アプリケーションノート

Determination of Anionic Polar Pesticides in Wheat Flour Extracts Using UPLC-MS/MS with the Torus DEA Column

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Abstract

The use of the Torus DEA Column provides excellent chromatographic performance for anionic polar pesticides and metabolites, even for the analysis of a complex and difficult matrix such as wheat flour. In this application note, we report the performance data from an assessment of the slightly modified LC-MS/MS method for the determination of relevant anionic polar pesticides in wheat flour extracts, representative of commodities with high starch and/or protein content, low water and fat content.

Benefits

This method is suitable for the determination of a range of polar anionic pesticides in wheat flour extract to facilitate monitoring of MRL/tolerance compliance. The method offers excellent chromatographic retention, selectivity, peak shape, and stability coupled with sufficient sensitivity to determine residues at concentrations as low as 0.01 mg/kg (10 ppb) levels in crude extracts without cleanup.

Introduction

Although various multi-residue LC-MS/MS methods are available to analyze food for pesticide residues, polar, anionic pesticides and their metabolites remain a considerable challenge. The QuPPe (Quick Polar Pesticides) method[1] allows the simultaneous extraction of many of these highly polar compounds. QuPPe is typically used with LC-MS/MS instruments offering high sensitivity in order to deal with the significant matrix effects associated with the crude extracts (no cleanup).

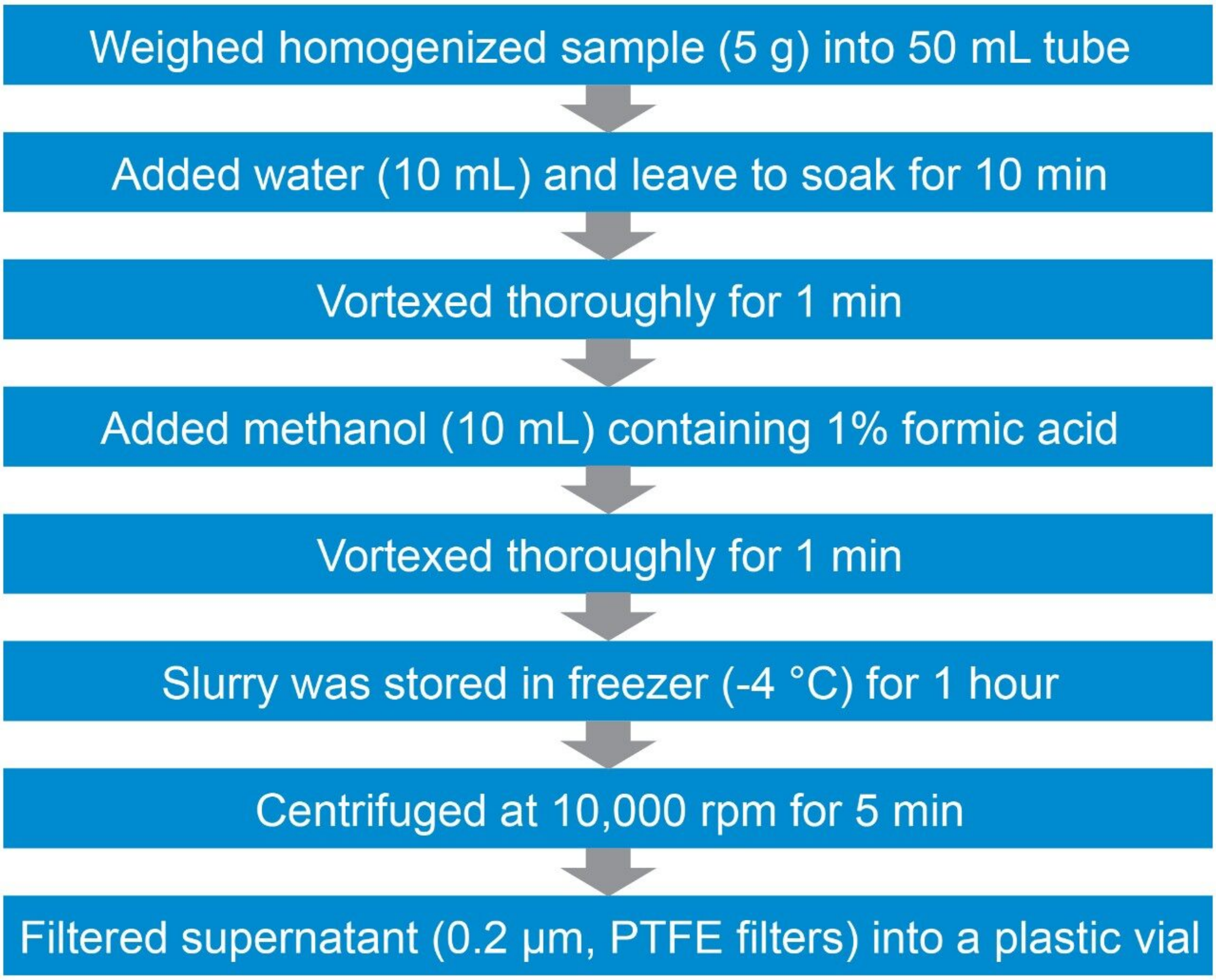
Previously, we have reported the results of the validation of a method based on QuPPe using Waters Torus DEA Column (p/n: 186007616) for the determination of polar pesticides and their metabolites in spinach.[2] The Torus DEA Column provides HILIC and WAX interactions, which has been shown to offer sufficient retention of these highly polar and ionic compounds while providing excellent retention time stability, selectivity, and peak shape.

