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Improved Multi-Analyte Method for the Underivatized Analysis of Anionic Pesticides in Food by LC-MS/MS

Benjamin Wuyts,[1] Euan Ross,[1] Simon Hird,[1] Dimple Shah[2]
[1]Waters Corporation, Wilmslow, UK
[2]Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Underivatized analysis of anionic polar pesticides in food
- Excellent sensitivity and precision
- Satisfactory chromatographic performance on routine LC-MS/MS system
- Accurate quantitation of residues in the absence of isotopically labeled internal standards

WATERS SOLUTIONS

Xevo® TQ-XS Triple Quadrupole Mass Spectrometry

ACQUITY® UPLC® H-Class Bio System

MassLynx® MS Software

TargetLynx™ XS Application Manager

KEYWORDS

TQ MS, polar pesticides, food safety, underivatized, MRL

INTRODUCTION

Pesticides are used to protect crops from infestation by pests and plant diseases before and after harvest. They provide multiple benefits to consumers and producers alike. However, a possible consequence of pesticide use may be the presence of residues in food. Where pesticides are approved for use, Maximum Residue Levels (MRLs) have been set at the highest level of pesticide that the relevant regulatory body would expect to find in that crop when it has been treated in line with good agricultural practice. In the European Union (EU) a default MRL, equal to the limit of quantification (LOQ) achievable with analytical methods used for MRL enforcement, is applicable for pesticide/commodity combinations not explicitly mentioned in the MRL legislation.

Expanding on previous work,[1] this application note describes the quantitative determination of an extended suite of eight anionic polar pesticides, which are not amenable to conventional reverse-phase chromatography by LC-MS/MS. A hydrophilic interaction chromatography (HILIC)-based analytical column was used without the need for derivatization or specialty ion chromatography equipment.

EXPERIMENTAL

UPLC conditions

UPLC system:	ACQUITY UPLC H-Class Bio
Column:	Shodex HILICpak VT-50 2D 5 µm, 2 x 150 mm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	0.2 mL/min
Mobile phase A:	68: 12: 20 Water: 45 mM ammonium bicarbonate: acetonitrile
Mobile phase B:	50 mM Ammonium bicarbonate
Wash:	Acetonitrile
Purge:	80: 20 Water: methanol

Gradient method:

Time	%A	%B
0.00	100	0
6.50	55	45
18.00	0	100
21.00	0	100
21.01	100	0
24.00	100	0

MS conditions

MS system:	Xevo TQ-XS
Acquisition mode:	MRM
Ionization mode:	ESI–
Source temp.:	150 °C
Capillary voltage:	2.4 kV
Cone gas flow:	300 L/Hr
Desolvation temp.:	600 °C
Desolvation gas flow:	1000 L/Hr
Nebulizer:	7 bar

Table 1. MRM transitions and parameters for the anionic polar pesticides.

Compound	Transitions	Cone voltage (V)	Collision energy (eV)
Glyphosate	**167.85>62.85** 167.85>80.85	30	16 15
AMPA	**109.85>62.85** 109.85>80.85	30	15 15
Glufosinate	**179.9>62.85** 179.9>84.85	30	25 16
Ethephon	**142.85>106.8** 142.85>78.8	20	10 15
Fosetyl-al	**108.85>62.85** 108.85>80.8	20	15 10
Phosphonic acid	**80.8>78.8** 80.8>62.8	20	14 12
Chlorate	**82.8>66.8** 84.8>68.9	25	15 15
Maleic hydrazide	**110.85>81.85** 110.85>54.9	20	15

*Quantitative transition in **bold**.*

Sample extraction

Analytical standards, as detailed in Table 1, were purchased from Sigma Aldrich. All food samples (tomato juice, apple juice, and beer) were purchased from local retail outlets. Samples and standards were prepared in accordance with the QuPPe v9 method.[2] All data were acquired and processed using MassLynx MS Software.

RESULTS AND DISCUSSION

Various mobile phase compositions and gradients were evaluated, and MRM transitions were selected and conditions optimized. The conditions detailed in the Experimental section provided the best overall performance, in terms of sufficient retention, peak shape, practical run time, and for separation of the critical isobaric pairs: AMPA/fosetyl and fosetyl/phosphonic acid, as discussed in the EURL's QuPPe method.

A common challenge encountered when analysing polar pesticides such as glyphosate is peak shape reproducibility. There are several factors that can affect peak shape such as matrix effects, secondary interactions with column stationary phases, and interactions with metal ions (e.g. calcium, manganese and iron). Tailing peaks, as shown in Figure 1A, are a frequent occurrence, observed in many previously published methods for these analytes.[2] The combination of the ACQUITY H-Class Bio and newly developed method described in this application note provided Gaussian peak shapes (shown in Figure 1B) for the tested matrices, thus increasing sensitivity and reproducibility.

To evaluate the performance of the method, all food samples were spiked at three concentrations (0.01, 0.05, and 0.1 mg/kg) prior to extraction using the QuPPe method and replicate injections were made to provide information on sensitivity, accuracy, and precision of measurements and overall robustness.

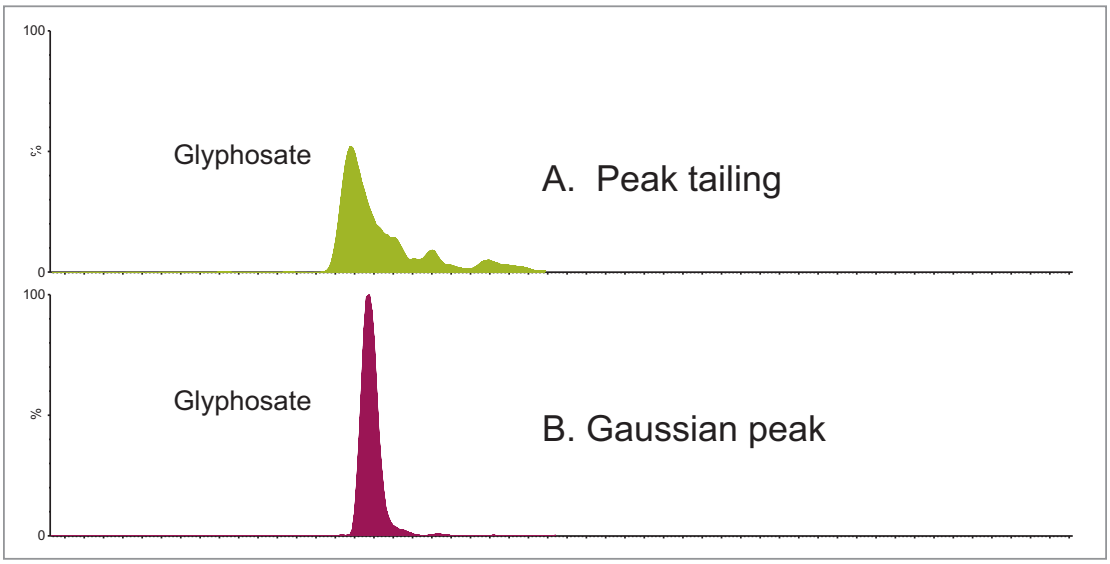


Figure 1. An example of chromatographic peak performance observed is shown for glyphosate, where significant peak tailing (A) was reduced by cleaning column and LC (B). Gaussian peak shape was maintained over long analytical runs using the inert ACQUITY UPLC H-Class Bio System.

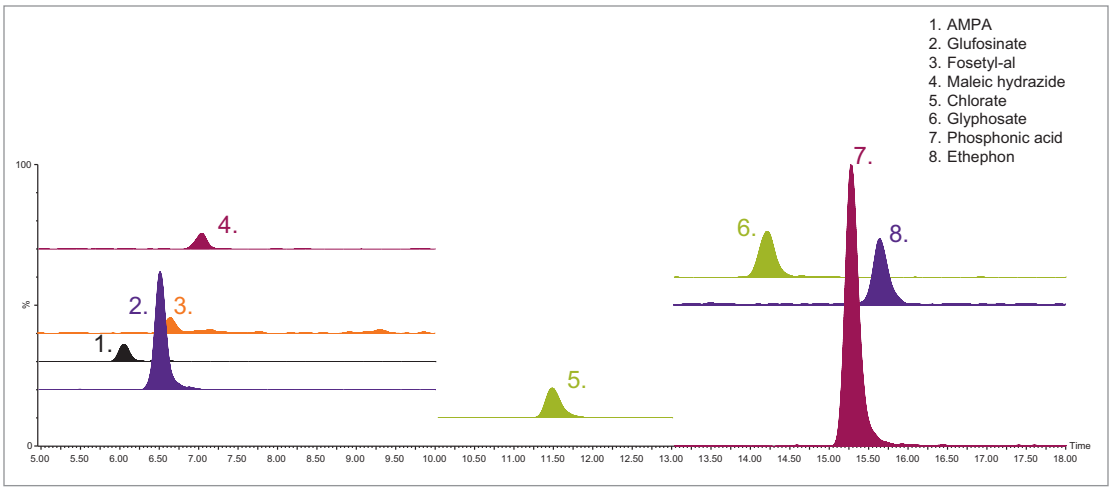


Figure 2. Example of chromatographic separation of anionic pesticides, spiked to 0.01 mg/kg in beer, plotted relative to the most abundant intensity. Due to incurred residues phosphonic acid detected, the spiked analytes appear as low traces and so are offset.

An example of the chromatographic performance and sensitivity observed from analysis of a beer sample spiked at 0.01 mg/kg is shown in Figure 2. The orange trace in Figure 3 shows concurrent RADAR™ acquisition (ESI-, full scan *m/z* 50–300) for the chromatography of apple juice matrix over the entire LC run. This RADAR scan illustrates the complexity of the injected extract, even for this relatively simple commodity, where the y-axis for the RADAR full scan and MRMs are not linked due to the significant intensity of co-extractives.

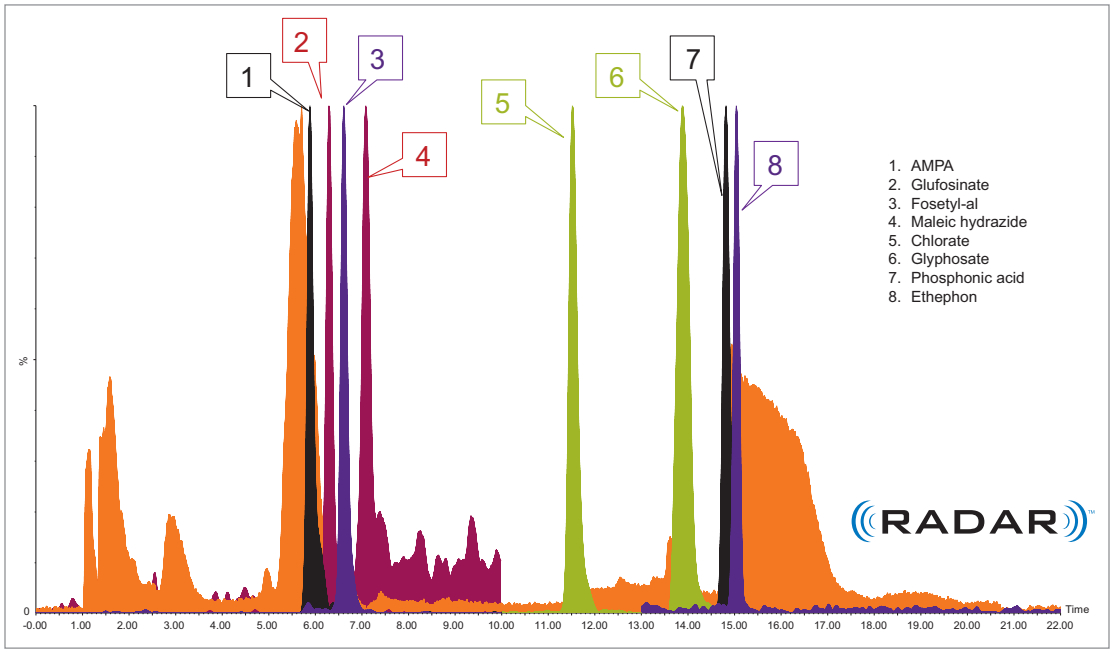
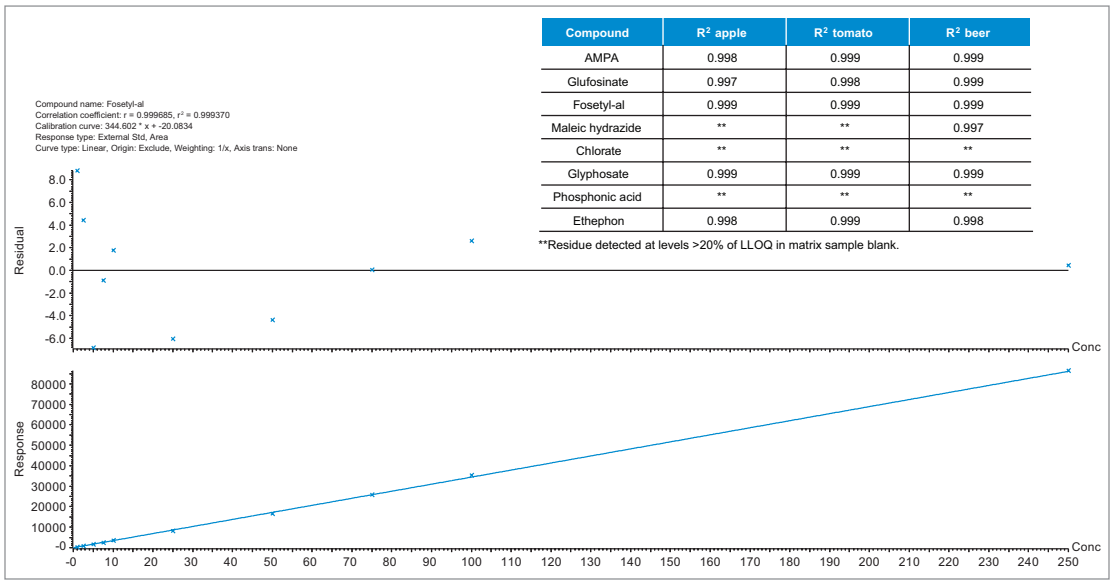


Figure 3. Example of the chromatographic separation of anionic pesticides, spiked to 0.01 mg/kg in apple. The orange trace shows a full scan (RADAR) acquired throughout the full chromatographic method illustrating background contamination from matrix.

The response for all eight anionic polar pesticides were linear over the range investigated (0.001 to 0.25 mg/kg) with good residuals, even in the absence of isotopically labeled internal standards (Figure 4). Due to presence of incurred residues of maleic hydrazide, chlorate, and phosphonic acid detected in some samples used as blanks, an offset on the X-axis for their matrix-matched calibration graph was observed. The concentration of these incurred residues was determined in samples of apple juice, tomato juice, and beer by standard addition. An example is shown in Figure 5, where phosphonic acid was quantified using the standard addition processing functionality within TargetLynx XS.



Compound	R^2 apple	R^2 tomato	R^2 beer
AMPA	0.998	0.999	0.999
Glufosinate	0.997	0.998	0.999
Fosetyl-al	0.999	0.999	0.999
Maleic hydrazide	**	**	0.997
Chlorate	**	**	**
Glyphosate	0.999	0.999	0.999
Phosphonic acid	**	**	**
Ethephon	0.998	0.999	0.998

**Residue detected at levels >20% of LLOQ in matrix sample blank.

Figure 4. Example of calibration curve prepared in beer over a range of 0.001 to 0.25 mg/kg, where all residuals are <15%. Inset shows a summary table with the minimum R^2 achieved for each analyte in all calibration curves, where all residuals were <25%.

