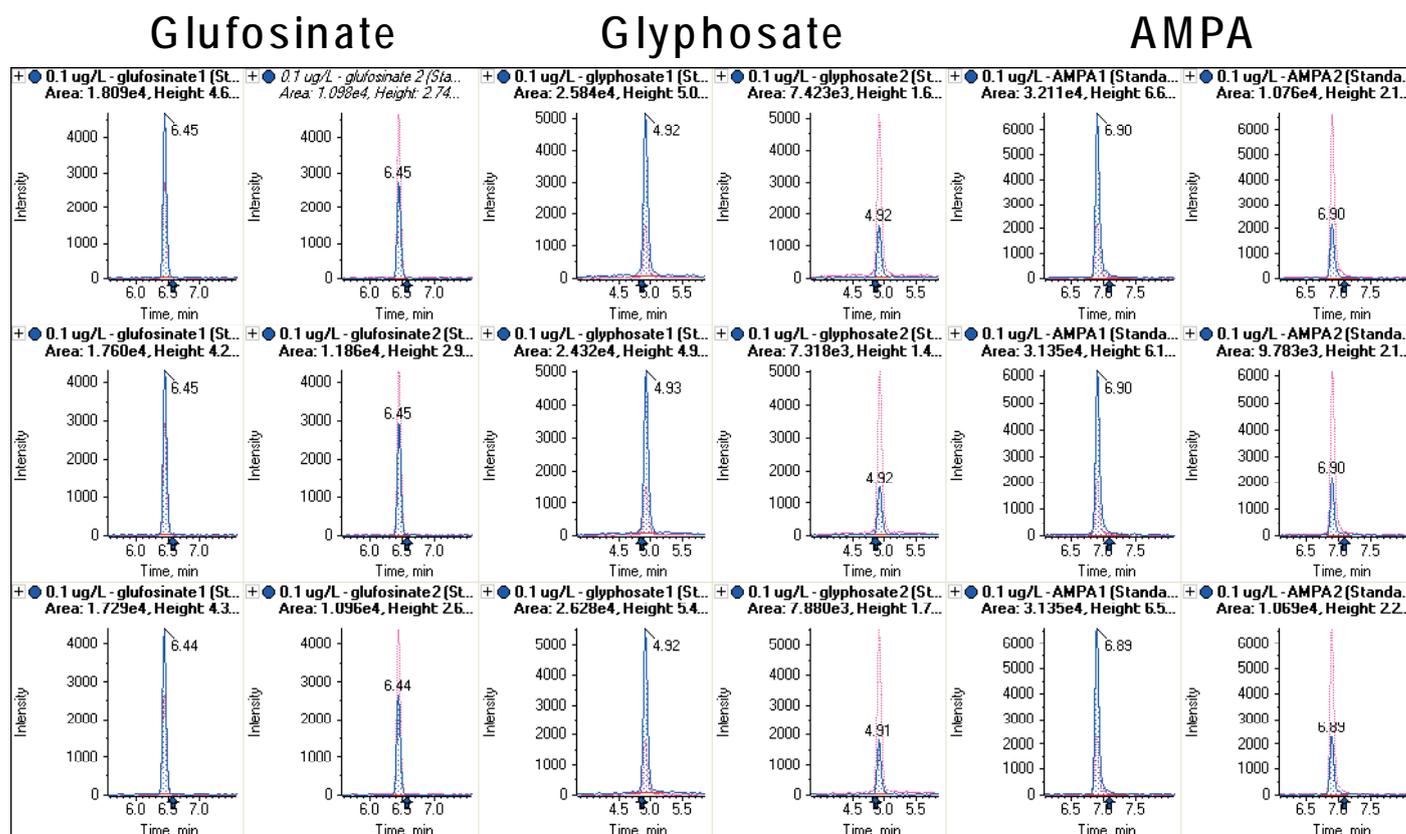


Figure 7. Triplicate analysis of polar pesticides in a spiked water sample at 0.1 $\mu\text{g/L}$ (injection volume 10 μL), ion ratios for compound identification were calculated automatically in MultiQuant™ software version 3.0.