In this application note, we report the performance data from an assessment of the slightly modified LC-MS/MS method for the determination of relevant anionic polar pesticides in wheat flour extracts, representative of commodities with high starch and/or protein content, low water and fat content. The previous LC-MS/MS method was developed on I-class. For similar chromatographic performance, method was transfer for H-class. Along with evaluating performance in a more complex and difficult matrix, this work also demonstrates how the method can be transferred to the ACQUITY UPLC H-Class Bio System. This system has the advantage of an inert flow path which reduces the unwanted interactions between some of these anionic analytes and metals in the UPLC system.

Experimental

Sample preparation and extraction

A sample of wheat flour was purchased from a retail outlet and stored frozen. Test portions were extracted using the EURL Quick Polar Pesticides (QuPPe) method,[1] modified by the addition of a freezing out step, prior to centrifugation. The details of the method are summarized in Figure 1.



Weighed homogenized sample (5 g) into 50 mL tube

Added water (10 mL) and leave to soak for 10 min

Vortexed thoroughly for 1 min

Added methanol (10 mL) containing 1% formic acid

Vortexed thoroughly for 1 min

Slurry was stored in freezer (-4 °C) for 1 hour

Centrifuged at 10,000 rpm for 5 min

Filtered supernatant (0.2 µm, PTFE filters) into a plastic vial

Figure 1. Schematic showing the modified Quick Polar Pesticides (QuPPe) method.

The performance of the overall method was assessed as per in accordance with SANTE guidelines.[3] Solutions of matrix-matched standards were prepared over the range 0.005 to 0.250 mg/kg (5 to 250 ppb) and analyzed to determine the concentration of the anionic pesticides and metabolites in replicate injections of the standards at 0.1 mg/kg (using bracketed calibration) to realistically mimic the impact on the instrument of a routine batch of samples. Replicate injections of the 0.1 mg/kg spiked samples (n=15) were run to determine the reproducibility of the LC-MS/MS method.

UPLC conditions

Before use, the LC system and column requires simple cleaning and conditioning steps to remove metal ions that have been shown to interact with polar pesticides and cause poor peak shapes. Details can be found in the Waters Start-Up Guide[4] (p/n: 720006156EN).

UPLC system:	ACQUITY UPLC H-Class Bio with FTN Sample Manager
Column:	Torus DEA 1.7 µm, 2.1 × 100 mm
Mobile phase A:	50 mM Ammonium formate + 0.9% formic acid
Mobile phase B:	Acetonitrile + 0.9% formic acid
Flow rate:	0.5 mL/min
Injection volume:	10 µL
Weak wash solvent:	90:10 acetonitrile:water
Strong wash solvent:	10:90 acetonitrile:water
Column temp.:	50 °C
Sample temp.:	10 °C
Run time:	20 min

Gradient

Time (min)	%A	%B	Curve
0.00	10	90	–
4.00	60	40	2
5.00	90	10	6
8.5	90	10	1
15.5	10	90	1

MS conditions

MS system:	Xevo TQ-XS
Ionization:	ESI
Capillary voltage:	2.5 kV
Ion counting threshold:	250
Desolvation temp.:	600 °C
Desolvation gas flow:	1000 L/Hr
Source temp.:	150 °C
Cone gas flow:	300 L/Hr
Collision gas flow:	0.14 mL/min
Nebulizer gas pressure:	7 Bar

