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应用纪要

Improved Chromatographic Retention and Resolution for the Analysis of Anionic Polar Pesticides and Plant Growth Regulators in Food Commodities Using the Torus DEA Column

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief outlines a modern, alternative chromatographic approach, which provides excellent retention and separation of a range of polar anionic pesticides and plant growth regulators, using Waters Torus DEA* chemistry.

Benefits

- Column suitable for routine LC-MS/MS analysis of polar, anionic pesticides on a standard UPLC platform
- Retention >2X column void volume as specified in the SANTE guidelines[2]
- Excellent separation of critical pairs of isobaric compounds
- Separation of target analytes from some major food co-extractives; organic acids

- Mobile phase conditions optimized for sensitivity
- Reproducible retention times in food samples, extracted using the Quick Polar Pesticides Method (QuPPe)[3]

Introduction

Interest in the determination of highly polar, anionic pesticides in foodstuffs has increased noticeably in the last five years due to concerns over the potential safety of Glyphosate.[1] Consequently, demand for surveillance has increased. Because of the physiochemical properties of highly polar, anionic compounds such as glyphosate and ethephon, standard analytical methods using reversed phase chemistries such as C_{18} are not applicable, due to insufficient retention and problems with separation. With the highly polar nature of these compounds, derivatization with 9-fluorenylmethyl chloroformate (FMOC-CL) has been used, where hydrogen atoms on the amino groups of compounds such as glyphosate are substituted by the FMOC compound, facilitating reversed phase retention as the aromatic rings of the FMOC reagent provide hydrophobic properties to the derivatized compounds. Although this method allows for retention on traditional C_{18} columns, it is undesirable for many labs to use this method, due to time constraints, need for multiple clean-up steps, additional costs, and severely restricting the scope of analysis to those compounds with amino functionality.

Alternative approaches to allow for the direct analysis of highly polar, anionic pesticides in food commodities have been sought by many pesticide residue laboratories for years. Several recent developments have provided improvements in chromatographic retention and separation that avoid the need for a number of different single-residue methods using different chromatographic conditions and eliminate derivatization or ion-pairing. Ion chromatography, with chemical suppression, does offer some potential advantages but requires the use of a separate, dedicated ion chromatography systems. The non-suppressed ion chromatography approach uses high concentrations of volatile buffers which can lead to ion suppression of some analytes, negatively impacting limits of quantification (LOQ).

This application brief outlines a modern, alternative chromatographic approach, which provides excellent retention and separation of a range of polar anionic pesticides and plant growth regulators, using Waters Torus DEA chemistry. The Torus DEA stationary phase consists of ethylene

bridged hybrid (BEH) particles with tri-functionally bonded diethylamine (DEA) ligands. The combination of the hydrophilic surface and the anion exchange properties of the ligands provide chromatographic characteristics well suited to the retention and separation of polar anionic compounds. The use of BEH particles provides mechanical robustness to the columns, allowing for the use of this stationary phase in small particle size column chemistries (sub-2-µm), thus facilitating the benefits of UltraPerformance LC (UPLC) for this application, such as increased chromatographic resolution.

Results and Discussion

The chromatographic method used for the retention and separation of polar anionic pesticides and plant growth regulators consists of the mobile phase and gradient conditions as shown in Table 1. The mobile phase composition differs from that reported in earlier application notes, as this method has been optimized for maximum sensitivity of the target analytes, whereas the previous method is optimized for maximum compound coverage.

Column	Torus DEA Column (130Å, 1.7 µm, 2.1 x 100 mm) [p/n 186007616]		
LC system	ACQUITY UPLC I-Class (FL)		
Solvent A	LCMS grade water + 0.9% LCMS grade formic acid		
Solvent B	LCMS grade acetonitrile + 0.9% LCMS grade formic acid		
Column temp.	50 °C		
Sample temp.	10 °C		
Injection volume	10 µL		
Flow rate	0.5 mL/min		
Time (min)	**%A**	**%B**	**Curve**
0	10	90	-
4.0	85	15	2
12.0	85	15	6
18.0	10	90	1

Table 1. Chromatographic conditions.

