DIRECT ANALYSIS OF GLYPHOSATE AND OTHER POLAR PESTICIDES IN VARIOUS FOOD MATRICES

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INTRODUCTION

A new, sensitive method has been developed to demonstrate the applicability of analyzing a panel of eight polar pesticides without the need for derivatization in a single liquid chromatography, tandem quadrupole mass spectrometry method. Targeted compounds include aminomethylphosphonic acid (AMPA), chlorate, ethephon, fosetyl aluminium, glufosinate, glyphosate, maleic hydrazide and phosphonic acid. Extracts of various foodstuffs were prepared in accordance with the Quick Polar Pesticides Method (QuPPe)[1]. Chromatographic separation was achieved on a hydrophilic interaction liquid chromatography (HILIC) column, applying a mobile phase gradient with ammonium bicarbonate. Targeted multi-reaction monitoring (MRM) methods were used to detect and quantify residues, with a minimum of two transitions per compound to meet identification criteria. The developed method was found to give retention of all analytes greater than two times the void volume. Overall method performance, in the absence of isotopically labeled internal standard (IS), was evaluated by assessing linearity, accuracy and sensitivity.

METHODS

SAMPLE PREPARATION

Analytical standards, were purchased from Sigma Aldrich. All samples and standards were prepared in accordance with the QuPPe v9.2 method[1].

All food samples (tomato, apple juice and beer) were purchased from local retail outlets.

MS CONDITIONS:

Mass spectrometer:	Xevo® TQ-XS
Ionisation Mode:	ESI -
Acquisition mode:	MRM
Capillary voltage:	2.4 kV
Source temp:	150 °C
Desolvation temp:	600 °C
Desolvation gas flow:	1000 L/Hr
Cone gas flow:	30 L/Hr
Data management:	MassLynx® v4.2, TargetLynx XS

Table 1: Overview of MS/MS transitions for the analytes of interest, where the transition in bold font was used as the quantifier trace.

	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 > 78.8 **142.85 > 106.8**	20	15 10
Fosetyl aluminium	**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 hydrazyde	110.85 > 54.9 **110.85 > 81.85**	20	15 15

UPLC CONDITIONS:

System:	ACQUITY® UPLC® H-Class Bio
Column:	Shodex HILICpak VT-50 2D (2 x 150 mm, 5 µm)
Mobile phase: A:	68: 12: 20 water: 45 mM ammonium bicarbonate: acetonitrile
Mobile phase: B:	50 mM ammonium bicarbonate
Needle wash:	Acetonitrile
Purge:	80: 20 water: methanol
Seal wash:	10:90 methanol: water
Injection vol:	10 µL
Column temp:	40 °C
Sample temp:	10 °C
Flow rate:	0.2ml/min
Gradient tablet:	

Time	% A	% B
Initial	100	0
6.5	55	45
18.0	0	100
21.0	0	100
21.1	100	0
24.0	100	0

RESULTS AND DISCUSSION

Method performance: An example of the chromatographic separation for all 8 analytes spiked into apple juice (100% pressed) is shown in **Figure 1**, at 0.01 mg/kg and prepared as per the QuPPe v9.2 method. The red trace shows the background matrix acquired as RADAR™ (full scan m/z 50 to 300) throughout the entire LC run acquired under ESI negative. This RADAR scan shows the complexity of sugars and other co-extractives in the matrix, while still achieving the required quantitative MRM information. The baseline separation of AMPA, phosphonic acid and fosetyl aluminum was achieved through mobile phase optimization. Baseline separation for these compounds is important to avoid possible interferences, as discussed in the EURL's QuPPe v9.2 method[1].

To evaluate the method's performance, all studied food samples were spiked at 0.01, 0.05 and 0.1 mg/kg level prior to extraction and prepared with the QuPPe v9.2 method[1]. Excellent recovery and precision was achieved for 6 out of the 8 analytes and these results are shown in, **Figure 2 (A., B. and C.)**. However, due to incurred residues of chlorate and phosphonic acid detected above 10 % of the 0.01 mg/kg spike in beer, apple and tomato, along with maleic hydrazide in apple and tomato juice, the accuracy could not be readily determined. These results have, therefore been omitted from the summarised results. Due to the poor response achieved for maleic hyrazide by LC-ES-MS/MS , also stated in the QuPPe v9.2 method[1], recovery data at the 0.01 mg/kg is also omitted from the results, while excellent recovery and repeatability is reported for 0.05 and 0.1 mg/kg in beer.

The Xevo TQ-XS showed high sensitivity for the pesticides, demonstrated by LLOQ values (S/N = 10) below 1 ng/mL (**Table 2**), in all matrices (except for ethephon in tomato juice, LLOQ = 2.5 ng/mL).

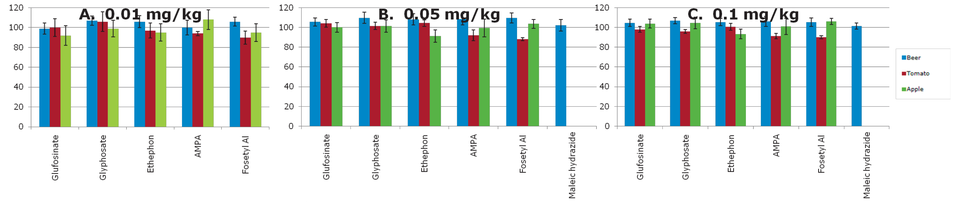

Figure 2 showing recoveries and precision (n=9) for each analyte in a selection of foods at A. 0.01 mg/kg ; B. 0.05 mg/kg and C. 0.1 mg/kg.

Quantitation and identification of residues: Excellent linearity (R^2> 0.997, residuals < 20%) was achieved for all analytes over an appropriate working range (0.001 to 0.25 mg/kg), in the absence of internal standard (IS). An example of this calibration is shown for fosetyl aluminium in **Figure 3**.

In the absence of IS, alternative calibration types were investigated to provide accurate quantification of incurred residues. Using standard addition calibration, incurred residues of phosphonic acid and chlorate were quantified in samples of apple juice, tomato juice and beer. An example of this calibration is shown in **Figure 4**. The standard addition processing functionality within TargetLynx XS calculated the concentration of phosphonic acid to be 34.7 mg/kg in beer. The data meet the acceptance criteria in SANTE guidelines 11495/2015[2] (at least 2 transitions acquired, ion ratio ±30% and retention time ±0.1%), as can be seen in **Figure 4**.

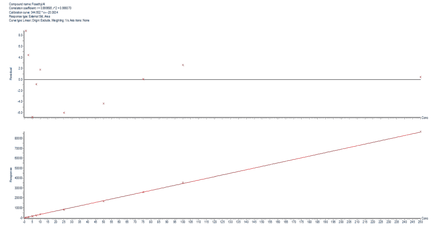

Figure 3: Example of calibration curve prepared in beer over a range of 0.001 to .25 mg.kg^{-1}, where all residuals are < 15 %..

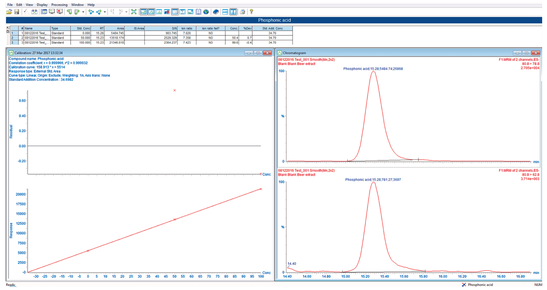

Figure 4: Example of standard addition calibration, accurately quantifying and confirming the residue detection of phosphonic acid in beer.

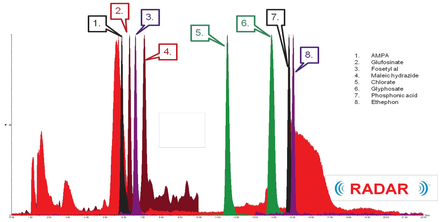

Figure 1. Example of the chromatographic separation of anionic pesticides, spiked to 0.01 mg/kg in apple juice. The red trace shows a full scan (RADAR) acquired throughout the full chromatographic method showing background contamination from matrix.

Table 2: Calculated LLOQs (S/N = 10) for each matrix, tested as part of the method performance evaluation.

	Apple juice LOQ (µg/kg)	Tomato juice LOQ (µg/kg)	Beer LOQ (µg/kg)
Glyphosate	0.04	0.3	0.1
AMPA	0.3	0.1	0.1
Glufosinate	0.1	0.4	0.2
Ethephon	0.7	2.5	0.7
Fosetyl Aluminium	0.2	0.8	0.1

CONCLUSIONS

- **This method has been developed for the underivatised analysis of eight anionic polar pesticides across a variety of foods.**
- **Utilising UPLC and MS/MS technologies, a robust and sensitive method has been developed**
- **Excellent levels of sensitivity, relative to the enforced MRLs, have been demonstrated**
- **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) http://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

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