Compound	Retention time (min)	MRM	Cone (V)	CE (eV)	Dwell time (s)
Aminomethyl-phosphonic acid (AMPA)	2.02	**110>63**	35	13	0.125
		110>79	35	14	0.125
3-Methylphosphinico-propionic acid (MPPA)	2.44	**151>133**	20	11	0.050
		151>107	20	14	0.050
Glufosinate	2.58	**180>85**	30	16	0.050
		180>95	30	16	0.050
N-Acetyl glufosinate (NAG)	2.98	**222>136**	30	20	0.020
		222>69	30	15	0.020
Fosetyl aluminium	3.01	**109>81**	20	10	0.010
		109>63	20	16	0.010
Ethephon	3.03	**143>107**	15	7	0.090
		143>79	15	7	0.090
Glyphosate	3.16	**168>63**	25	18	0.120
		168>150	25	9	0.120
Phosphonic acid	3.56	**81>79**	25	11	0.010
		81>63	25	13	0.010
N-Acetyl glyphosate	5.59	**210>150**	25	13	1.100
		210>192	25	9	1.100

Table 1. MRM parameters for anionic polar pesticides (quantitative transitions in bold).

Data was acquired using MassLynx MS Software v.4.2 and processed using TargetLynx XS Application Manager. The selection of MRM transitions and optimization of critical parameters was performed by infusion of individual solutions of each of the analytes and evaluation of the data by IntelliStart Software to automatically create acquisition and processing methods. Soft ionization mode was enabled for ethephon. Soft ionization mode is a function in the MS acquisition file that applies a shallower gradient of voltages to the ion transfer optics and improves transmission. Soft ionization is designed to reduce fragmentation and improve transmission of (fragile) compounds. As shown in Figure 2, increases in sensitivity (2X) and peak area (1.8X) were observed for Ethephon under soft ionization mode which ultimately improves sensitivity.

