Poster Reprint

ASMS 2018
MP-264

Analysis of glyphosate, AMPA and 7 other polar pesticides in food and water by SAX chromatography with MS/MS detection

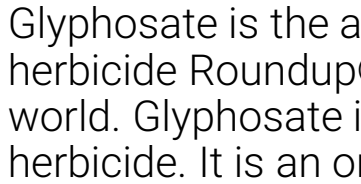
Jerry Zweigenbaum, Tarun Anumol, and Derick Lucas

Agilent, Wilmington, Delaware, USA

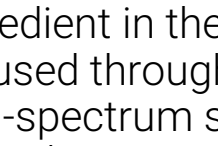
Introduction

Glyphosate is the active ingredient in the popular herbicide Roundup® and is used throughout the world. Glyphosate is a broad-spectrum systemic herbicide. It is an organophosphorus compound, specifically a phosphonate. Recently, its safe use has come into question. This has heightened the demand for a sensitive method at the low ppb level for food and even lower levels for environmental water analysis. Reliable sample preparation and analysis is needed to routinely analyze glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA). However, glyphosate and its metabolite's high polarity can be quite challenging not only extraction from food but in its analysis. Of concern is the affinity of these compounds to stainless steel and other surfaces making system-to-system reproducibility difficult. This shows the performance of the extraction of glyphosate, its major metabolite and seven other metabolites and polar pesticides.

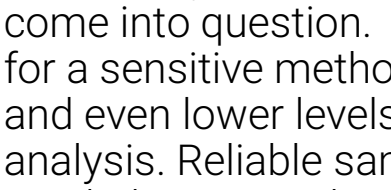
Polar Pesticides Analyzed



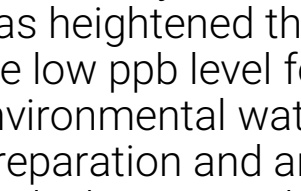
Glyphosate *m/z* 168→63/150



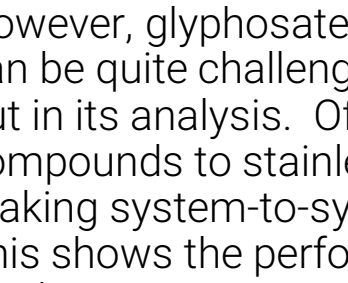
Aminomethylphosphonic acid (AMPA) *m/z* 110 →63/79



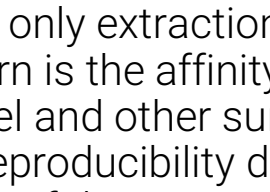
Glufosinate *m/z* 180 →134/63



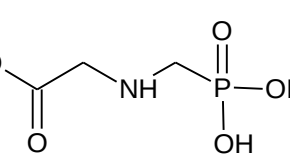
Fosetyl (Al^{+3} salt) *m/z* 109 →79/63



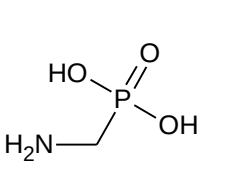
N-acetylglufosinate *m/z* 222 →136/59



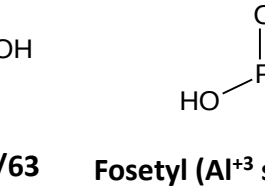
2-hydroxyethyphosphonic acid (HEPA) *m/z* 125 →95/79



Ethephon *m/z* 145 →107
***m/z* 143 → 107**



3-methylphosphinicoproprionic acid (MPPA) *m/z* 151 →133/63



maleic hydrazide *m/z* 111 →82/42

Experimental

Sample Preparation

At least 2 g of sample is weighed to a 15 mL VWR plastic centrifuge tube. The sample is then extracted following the QuPPE method where high water content samples (<80%) are extracted with an equal amount of acidified methanol and lower water content samples have added water to equal the amount of acidified methanol.[1]

Instrumental Conditions

Figure 1. Agilent 1260 Infinity Bio-inert Quaternary LC with 6495A Triple Quadrupole LC/MS System.