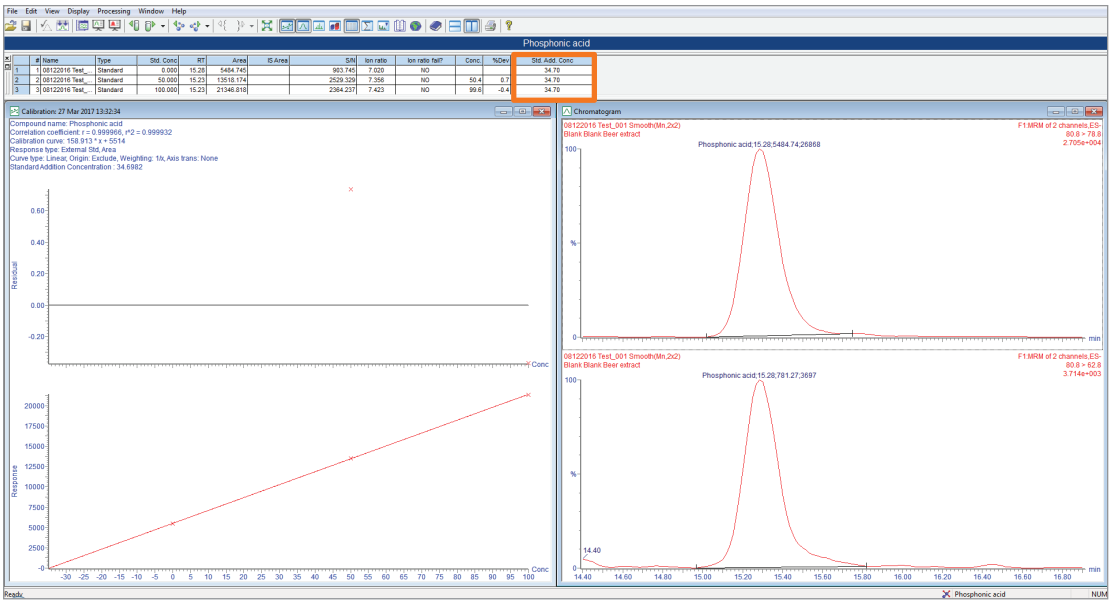


Figure 5. Example of standard addition calibration, accurately quantifying and confirming the residue detection of phosphonic acid.

Table 2. LLOQs calculated (S/N>10) for each of the commodities.

	Apple juice (mg/kg)	Tomato juice (mg/kg)	Beer (mg/kg)
Glyphosate	0.00004	0.0003	0.0001
AMPA	0.0003	0.0001	0.0001
Glufosinate	0.0001	0.0004	0.0002
Ethephon	0.0007	0.0025	0.0007
Fosetyl-al	0.0002	0.0008	0.0001

The method's accuracy and precision were determined by analyzing the spikes (n=9). Excellent recovery and precision were observed for five of the eight analytes at all three concentrations (Figure 6), but the presence of incurred residues in the blanks limited the results for maleic hyrazide to 0.05 and 0.1 mg/kg in beer and prevented any results being reported for chlorate and phosphonic acid or maleic hyrazide in the other commodities.

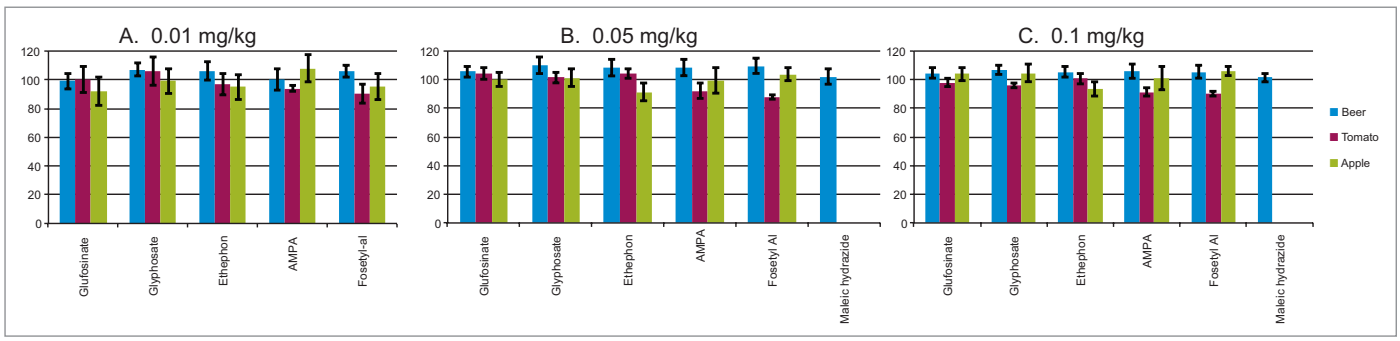


Figure 6. Recoveries and precision (n=9) for each analyte in a selection of foods at A. 0.01 mg/kg; B. 0.05 mg/kg and C. 0.1 mg/kg. Residues >0.01 mg/kg were detected for maleic hydrazide, chlorate, and phosphonic acid and so are omitted from this data.

CONCLUSIONS

This method has been developed for the direct analysis of a selection of anionic polar pesticides across a variety of foods. Utilizing routine LC and MS/MS technologies, a robustly sensitive method has been established that achieves excellent levels of sensitivity, relative to the enforced MRLs. In the absence of costly deuterated or isotopically labeled internal standards, accurate quantitation of residues in foods was readily achieved by standard addition, in compliance with SANTE guidelines 11495/2015.

References

1. B Wuyts, D Shah, E Ross, J Fox, E McCall. Highly Sensitive Analysis of Polar Pesticides in Food. Waters Technology Brief No. 720005822en. October, 2016.
2. M Anastassiades 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). EURL-SRM. Version 9.2, October, 2016.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com