The SANTE guidelines specify that "the minimum acceptable retention time for the analyte(s) under examination should be at least twice the retention time corresponding to the void volume of the

column” (SANTE 2018). The Torus DEA Column provides excellent retention of all compounds. Figure 1 shows the retention time corresponding to the void volume (t0) for the column and flow rate specified and the first eluting peak AMPA. The retention time of AMPA is 3.5 times t_0. An example of the chromatography for the full separation of several key compounds spiked into tomato at 0.050 mg/kg can be seen in Figure 2.

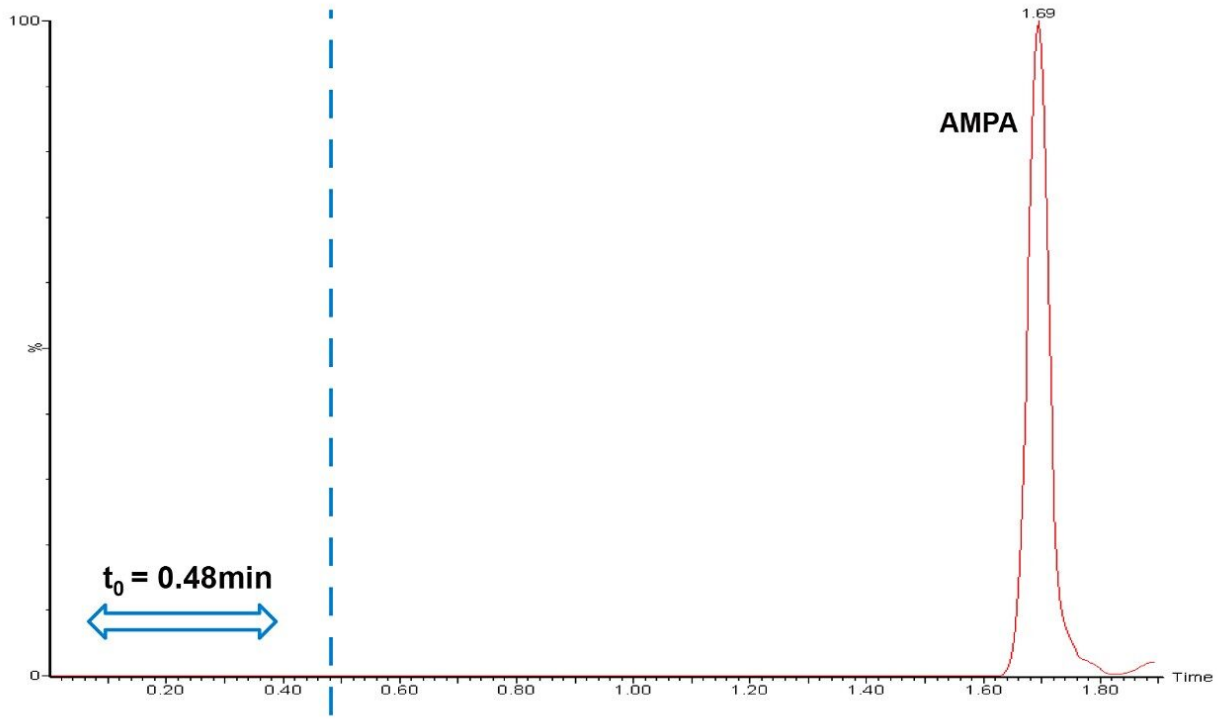

Figure 1. Area of no retention (t0) equivalent to 2x the column void volume as indicated in the SANTE guidelines.[1]