HPLC Condition			
Column: Shodex HILIC VT50 2D			
Injection volumn	10 uL		
Mobile Phase Initial			
A:50% UHP H2O, B: 10% NH4HCO3, C: 40% Acetonitrile			
Gradient			
Time	**A**	**B**	**C**
6.50 min	40.0 %	50.0 %	10.0 %
10.00 min	15.0 %	85.0 %	0.0 %
18.00 min	0.0 %	100.0 %	0.0 %
Flow	0.2 mL/min		
MS Conditions			
ESI	negative		
Source Parameters			
		Value (-)	
Gas Temp (°C)		140	
Gas Flow (l/min)		18	
Nebulizer (psi)		30	
SheathGasHeater		375	
SheathGasFlow		12	
Capillary (V)		3000	
VCharging		500	
Ion Funnel Parameters			
Neg High Pressure RF		110	
Neg Low Pressure RF		60	

Chromatographic Separation of standards in water and in QuPPE extraction solvent

Figure 2 shows the separation and sensitivity obtained with a standard in water. There is excellent separation of AMPA and fosetyl. However, the same separation is altered by dissolving the standard in the extraction solvent. Figure 3 shows AMPA and fosetyl much closer together while MPPA and ethephon actually switch in elution.

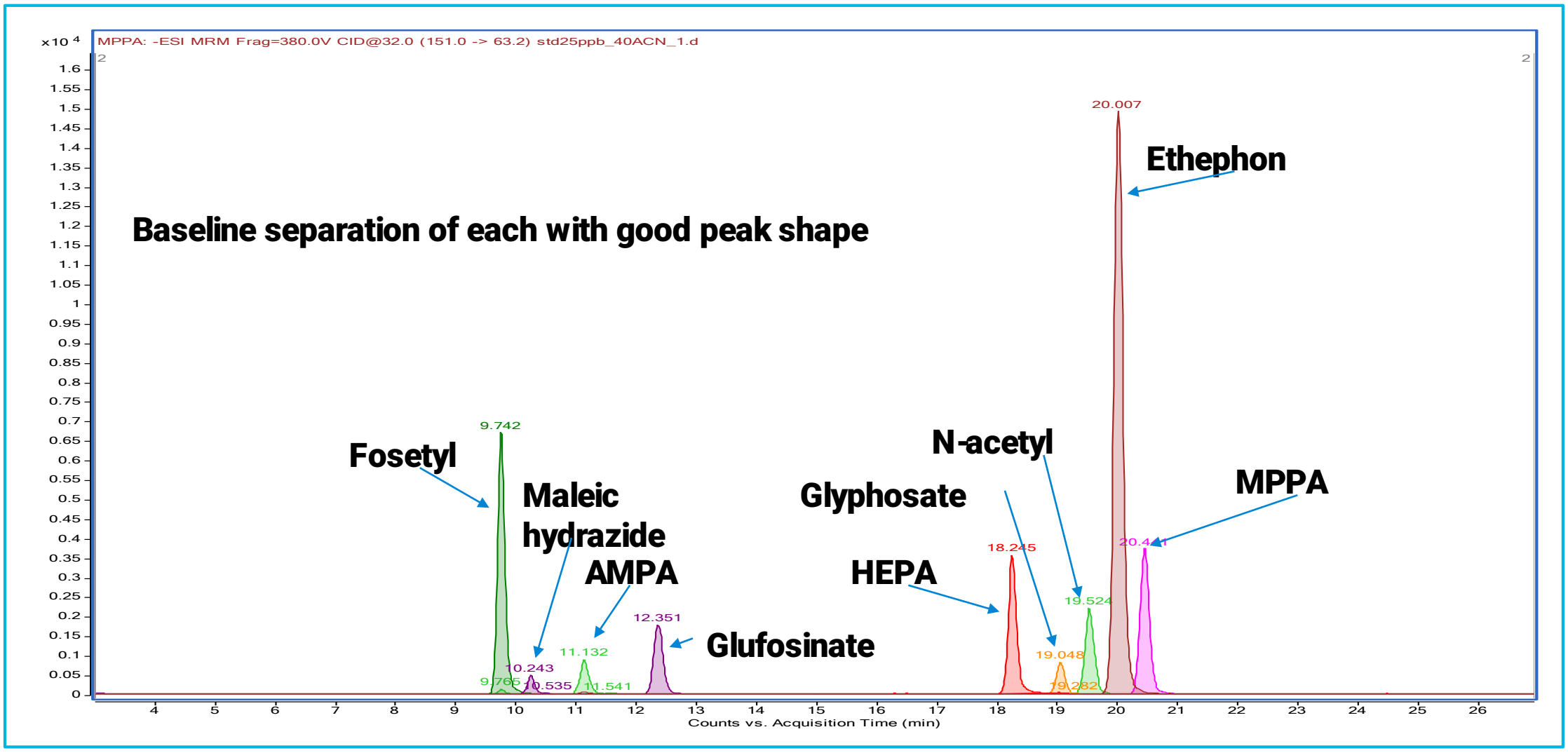


Figure 2. LC/MS/MS chromatogram of nine polar pesticides and metabolites of 10 ppb standard in water .