Table 2. Triplicate analysis of polar pesticides in a spiked water sample at 0.1 $\mu\text{g/L}$ (injection volume of 10 μL).

| Compound | Concentration ($\mu\text{g/L}$) | % 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 |

Food sample testing. Corn and soy samples were spiked at 10 and 100 $\mu\text{g/kg}$ and analyzed in triplicate using the automated derivatization and cleanup procedure. The method enabled accurate quantitation of all target compounds well below the target concentration of 100 $\mu\text{g/kg}$ with excellent repeatability (Table 3, Figures 8 and 9).

Table 3. Triplicate analysis of polar pesticides spiked into corn and soy samples 100 $\mu\text{g/kg}$.

| Compound | Concentration ($\mu\text{g/kg}$) | % 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 %) |

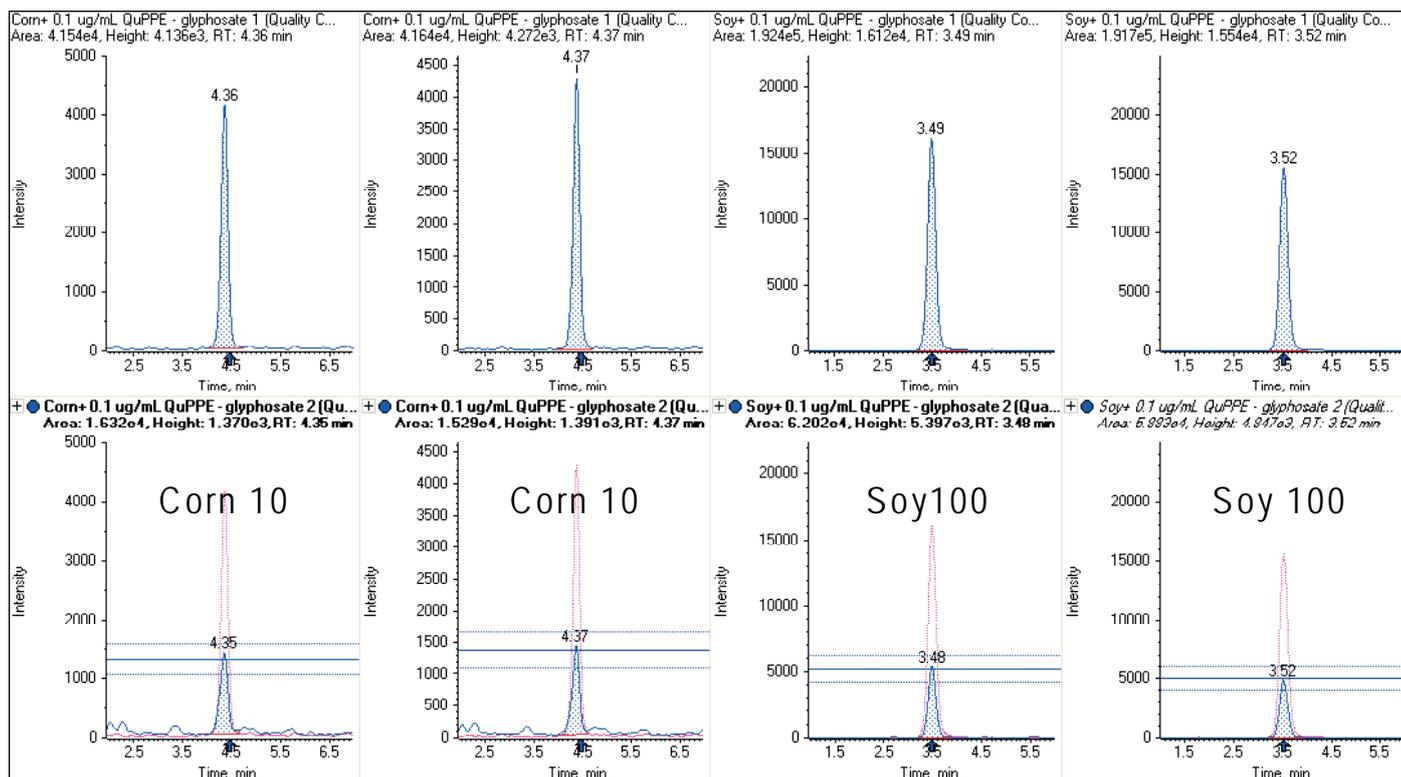


Figure 8. 10 and 100 $\mu\text{g}/\text{kg}$ of glyphosate spiked into corn and soy and analyzed using automated derivatization, dilution, and online SPE cleanup followed by LC/MS/MS.

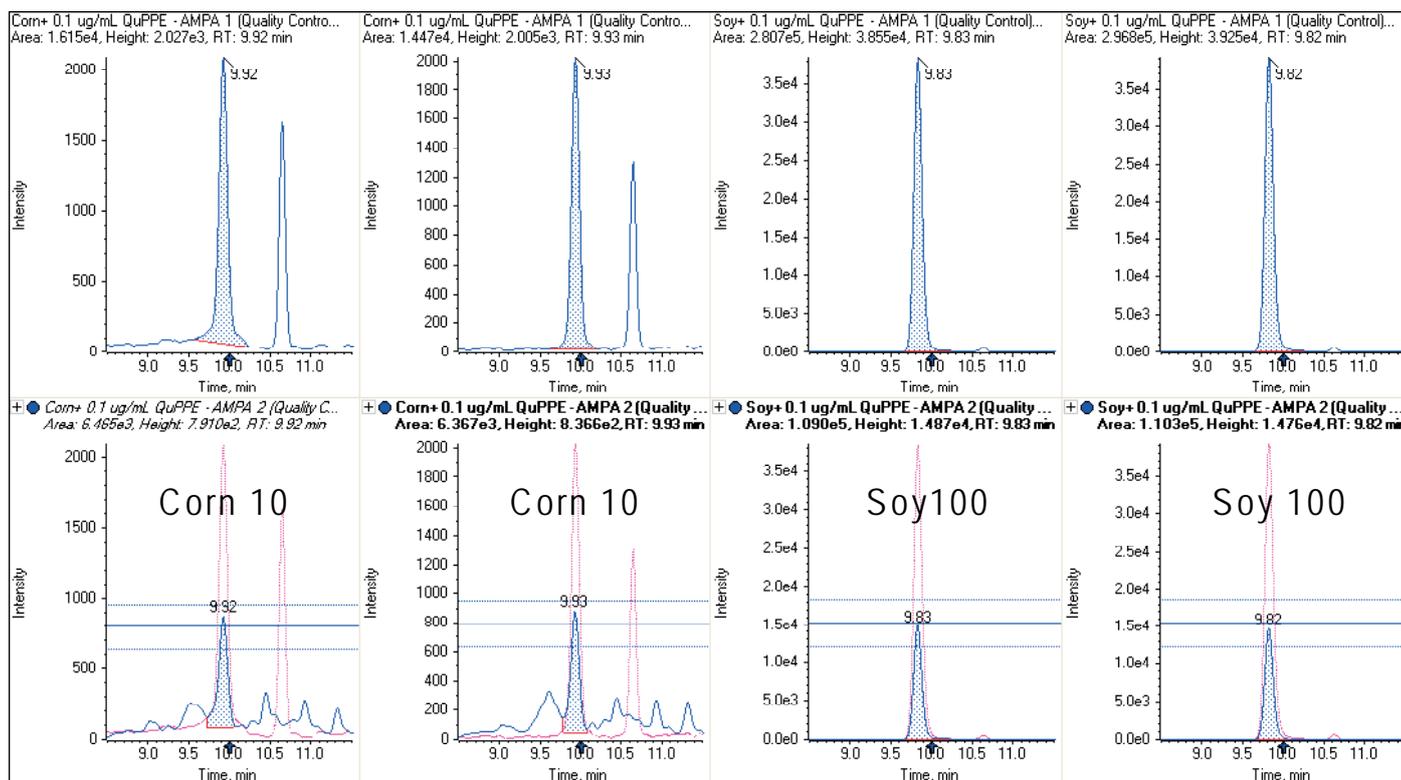


Figure 9. 10 and 100 $\mu\text{g}/\text{kg}$ of AMPA spiked into corn and soy and analyzed using automated derivatization, dilution, and online SPE cleanup followed by LC/MS/MS.

Ion ratios for compound identification were automatically calculated in the result table in MultiQuant™ software version 3.0. The quantifier and qualifier ratio was found to be a valuable tool to identify all target pesticides in matrix samples with excellent reproducibility and values well within the $\pm 20\%$ range.

The slightly higher % RSD of the ion ratio of glufosinate in corn can be explained by interfering matrix signals (Figure 10). Stable LC separation was essential for confident identification and accurate quantitation of glufosinate.

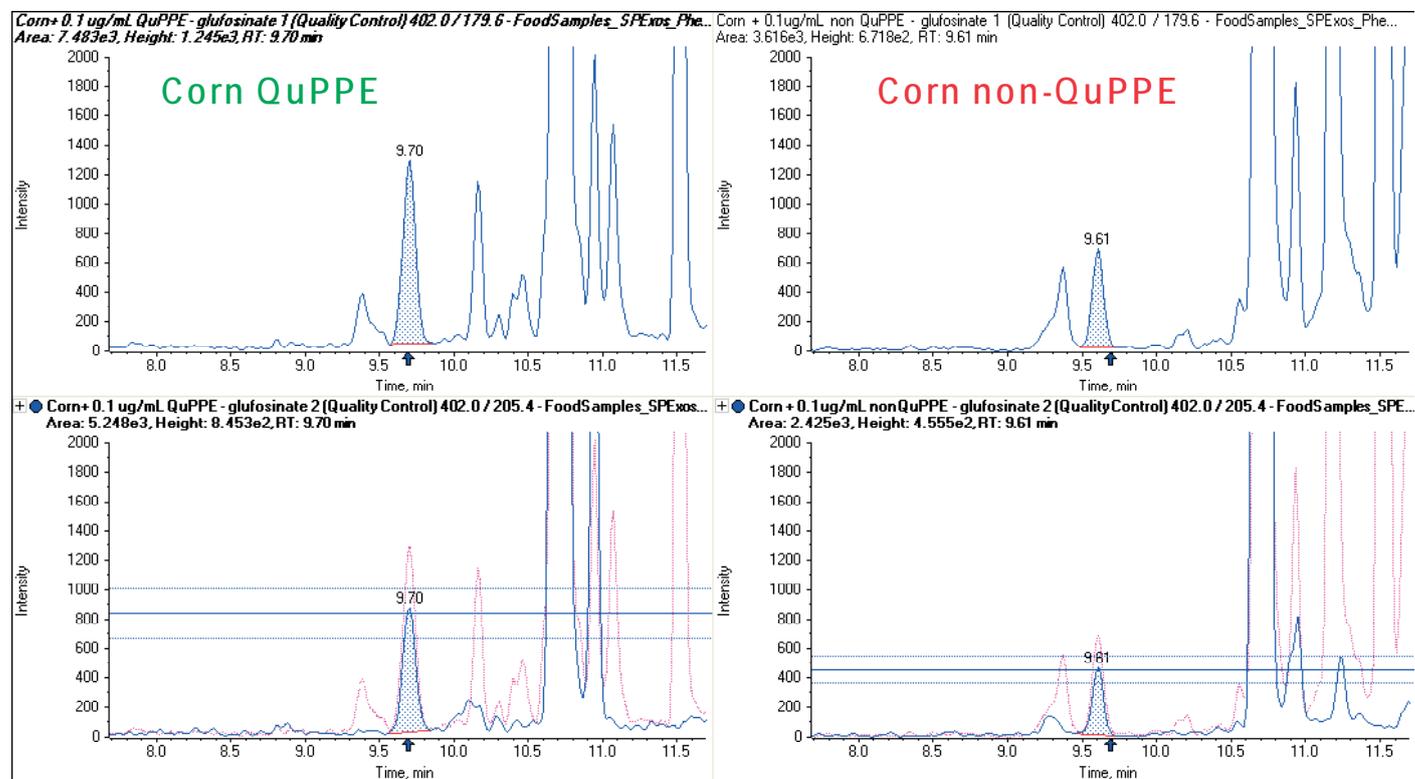


Figure 10. Corn analyzed for glufosinate using the QuPPE and a non-QuPPE extraction procedures respectively. The QuPPE protocol results in higher recoveries, but more matrix effect.

The results using the QuPPE extraction were compared to results obtained when using the batch equilibrium procedure based on extraction with 0.1 M HCl. In general, recoveries were between 70-120 % for both matrices

Extraction recoveries using the non-QuPPE extraction were found to be lower in all cases. However, in the case of corn this extraction resulted in cleaner MRM chromatograms for glufosinate (Figure 10).

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. When using this automated procedure for derivatization, extraction and analysis, more than 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.

CONCLUSIONS

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 concentrations [7-9] in drinking water and food samples
- The described workflow using the GERSTEL MPS XL autosampler 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 determination 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 to online SPE cleanup / analyte concentration to monitor at 10 µg/kg.

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