LC-MS/MS Analysis of Emerging Food Contaminants

Detection of Glyphosate in Food Samples using QuPPe-LC-DMS-MS/MS

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Overview

Here we present results of using QuPPe extraction (Quick Polar Pesticides), Liquid Chromatography, Differential Mobility Spectrometry and tandem Mass Spectrometry (LC-DMS-MS/MS) to identify and quantify underivatized glyphosate, AMPA, glufosinate, and MMPA in food samples.

The enhanced sensitivity of the SCIEX QTRAP® 6500+ LC-MS/MS system and selectivity of SelexION®+ DMS technology resulted in limits of quantitation (LOQ) for all target compounds of 100 µg/kg in food samples. Excellent repeatability and linearity was observed. High confidence in identification was achieved by monitoring 4 MRM transitions per compound.

Introduction

Glyphosate (N-(phosphonomethyl)glycine) is a widely used broad-spectrum systemic herbicide and crop desiccant. Generally, glyphosate is considered as safe and not toxic to humans.[1-3]

However, Glyphosate is a topic with an extraordinary degree of public attention and concerns since the International Agency for Research on Cancer (IARC), a branch of the World Health Organization, classified glyphosate as a probable human carcinogen.[4]

Traces of glyphosate have been found in surface water, many foods (i.e. bread, breakfast cereals, dairy, and beer) and also in human urine and breast milk.[5-9]

Glyphosate can be analyzed using Enzyme-linked immunosorbent assays (ELISA). Although relatively quick and simple to perform, ELISA tests are limited in selectivity and susceptible to cross-reactivity, which can lead to false positive or false negative results.

When analyzed using LC, Glyphosate is derivatized with FMOC to improve its retention, as it is very polar. This derivatization step complicates the analysis and there is a growing need for a method which can detect Glyphosate and AMPA in their underivatized forms. Anion exchange, HILIC, porous graphitized carbon and mixed-mode columns were used with LC-MS/MS to determine underivatized polar pesticides with limited success.[7, 10-12]

Here we used an LC method using a mixed-mode column and a mobile phase at pH 2.9. LOQs as low as 100 µg/kg were achieved for glyphosate, glufosinate and their metabolites AMPA and MMPA using high sensitivity detection with the SCIEX QTRAP® 6500+ system. High confidence in identification was achieved by monitoring 4 Multiple Reaction Monitoring (MRM) transitions. In matrix samples few transitions experienced interferences. SelexION®+ DMS technology was successfully used to remove interferences to improve Signal-to-Noise (S/N) and confidence in results.

Experimental

Sample Preparation

- Corn and soy beans purchased from a local supermarket
- QuPPe extraction: 10 g sample extracted after adjustment of water content with 10 mL methanol + 1% formic acid[12]
- Centrifugation
- 10x dilution with LC grade water to minimize potential matrix effects

LC Separation

- ExionLC™ AD system
- Acclaim Trinity Q1 (100 x 3 mm, 3µm)
- Gradient of water + 50 mM ammonium formate/formic acid (pH=2.9) and acetonitrile
- Injection of 10 µL

MS/MS Detection

- SCIEX QTRAP® 6500+ system and IonDrive Turbo V™ source
- Electrospray Ionization (ESI) probe, TEM = 700ºC
- Negative polarity
- SelexION®+ DMS technology, SV = 3800 V
- Multiple Reaction Monitoring (MRM) of 4 transition per analyte (Table 1) and *Scheduled* MRM™ algorithm
- Data acquisition using Analyst® software version 1.6.3
- Data processing in MultiQuant™ software version 3.0.2

Table 1. MRM transitions to detect Glyphosate and other polar pesticides with compound dependent parameters, Declustering Potential (DP), Collison Energy (CE), and Compensation Voltage (CoV)

Compound	Q1	Q3	DP (V)	CE (V)	CoV (V)
Glyphosate	168	63	-30	-26	8.2
	168	150	-30	-14	8.2
	168	124	-30	-16	8.2
	168	81	-30	-20	8.2
AMPA	110	63	-15	-26	-1.8
	110	79	-15	-36	-1.8
	110	81	-15	-16	-1.8
	110	80	-15	-24	-1.8
Glufosinate	180	63	-50	-66	9.2
	180	95	-50	-24	9.2
	180	136	-50	-22	9.2
	180	85	-50	-24	9.2
MMPA	151	133	-10	-18	7.0
	151	63	-10	-44	7.0
	151	107	-10	-20	7.0
	151	78	-10	-28	7.0

Results and Discussion

Three different LC Methods were evaluated in their performance with respect to selectivity, sensitivity, and ease of use for routine food analyses. The LC method using a mixed-mode column and a mobile phase at pH 2.9 was selected because of good peak shape, best signal-to-noise (S/N), and good separation to allow use of MRM scheduling.[13]

Figure 1 shows an example chromatogram of all 4 target compounds at a concentration of 100 ng/mL.

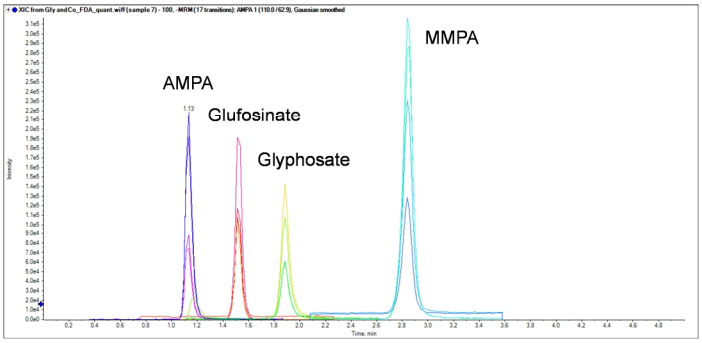

Figure 1. MRM chromatograms of 100 ng/mL of Glyphosate, AMPA, Glufosinate and MMPA using a mixed-mode column and a mobile phase at pH 2.9

The LOQ was evaluated by repeat analysis of low level solvent standards. Figures 2a to d show the 4 MRM transitions of all target compounds at a concentration of 10 ng/mL. After 5 injections the coefficients of variation (%CV) were between 2.4% and 3.3%.

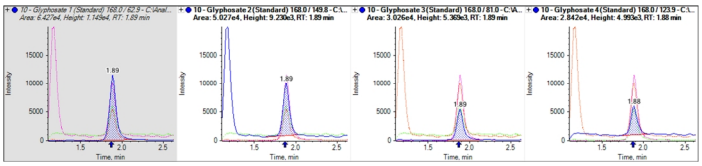

Figure 2a. 10 ng/mL Glyphosate with a %CV of 2.99% (n = 5)

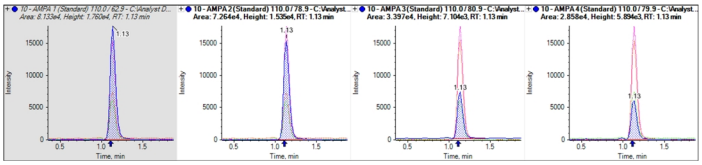

Figure 2b. 10 ng/L AMPA with a %CV of 2.85% (n = 5)

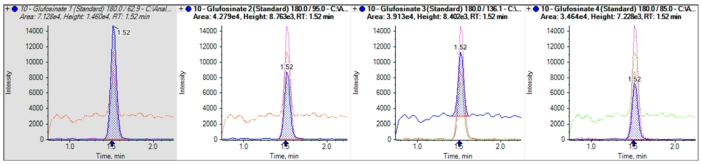

Figure 2c. 10 ng/mL Glufosinate with a %CV of 2.99% (n = 5)

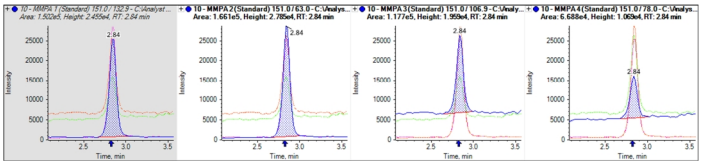

Figure 2d. 10 ng/L MMPA with a %CV of 2.85% (n = 5)

Linearity for quantitation was evaluated over a range from 1 to 1000 ng/mL. Linearity was excellent with coefficients of regression (r) better than 0.999 using linear fit and 1/x weighting (Figure 3). Accuracies were all well between 80 and 120% at all concentration levels.