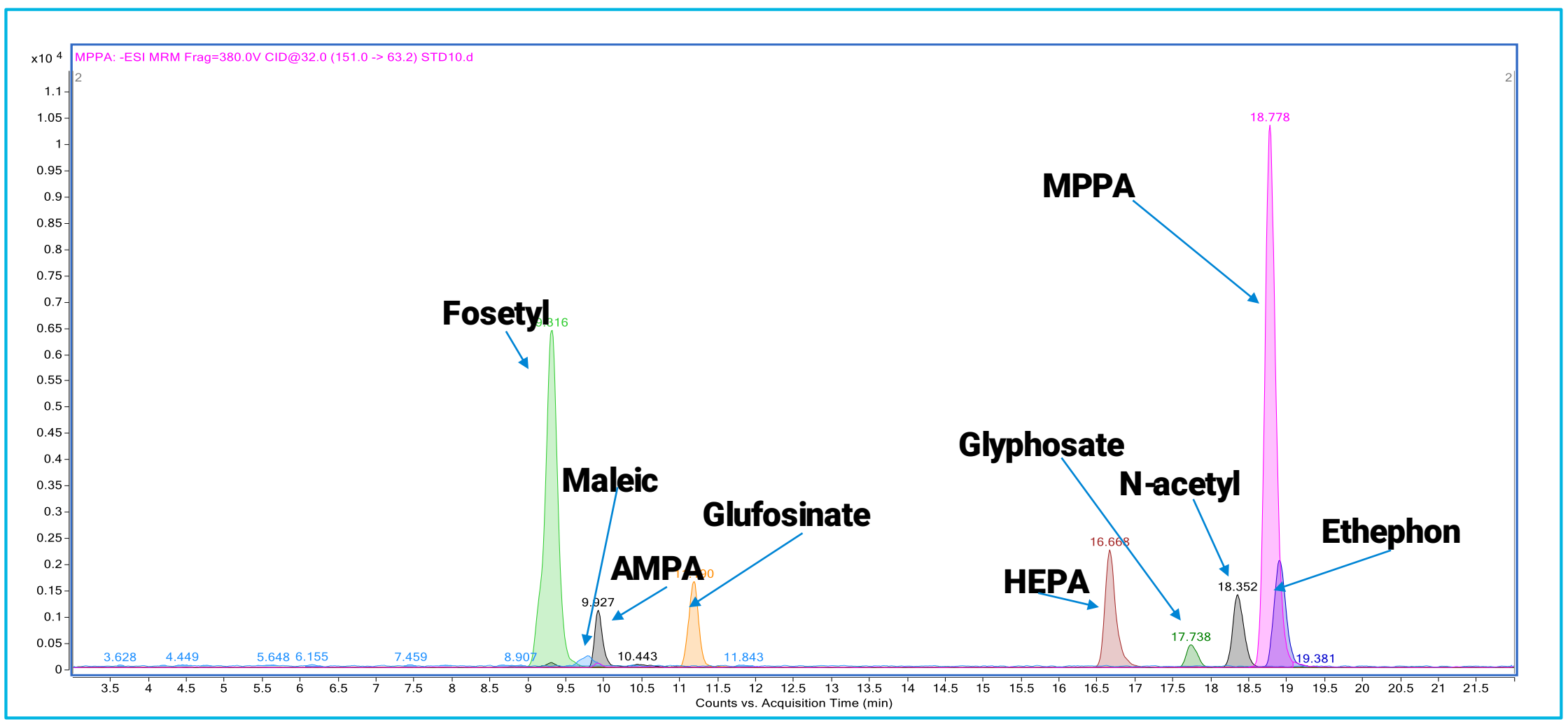


Figure 3. LC/MS/MS chromatogram of nine polar pesticides and metabolites of 10 ppb standard in 50:50 water:acidified methanol (QuPPE extraction solvent).

Results and Discussion

Need to separation fosetyl and AMPA

Figure 4 shows the MRM chromatograms for fosetyl and AMAP. A distinct peak appears in both AMPA MRMs that