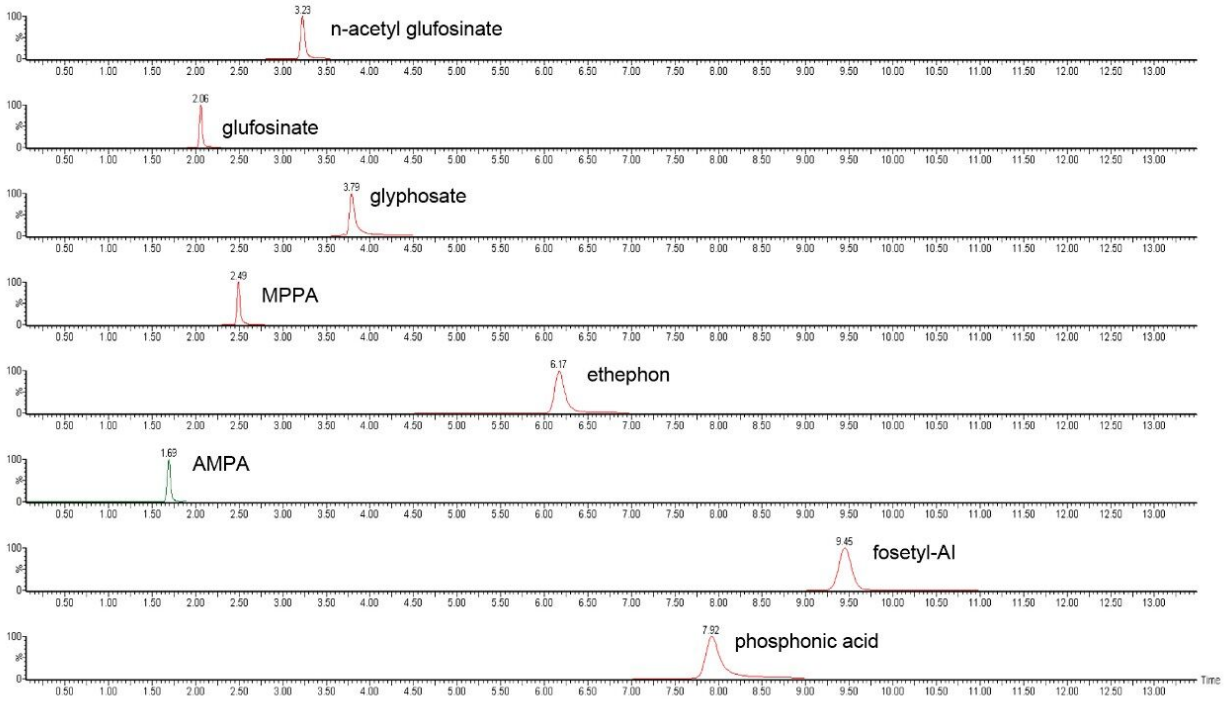

Figure 2. Example chromatography of 0.050 mg/kg spiked tomato matrix QuPPe extract.

The QuPPe document describes some key chromatographic separations required by any method which has been developed for this analysis. These separations include those between AMPA, fosetyl, and phosphonic acid. Fosetyl and AMPA both share the same multiple reaction monitoring (MRM) transition, *m/z* 110>81, so chromatographic separation is required to avoid false identification. Fosetyl also degrades to phosphonic acid in LC-MS/MS sources and good separation is also required between these two compounds. An example of this critical separation on the Torus DEA Column and the isobaric interferences in the MRM transitions of AMPA and phosphonic acid by fosetyl can be seen in Figure 3. Another critical pair that needs chromatographic separation is AMPA and N-acetyl AMPA, due to the potential for N-acetyl AMPA to be transformed in the source to AMPA. An example of the excellent separation between these two compounds and the isobaric interference of N-acetyl AMPA in the MRM traces of AMPA can be seen in Figure 4.

