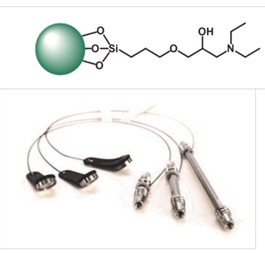
OVERCOMING THE CHALLENGES OF ANALYSING IONIC POLAR PESTICIDES IN FOOD

Benjamin Wuyts[1], Euan Ross[1], Dimple Shah[2], Simon Hird[1], Gareth Cleland[2]
[1] Waters Corporation, Wilmslow, SK9 4AX, UK; [2] Waters Corporation, Milford, MA 01757, U.S.A.

INTRODUCTION

Glyphosate, a non-selective broad spectrum herbicide, accounts for more than half of global herbicide sales. While discussions on the toxicological concerns of glyphosate and associated compounds continue, maximum residue limits (MRLs) are enforced globally, requiring continued analytical testing to ensure consumer safety. In previous work, various methodologies have been presented, looking at underivatised options for the direct analysis of polar pesticides in food. [Scan the QR code below to discover more] Here, the novel application of the Waters' Torus DEA column is presented, showing the improved performance of a UPLC-MS/MS method for the underivatised analysis of glyphosate and a selection of other anionic pesticides. Separation is achieved under Hydrophilic Interaction Chromatography (HILIC), after a simple column conditioning to activate the mechanism. Preliminary method performance, in the absence of isotopically labelled internal standard is summarised, in accordance with relevant SANTE 11945/2015 guidelines. [1]



METHODS

Samples of onion and spinach, labelled as organic, were purchased from retail outlets, homogenised and extracted using the EURL Quick Polar Pesticides Extraction method. [2]

Liquid chromatography

LC system:	ACQUITY UPLC I-Class
Column:	Torus DEA 2.1 x 100 mm
Mobile phase A:	50 mM ammonium formate pH 2.9
Mobile phase B:	0.9% formic acid in acetonitrile
Strong Wash:	10:90 acetonitrile : water
Weak Wash:	90:10 acetonitrile : water
Column temperature:	50 °C
Sample temperature:	10 °C
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Runtime:	20 minutes

Mass Spectrometry

MS system:	Xevo TQ-XS
Ionisation mode:	ESI negative
Capillary:	2.5 kV
Desolvation temp.:	600°C
Desolvation gas flow:	1000 L/hr
Source temp.:	150 °C
Acquisition:	MRM with at least 2 transitions per compound. Primary transition reported in Figure 1.

RESULTS AND DISCUSSION

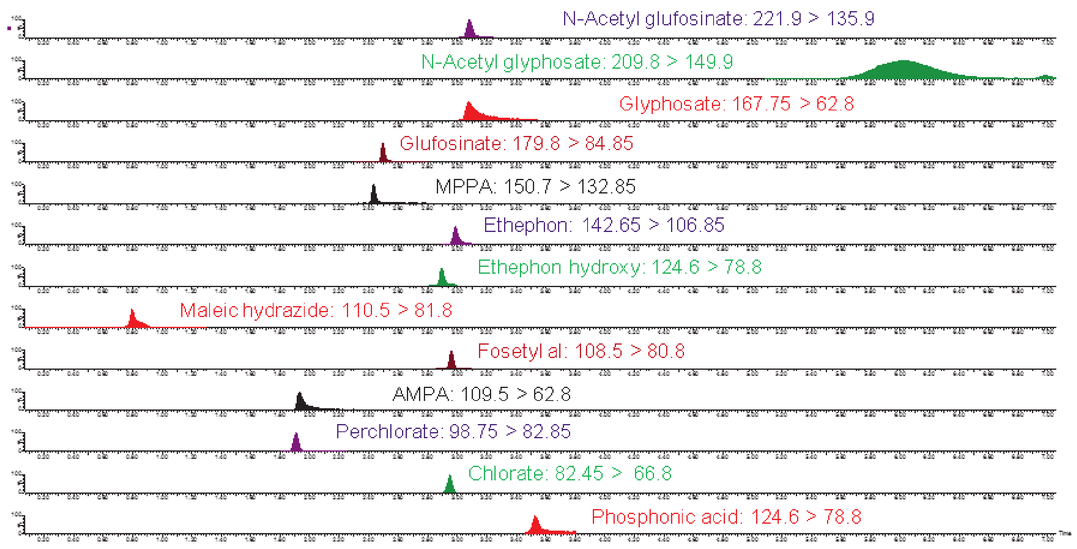


Figure 1: Example of chromatographic performance at 0.05 mg/L for the 13 analytes in extraction solvent.