exactly aligns with fosetyl. Figure 5 shows the fragmentation for the product ions of both compounds.[2] Because the fragments contain no carbon, the C^{13} isotope for fosetyl is producing the same transitions as AMPA and thus the two must be chromatographically separated.

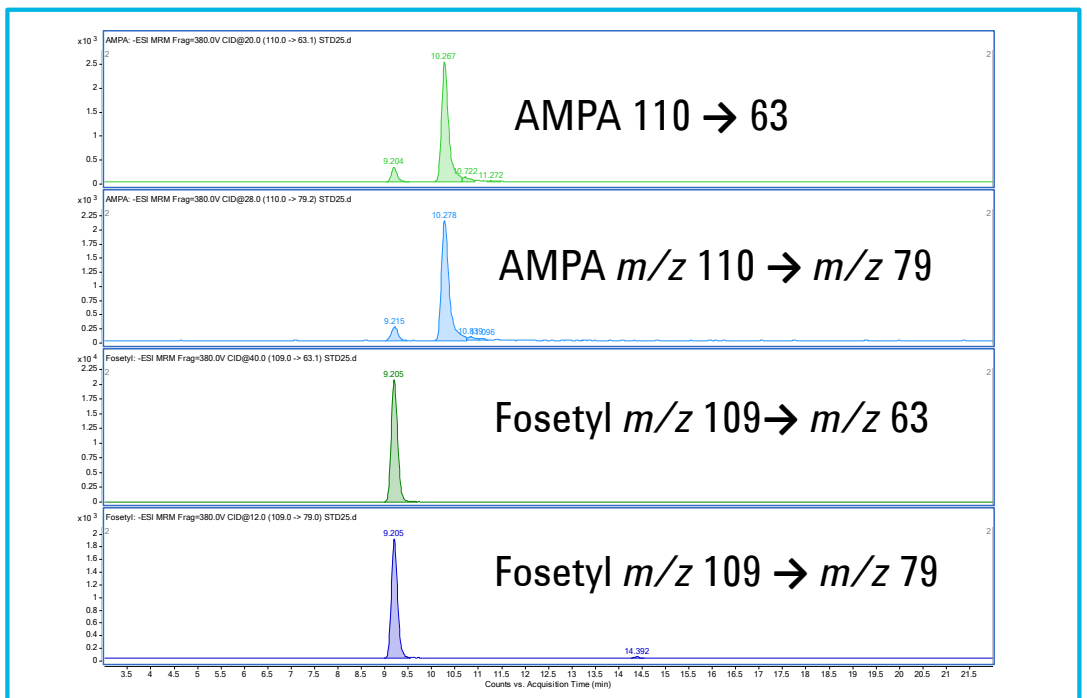


Figure 4. Transitions for AMPA and fosetyl.

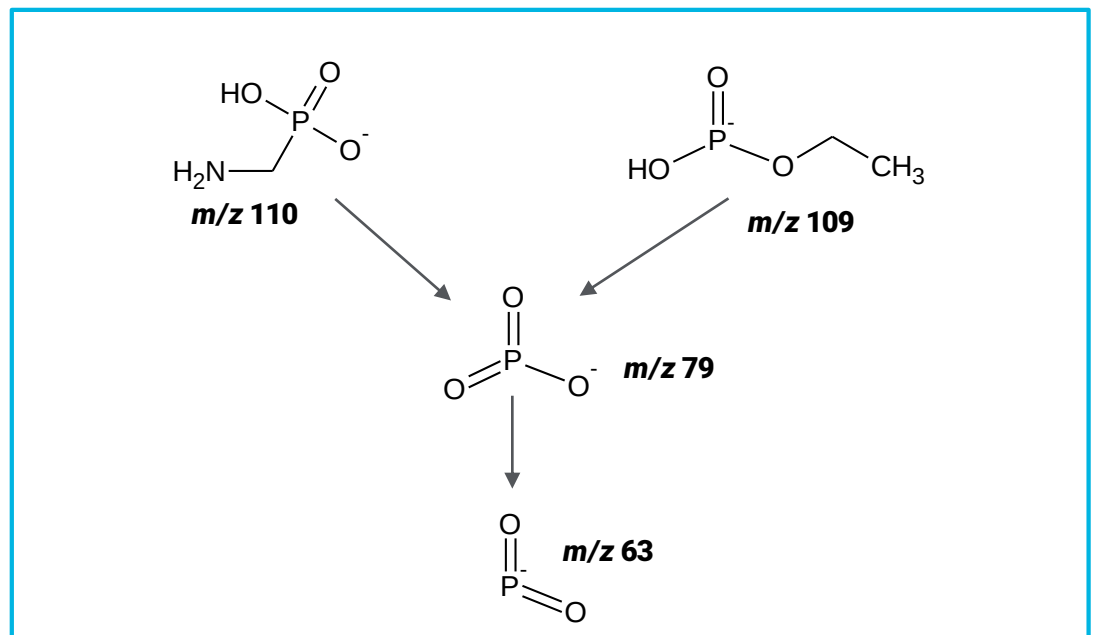


Figure 5. Fragmentation of AMPA and fosetyl.

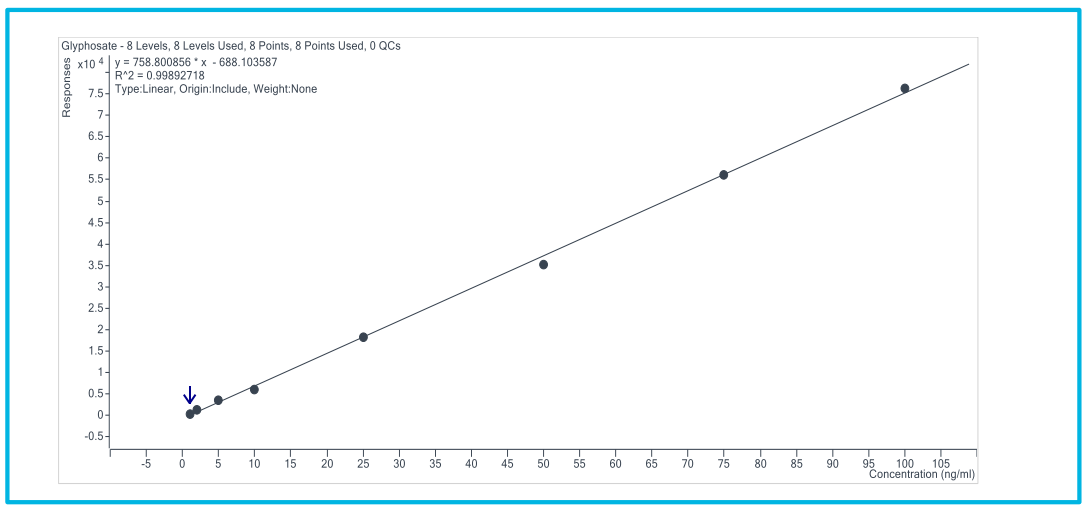


Figure 6. Calibration curve for glyphosate from 1 to 100 ppb

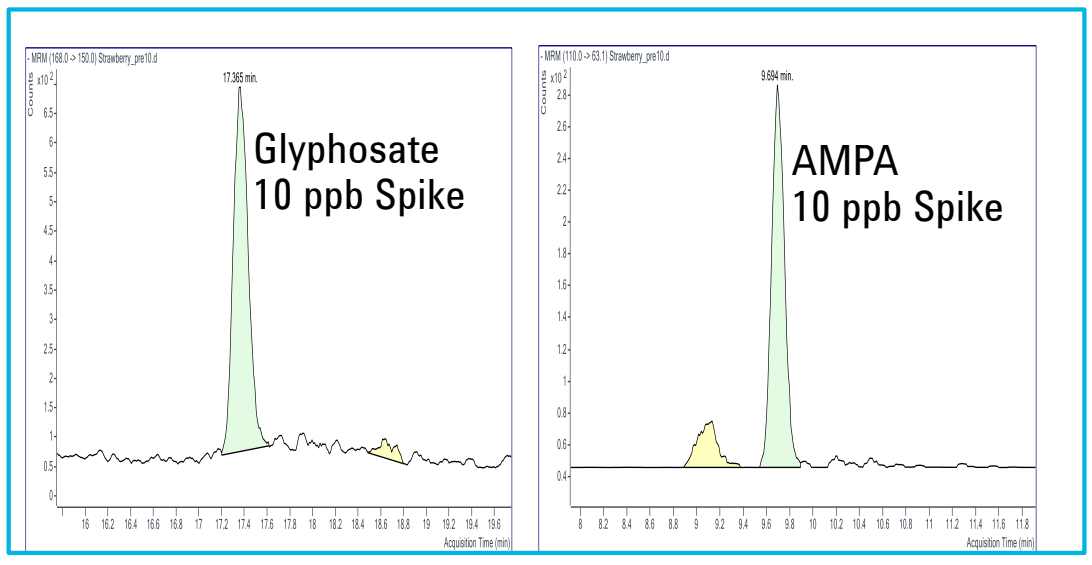


Figure 7. Quantitation transitions for glyphosate and AMPA extracted from strawberry at 10 ppb

Conclusions

Method for direct analysis of polar pesticides provides good recovery and detection for high water content low protein foods

- Not shown but recovery for strawberry, orange, bell pepper, spinach, and peach is greater than 80%. Corn is about 50%.
- Using all PEEK from injection loop to spray provides repeatable results even though sample and standard solvent impact chromatography, QuPPE extraction provides good reproducibility among different foods
- LOQs for high water content foods is below 10 ppb

References

1. Anastassiades, M., et al. "Quick method for the analysis of numerous highly polar pesticides in foods of plant origin via LC-MS/MS involving simultaneous extraction with methanol (QuPPe-Method)." EU Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM) (2016).

2. Goodwin, Lee, et al. "Tandem mass spectrometric analysis of glyphosate, glufosinate, aminomethylphosphonic acid and methylphosphinicopropionic acid." Rapid communications in mass spectrometry 17.9 (2003): 963-969.

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