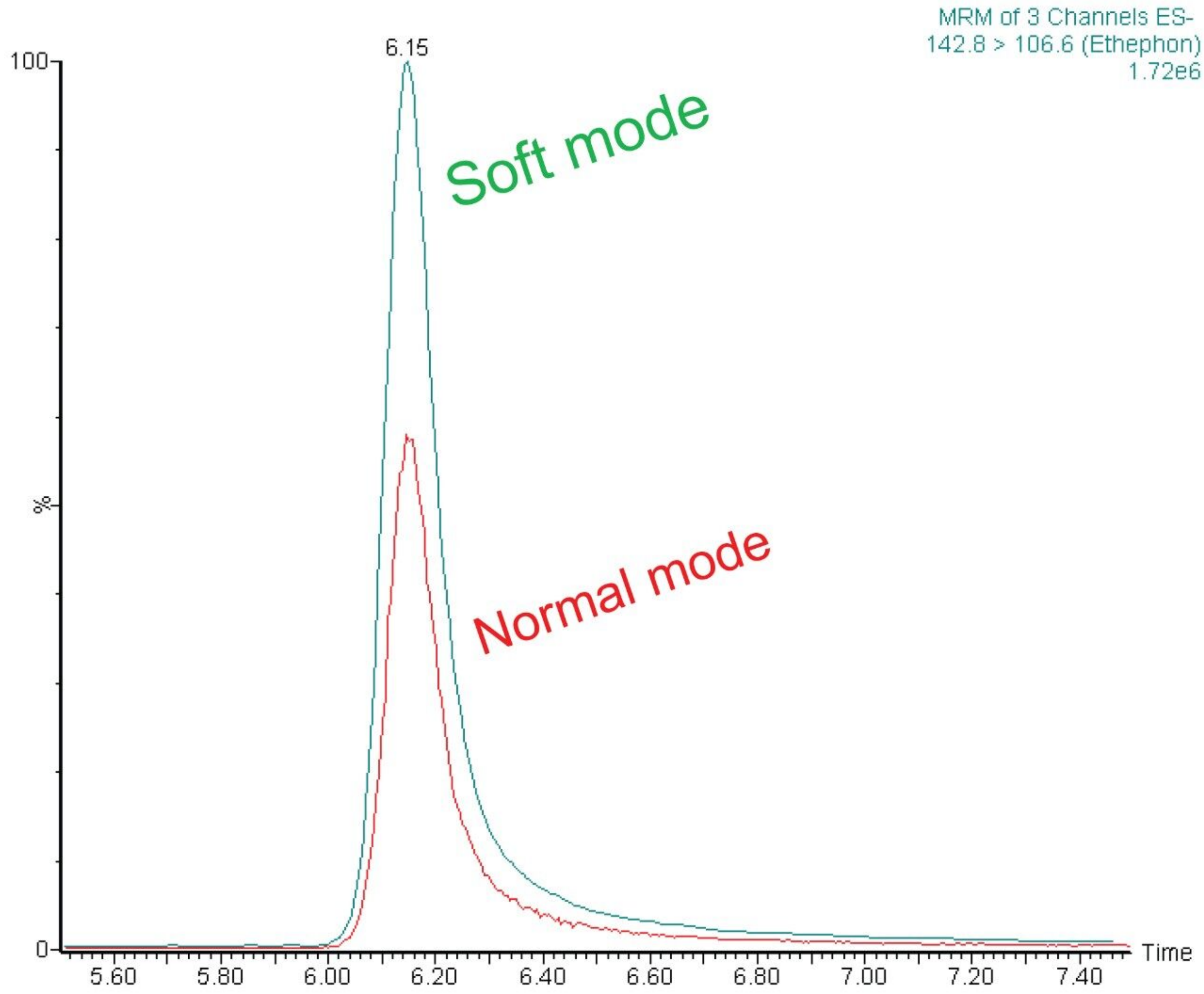


Figure 2. An overlay chromatograms of ethephon with soft ionization mode (green) and normal mode (red). Increased in intensity (2X) and peak area (1.8X) were observed for Ethephon with soft ionization mode.

Results and Discussion

Excellent sensitivity and selectivity was demonstrated from the analysis of matrix-matched standards. Figure 3 shows the chromatography and response for the analytes at 0.01 mg/kg. The chromatgraphic separation of AMPA from Fosetyl AL, and Fosetyl AL from phosphonic acid was vital due to isobaric compounds and degradation issue respectively.

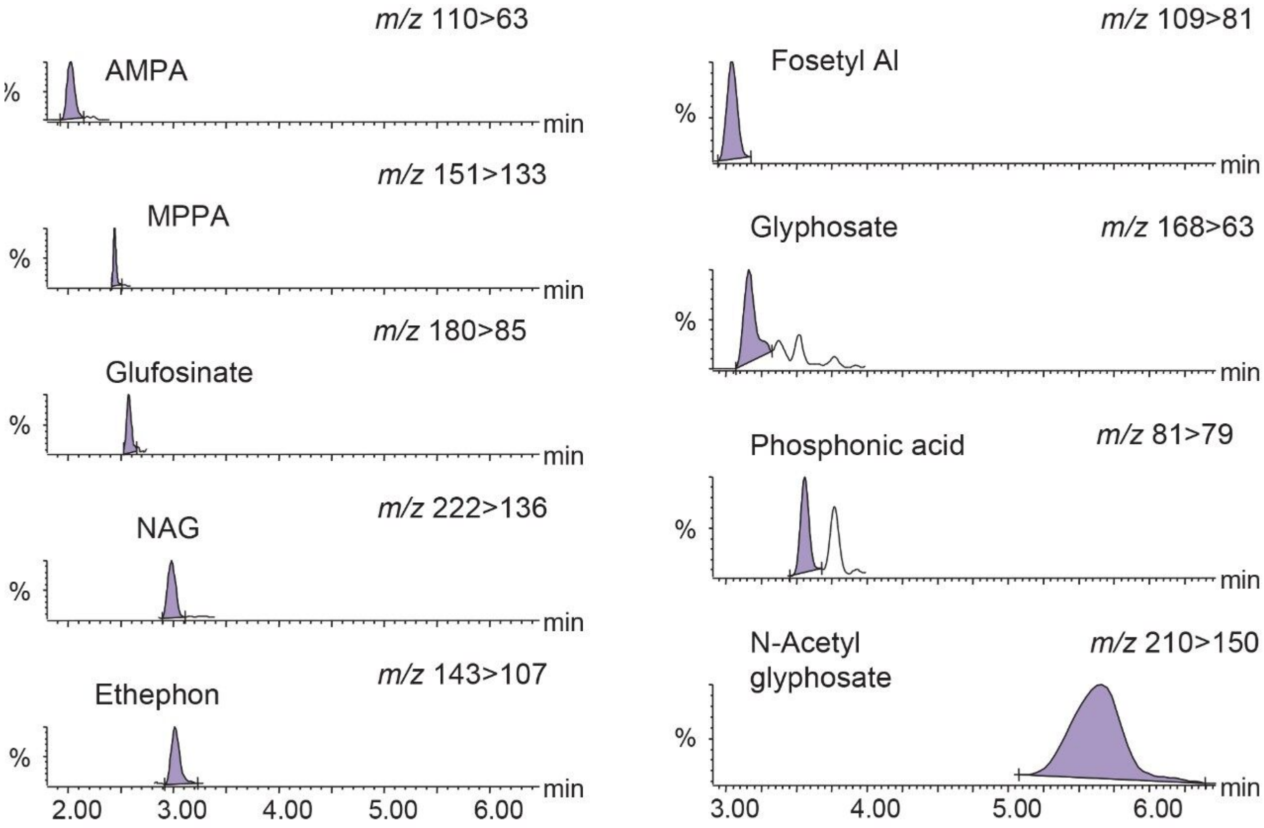
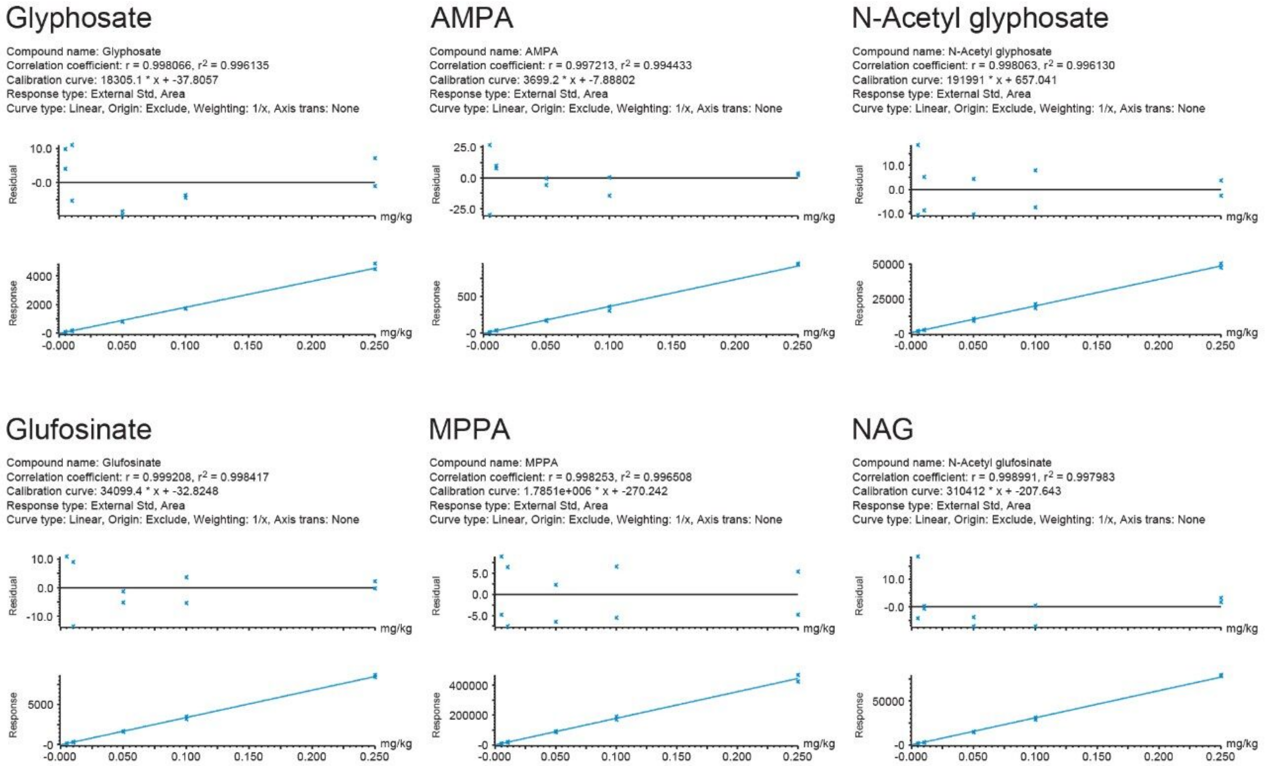


Figure 3. Typical chromatograms showing anionic polar pesticides from analysis of matrix-matched standard at 0.01 mg/kg (10 ppb) in wheat flour.

Calibration characteristics were assessed for the pesticides of interest through the use of bracketed calibration over a suitable concentration range, as shown in Figure 4. The coefficients of determination (r^2 >0.99) and the residuals (referred to in the SANTE document as back-calculated concentrations; <20%)* were excellent, demonstrating good repeatability of the measurements, in the absence of labeled standards. Peak shapes remained stable without deterioration throughout the run. Replicate (n=15) injections of the matrix matched standard at 0.10 mg/kg (100 ppb) showed good precision, with RSDs <5% for all but fosetyl-Al (5.4% RSD) and AMPA (9.6% RSD).



Compound name: Glyphosate
Correlation coefficient: r = 0.998066, r^2 = 0.996135
Calibration curve: 18305.1 * x + -37.8057
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Compound name: AMPA
Correlation coefficient: r = 0.997213, r^2 = 0.994433
Calibration curve: 3699.2 * x + -7.88802
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Compound name: N-Acetyl glyphosate
Correlation coefficient: r = 0.998063, r^2 = 0.996130
Calibration curve: 191991 * x + 657.041
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Compound name: Glufosinate
Correlation coefficient: r = 0.999208, r^2 = 0.998417
Calibration curve: 34099.4 * x + -32.8248
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Compound name: MPPA
Correlation coefficient: r = 0.998253, r^2 = 0.996508
Calibration curve: 1.7851e+006 * x + -270.242
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Compound name: N-Acetyl glufosinate
Correlation coefficient: r = 0.998991, r^2 = 0.997983
Calibration curve: 310412 * x + -207.643
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

Figure 4. Calibration graphs for a selection of anionic polar pesticides, over the range in 0.005–0.250 mg/kg (5–250 ppb), in wheat flour.

Retention times from the matrix matched standards agreed well with reference values and most were within the required tolerances(+/- 0.1). Ion ratios were also within required tolerance (+/- 30%) for all matrix-matched standards. Overall all injections, samples and standards were mostly compliant with relevant tolerances. However, sensitivity/selectivity for the second transition was compromised for some of the compounds at concentrations ⩽0.010 mg/kg (10 ppb).

Phosphonic acid was detected in the matrix used for preparation of the matrix matched standards. The concentration was calculated using standard addition feature of TargetLynx XS and found to be 0.002 mg/kg (2 ppb). Ion ratios and retention times were within the required tolerances of the SANTE guidelines.

The UPLC-MS/MS method performance has been determined to be suitable for monitoring MRL compliance of the target compounds in wheat flour. The scope of the analysis includes all of the components that make up the residue definition. Although the MRLs/tolerances for those pesticides approved for use on wheat vary across the globe, values tend to be in the ppm range (e.g. 10 and 30 mg/kg for glyphosate in the EU and U.S. respectively), so extracts can be diluted prior to analysis. This method also has been shown to have sufficient sensitivity to be used in combination with established extraction protocols for checking compliance with the EU default MRLs derived from the lower limit of analytical determination for these compounds (e.g. 0.03 mg/kg (30 ppb) for glufosinate in the EU).

Conclusion

The use of the Torus DEA Column provides excellent chromatographic performance for anionic polar pesticides and metabolites, even for the analysis of a complex and difficult matrix such as wheat flour. When coupled with the high sensitivity of the Xevo TQ-XS, these challenging compounds can be determined in a single analysis, without the need to use derivatization or specialized equipment. When used in combination with established extraction protocols, this method is suitable for checking MRL/tolerance compliance. Although we have shown data in a wheat flour extract that meet SANTE criteria, scientists must fully validate the method on their commodities of interest, in their own laboratories, to demonstrate that, when coupled with their extraction protocols, it is fit for purpose.

References

1. http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf
2. Wuyts B et al. Determination of Anionic Polar Pesticides in Spinach using a Novel Application of Torus DEA Column Chemistry by Liquid Chromatography-Tandem Quadrupole Mass Spectrometry. Waters application note no. 720006213EN, February, 2018.
3. European Union (2017). Document No. SANTE 11813/2017. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed.
4. Torus DEA Column Startup Guide for Polar Pesticide Separations. Waters support document no. 720006156EN (2017).

**Please note: This Application Note was developed on a Torus DEA Column, improved performance can be achieved using the Waters Anionic Polar Pesticide Column coupled with our most recent Application Notes please contact Waters Chemistry Technical Services with any questions www.waters.com/contact.*

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