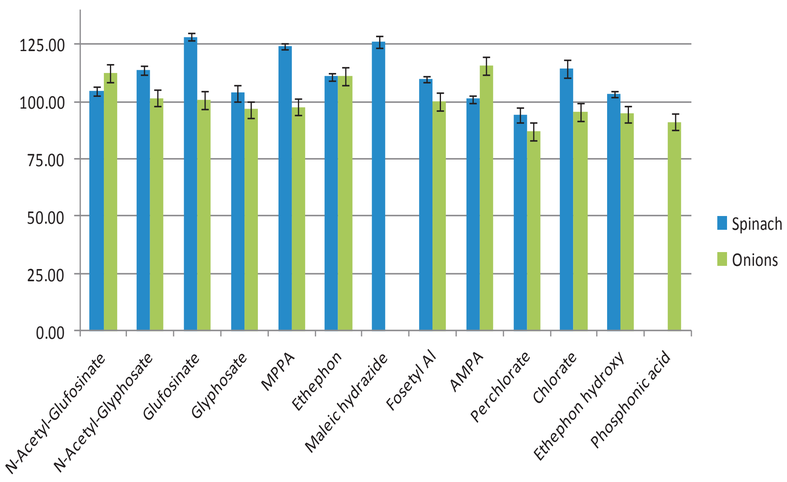


Figure 2: Summary of recoveries and repeatability achieved for QuPPe extracts run, spiked to 0.01 mg/kg in onion (n=5) and spinach (n=5).

Both food samples, spinach and onion were spiked to 0.01 mg/kg and extracted in accordance with the QuPPe method.

Five replicates were prepared for each sample. The recoveries and repeatability achieved is summarised in Figure 2, where excellent method performance is shown. Due to incurred residues detected in the samples, maleic hydrazide in onion and phosphonic acid in spinach, recoveries have been omitted.

For the remaining 11 analytes the recoveries, which were calculated against matrix matched calibration curve, fell within 80 and 125 %. Repeatability, reported as %RSD, was less than 20% for all 13 analytes across the 2 matrices.

Linearity of the 13 analytes was determined in solvent and matrix matched calibration curves. Excellent performance was demonstrated in solvent, over the range of 0.0005 to 0.2 mg/L for all analytes, where residuals were < 20%. Similar performance was observed in both spinach and onions matrix (residuals < 22%).

However, due to the presence of incurred residues in the samples, standard addition calibration curves were generated in TargetLynx XS to reliably quantify the maleic hydrazide and phosphonic acid residues in the absence of isotopically labelled internal standard. An example is shown in Figure 3, where an incurred residue of maleic hyrdazide was quantified at 0.072 mg/kg in onion.

All external calibration curves were also used to evaluate matrix effects, reported in Figure 4 and 5. Comparing the slope of all curves, a value > 100% signifies ion enhancement and < 100 % signifies suppression of ions due to matrix interferences. Acquiring a RADAR scan (full MS scan) simultaneously to the MRMs, provides additional information on matrix and background ions, as shown in Figure 4.

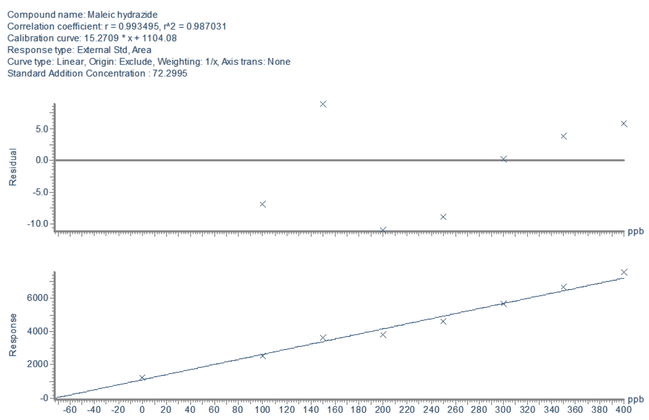


Figure 3: Standard addition plot quantifying incurred residue of maleic hydrazide in onion to 0.072 mg/kg

MATRIX EFFECTS

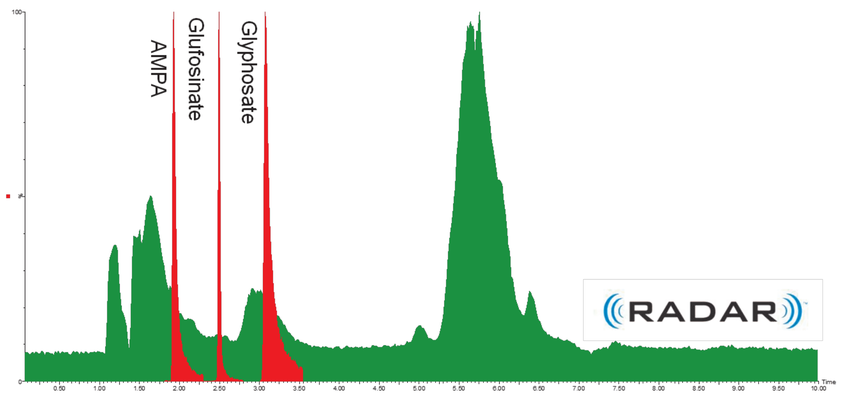
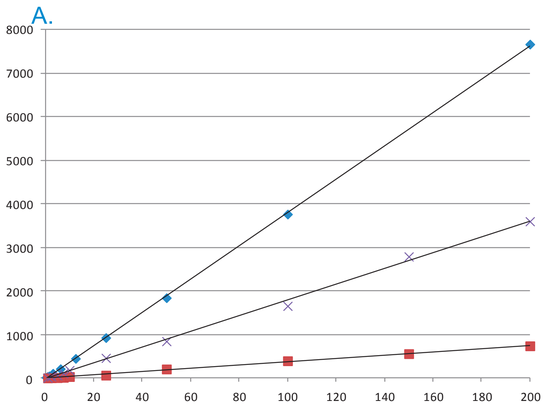


Figure 4: RADAR full scan acquisition showing the complexity of matrix ionised, which can impact ionisation efficiency and matrix effects of the analytes of interest.