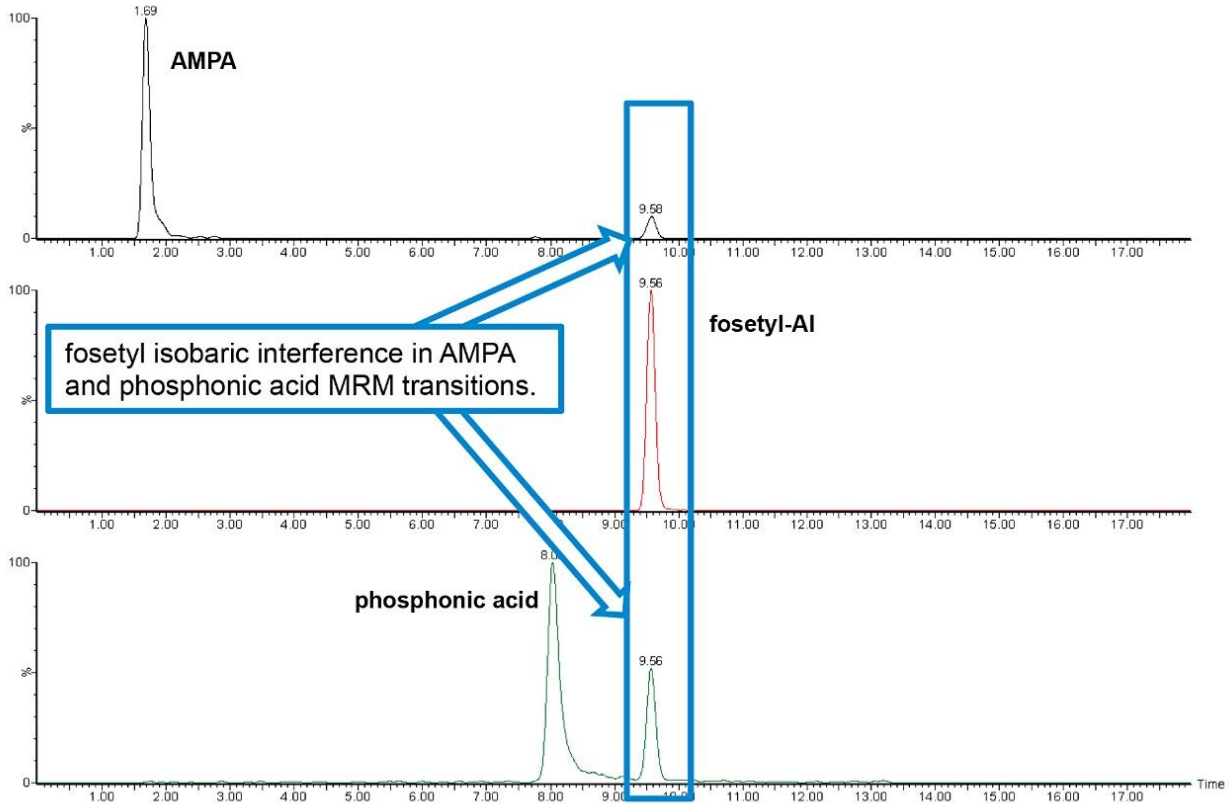

Figure 3. Critical separation between AMPA, fosetyl, and phosphonic acid in tomato matrix extracted using the QuPPe method.

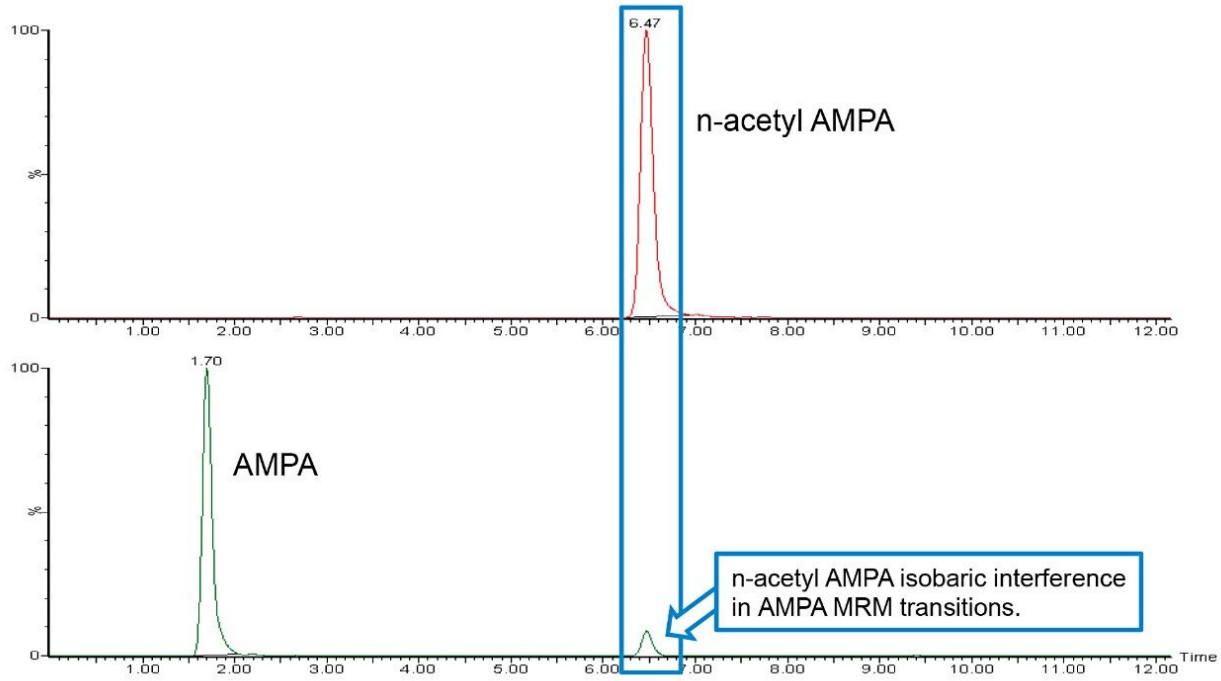

Figure 4. Critical separation between AMPA and n-acetyl AMPA in wheat flour matrix extracted using the QuPPe method.

An additional challenge associated with the analysis of QuPPe extracts is the potential for significant ion suppression from co-elution of significant levels of matrix co-extractives with certain polar anionic pesticides. Two major organic acids, citric acid and malic acid, are present in tomatoes at parts per million levels (ppm). Without chromatographic separation, the presence of these organic acids will suppress the signal from compounds such as AMPA and glufosinate, significantly reducing the sensitivity of the method. An example of the separation between the first two eluting polar anionic pesticides, AMPA and glufosinate, and malic acid in tomato can be seen in Figure 5.

The retention time tolerance in the SANTE guidelines is +/- 0.1 minutes. The Torus DEA Column exhibits very stable retention times during analysis, an example of 15 replicate, overlaid injections at the target LOQ of 0.010 mg/kg in a QuPPe extract of tomato for four representative compounds is shown in Figure 6.

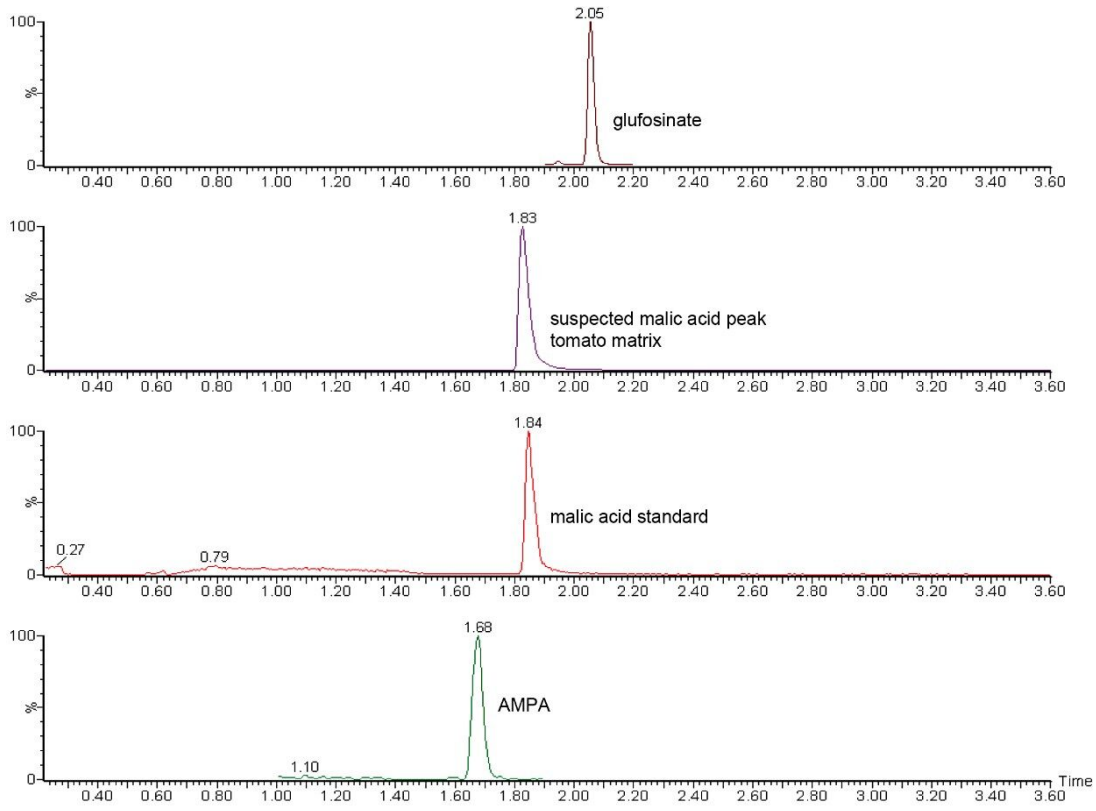

Figure 5. Separation between AMPA, glufosinate and, malic acid in QuPPe extract of tomato and a solvent standard of malic acid.

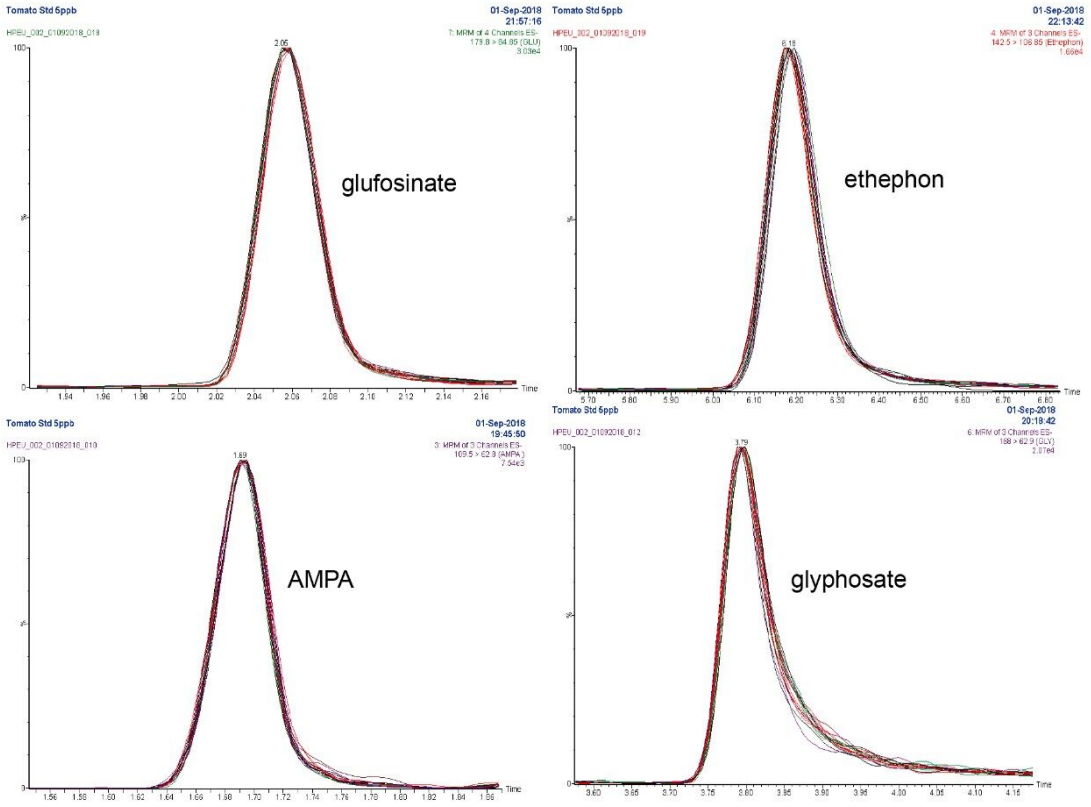

Figure 6. Overlay of n=15 injections of representative compounds at 0.010 (mg/kg) in tomato (5.0 ng/mL in vial), target LOQ level.

Conclusion

The Torus DEA Column allows for the direct analysis of QuPPe extracts of polar anionic pesticides and plant growth regulators. Torus DEA column technology requires only a standard UPLC inlet platform so the need for specialized ion chromatography systems is negated. The method described in this technical note uses a gradient of acetonitrile and water containing formic acid only, without the need for high amounts of buffers which can cause ion suppression or an increase of in source maintenance. The retention of the earliest eluting compound exceeds the requirements in the SANTE guidelines of two column void volumes of retention, with excellent peak shapes and stable retention times from analysis of QuPPe extracts. The selectivity of the TORUS DEA Column allows for the crucial separations indicated in the QuPPe document as essential for any chromatographic method

targeting these compounds.

References

1. European Food Safety Authority. (2017), EFSA statement addressing stakeholder concerns relating to EU assessment of glyphosate (Accessed 17 September 2018).
2. 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 (17 September 2018).
3. European Commission (2017) QuPPe Method [Online]. http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf. Accessed September 17, 2018.

**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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