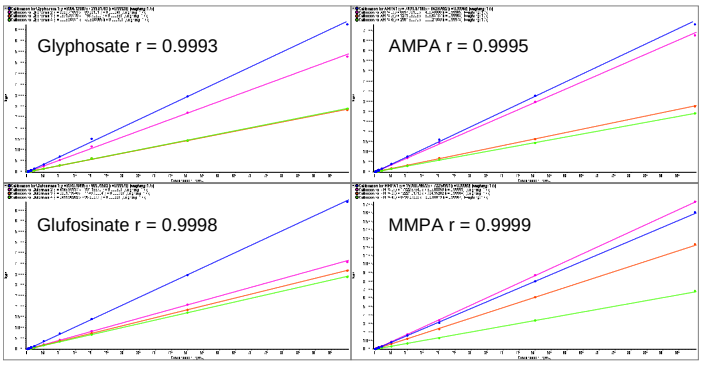

Figure 3. Linearity for all 4 target compounds from 1 to 1000 ng/mL using linear fit and 1/x weighting

After initial verification, the new LC-MS/MS method was applied to the analysis of glyphosate and other polar pesticides in food samples. Corn and soy were extracted following the QuPPe protocol, spiked at 100 ng/mL and diluted 10x to a final concentration of 10 ng/mL in the diluted extract. Chromatograms are shown in Figures 4a to d.

High confidence in identification was achieved by the detection of 4 MRM transitions and calculation of quantifier-qualifier ratios. An MRM ratio tolerance of 30% was applied to identification as specified in the guideline SANTE/11945/2015.[14]

At a concentration of 10 ng/mL Glyphosate and MMPA were identified using 4 MRM transitions in corn and soy extract. AMPA and Glufosinate experienced some interferences but confident identification based on 2 and 3 transitions, respectively, was still possible.

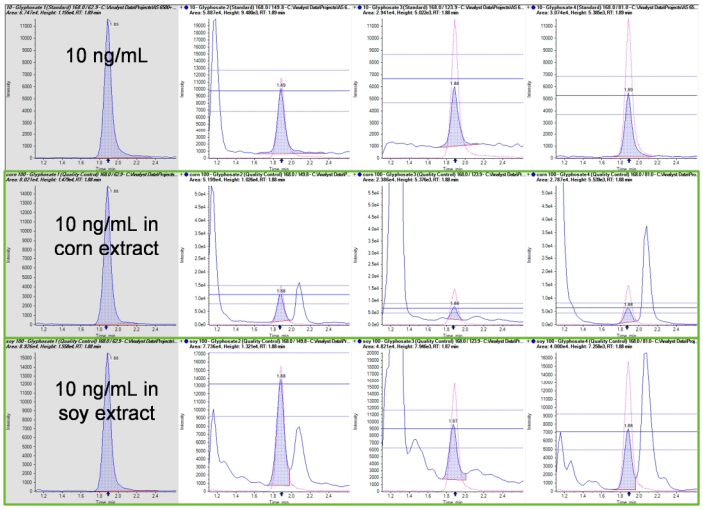

Figure 4a. 10 ng/mL Glyphosate in solvent, corn and soy extract, confident identification of Glyphosate based on 4 MRM transitions

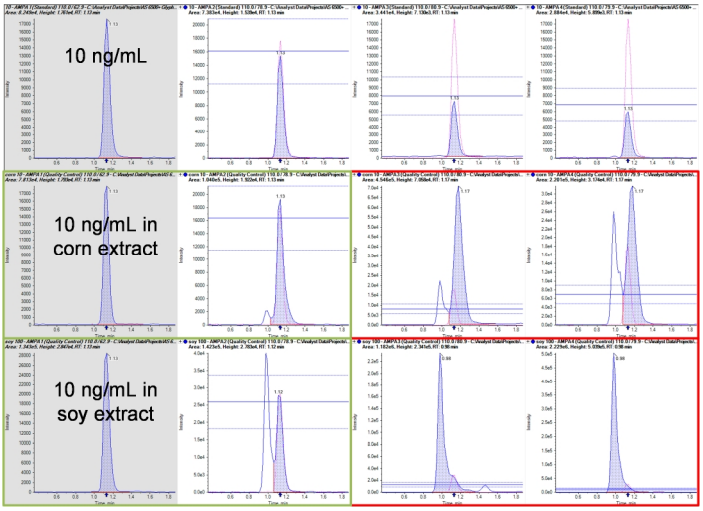

Figure 4b. 10 ng/mL AMPA in solvent, corn and soy extract, identification of AMPA based on 2 of 4 MRM transitions, the other 2 transitions had significant interferences in both matrices

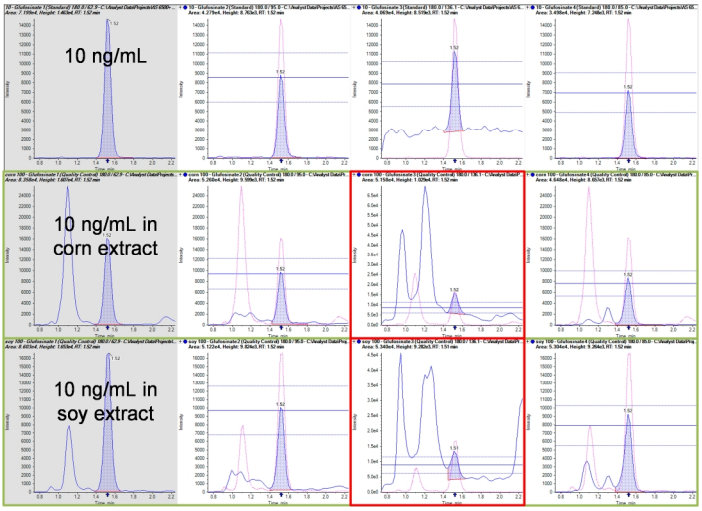

Figure 4c. 10 ng/mL Glufosinate in solvent, corn and soy extract, identification of Glufosinate based on 3 of 4 MRM transitions, one transition had significant interferences in both matrices

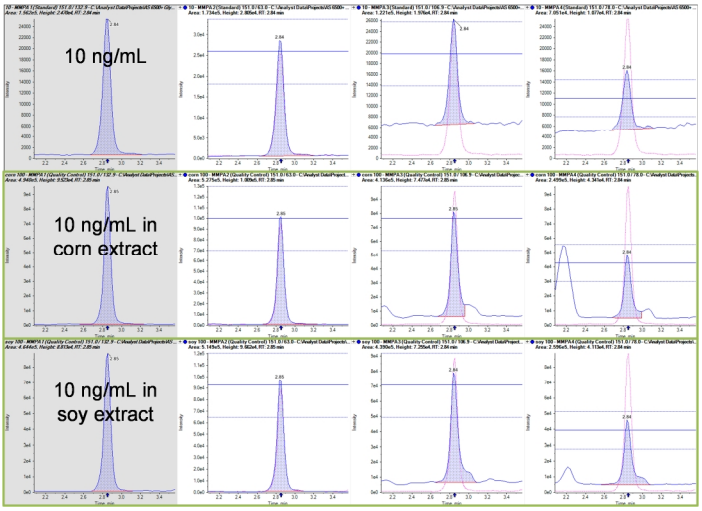

Figure 4d. 10 ng/mL MMPA in solvent, corn and soy extract, confident identification of MMPA based on 4 MRM transitions

In a next step SelexION®+ DMS technology was used to increase selectivity and confidence in identification.

The DMS cell is a planar differential mobility device that attaches between the curtain plate and orifice plate of the QTRAP® 6500+ system. Gas draws the ions through the DMS cell towards the orifice while an asymmetric waveform is applied to the plates, which alternates between a high and low field. Unlike traditional ion mobility, ions are not separated in time as they traverse the cell. They are separated in trajectory based on the difference in their mobility between the high field and low field portions of the applied Separation Voltage (SV). As the ions migrate towards the walls of the DMS cell at different rates, they will be separated. By applying a second voltage offset (the Compensation Voltage, CoV) the trajectory of a desired ion can be corrected along the axis of the DMS cell and transmitted to the mass analyzer (Figure 5).

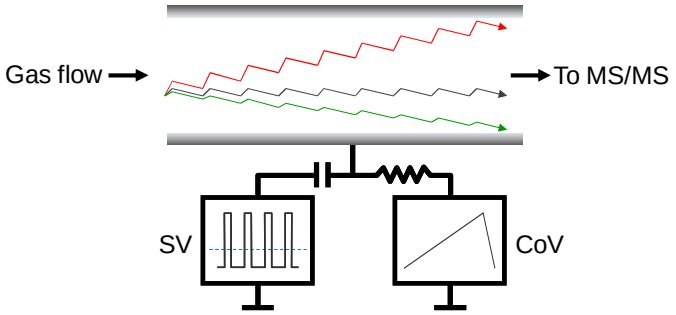

Figure 5. Differential Mobility Separation Process. Planar design of the DMS cell uses an asymmetric RF waveform (SV) to separate ions based on differential mobility between the high and low fields. The compensation voltage (CoV) is used to correct the trajectory of the ion of interest which traverses the cell and into the orifice while interferences are deflected into the cell walls.

In comparison to previous versions, the SelexION®+ cell uses an additional lens reducing ion transit times through detrimental fringing fields resulting in signal improvements.

Chromatograms of 10 ng/mL in solvent and diluted QuPPe extracts are shown in Figures 5a to d. Comparing Figures 4

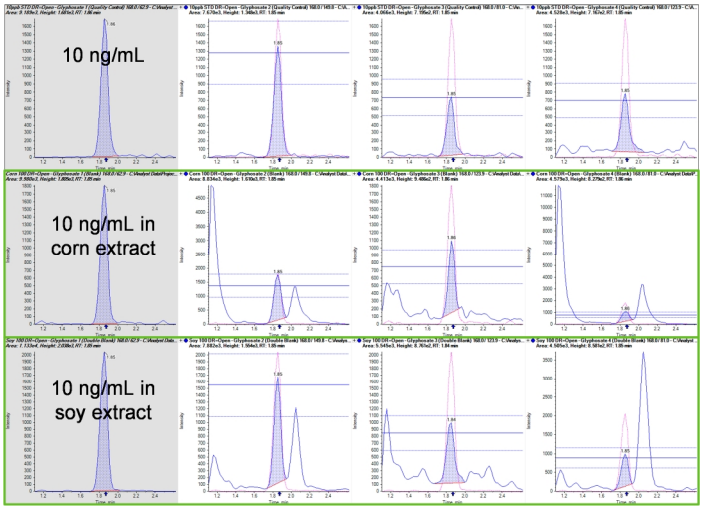

Figure 5a. 10 ng/mL Glyphosate in solvent, corn and soy extract, confident identification of Glyphosate based on 4 MRM transitions and enhanced selectivity using SelexION®+ DMS technology

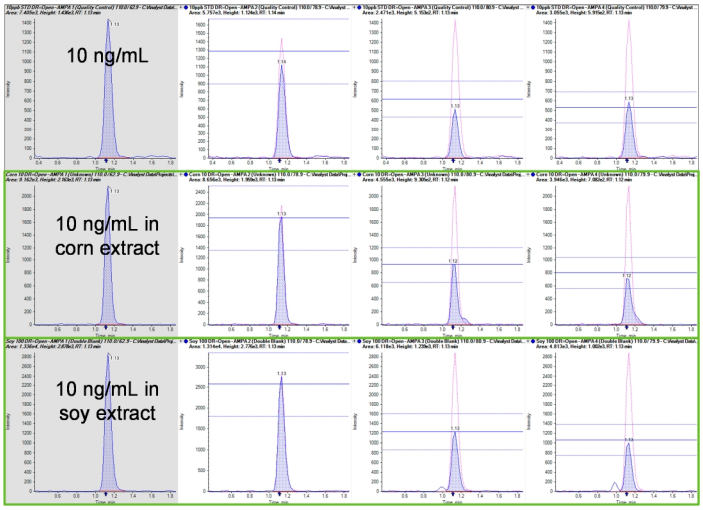

Figure 5b. 10 ng/mL AMPA in solvent, corn and soy extract, confident identification of AMPA based on 4 MRM transitions and enhanced selectivity using SelexION®+ DMS technology

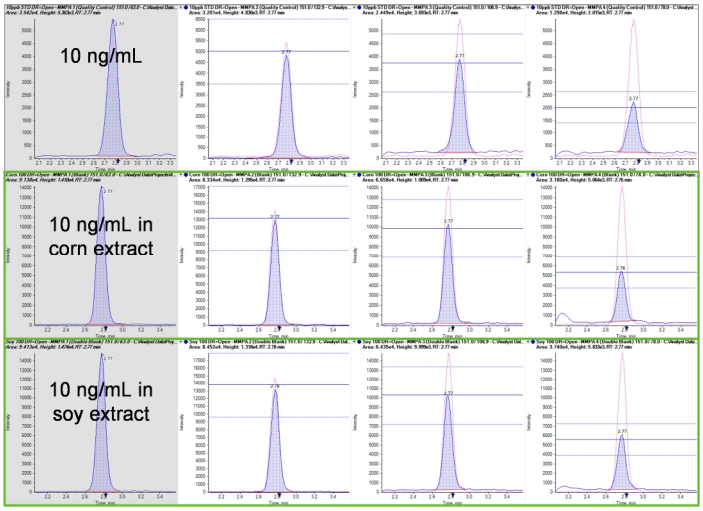

Figure 5d. 10 ng/mL MMPA in solvent, corn and soy extract, confident identification of MMPA based on 4 MRM transitions and enhanced selectivity using SelexION®+ DMS technology

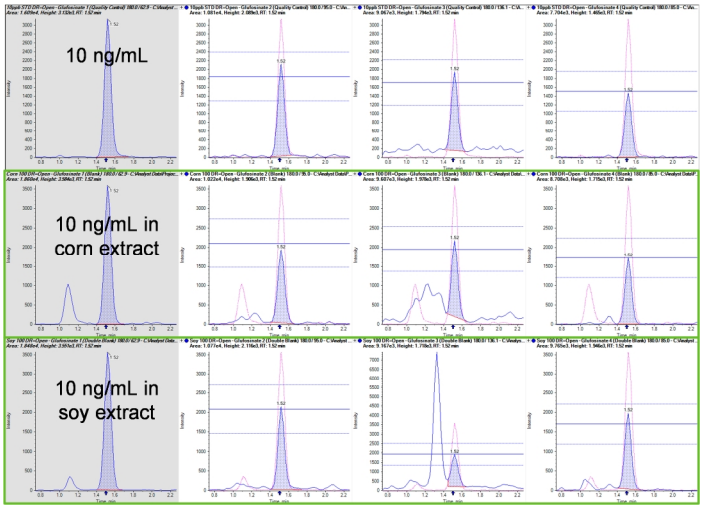

Figure 5c. 10 ng/mL Glufosinate in solvent, corn and soy extract, confident identification of Glufosinate based on 4 MRM transitions and enhanced selectivity using SelexION®+ DMS technology

and 5 it is obvious that enhanced selectivity provided by differential mobility spectrometry helped to remove background and matrix interferences. This is most notable for AMPA and Glufosinate and results in higher confidence in identification based on a total of 4 MRM transitions.

Summary

Here we presented results of using QuPPe extraction (Quick Polar Pesticides), Liquid Chromatography, Differential Mobility Spectrometry and tandem Mass Spectrometry (LC-DMS-MS/MS) to identify and quantify underivatized glyphosate, AMPA, glufosinate, and MMPA in food samples.

The method using a SCIEX QTRAP® 6500+ system with SelexION®+ DMS technology provided excellent sensitivity, repeatability, and linearity. Corn and soy samples were extracted and diluted 10x to minimize potential matrix effects. The LOQs for all 4 target compounds were below 100 µg/kg. High confidence in identification was achieved by monitoring 4 MRM transitions. DMS technology was used successfully to remove background and matrix interferences.

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Publication number: RUO-MKT-02-4544-A

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