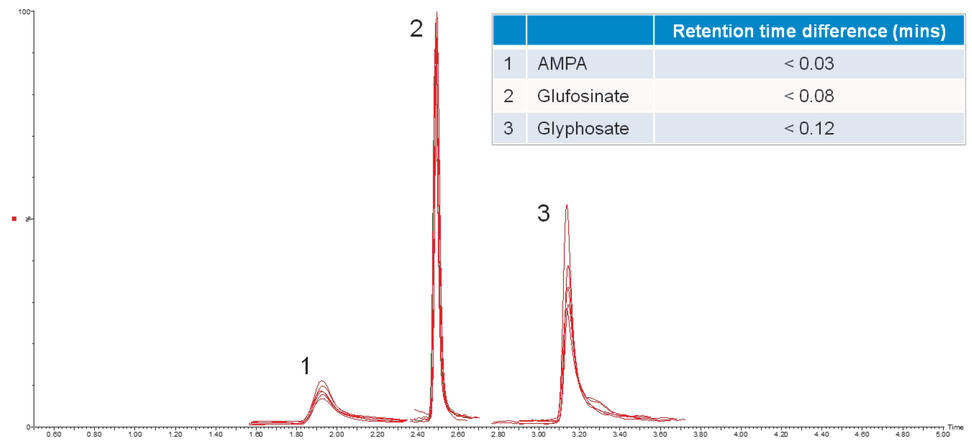
B.

	Onion (%)	Spinach (%)
N-Acetyl-Glufosinate	101	233
N-Acetyl-Glyphosate	105	370
Glufosinate	96	99
Glyphosate	109	104
MPPA	339	488
Ethephon	131	287
Maleic hydrazide	14	9
Fosetyl Al	100	107
AMPA	10	47
Perchlorate	59	494
Chlorate	278	245
Ethephon hydroxy	131	156
Phosphonic acid	149	151

Figure 5: Matrix effects determined by comparing the slopes of matrix matched and solvent calibration curves.
A. An example of AMPA curves show significant suppression by both matrices.
B. Table summarising enhancement (>50%) and suppression (<50%) for all analytes in onion and spinach.

TORUS DEA INTER-BATCH REPEATABILITY

Six batches of Torus DEA columns were evaluated to determine repeatability of the method across batch lots, focussing on AMPA, glyphosate and glufosinate. Replicate injections (n=6) were made on all 6 columns. Representative results are summarised in Figure 6, where one injection per column is overlaid. Excellent reliability was achieved for all injection across the six batches, in terms of retention time (< 0.12 minute difference) and peak area (%RSD < 20%).



		Retention time difference (mins)
1	AMPA	< 0.03
2	Glufosinate	< 0.08
3	Glyphosate	< 0.12

Figure 6: Excellent interbatch repeatability (%RSD $_{\text{retention time}}$ ≤ 2.2 % and %RSD $_{\text{peak area}}$ < 20 %) shown for AMPA, glufosinate and glyphosate, where 0.1 mg/L standards, acquired on 6 different Torus DEA column batches are overlaid.

CONCLUSIONS

- **Expanding on previous LC-MS/MS methods, initial work using the Torus DEA column has demonstrated excellent performance for the reliable analysis of anionic polar pesticides in food.**
- **Key benefits include:**
  - ✓ **Improved chromatographic performance for a broad scope of anionic pesticides in a single injection.**
  - ✓ **Maintained system sensitivity with LODs < 0.001 mg/kg for all analytes.**
  - ✓ **Repeatable quantitative analysis, with %RSD < 22 % achieved at 0.01 mg/kg in onion and spinach, without isotopically labelled internal standards.**
  - ✓ **Excellent repeatability across 6 batches of Torus DEA columns, where retention times were < 0.05 minute intra-batch and < 0.12 minute inter- batches for AMPA, glufosinate and glyphosate.**
  - ✓ **Incurred residues of analytes accurately quantified using standard addition calibration, in the absence of isotopically labelled internal standard.**

MORE INFORMATION

Scan the QR code to find material referenced in this poster or simply visit

www.waters.com/polarpesticides

Share your thoughts or follow us on

#PolarPesticides

References

1. www.eurl-pesticides.eu/docs/public/tmplt_article.asp? CntID=887&LabID=200&Lang=EN
2. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_11945.pdf

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS