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Determination of Anionic Polar Pesticides in High Water Foodstuffs

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The compact Xevo TQ-S micro demonstrated robust system and method performance in the analysis of polar, anionic pesticides on a routine-level UPLC-MS/MS platform.

GOAL

To evaluate the performance of the Xevo™ TQ-S micro for key representative compounds to determine whether the solution can be used to meet the default MRL for key representative compounds.

BACKGROUND

Interest in the determination of highly polar, anionic pesticides in foodstuffs has noticeably increased over the last five years, resulting from concerns regarding the potential safety of glyphosate.[1] Because of this, the demand for surveillance has increased, leading to a desire for underivatized analysis of highly polar anionic compounds by many food safety laboratories.

It is the aim of most of these laboratories to have detection limits at or below 0.010 mg/kg for all pesticide/commodity combinations, facilitating more efficient, simplified workflows to accommodate compounds/commodity combinations with default MRLs[2] (0.010 mg/kg) as well as organic and infant foods, which have lower MRLs.

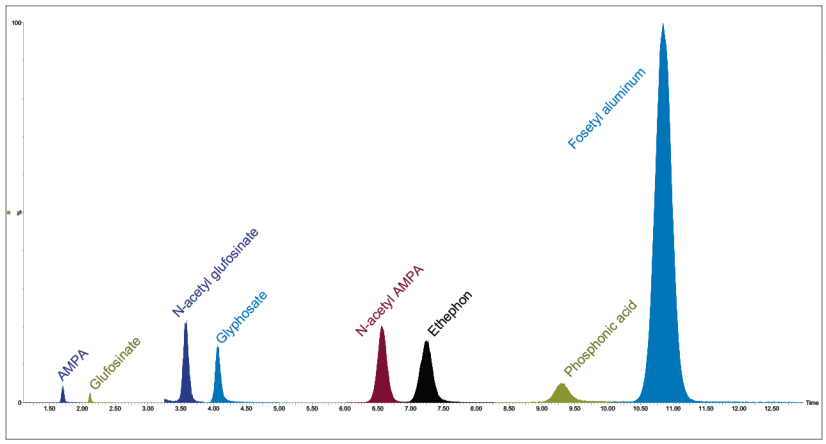

Figure 1. Example chromatography at 0.010 mg/kg spike level in a cucumber QuPPe extract sample.

The analysis of highly polar pesticides without derivatization typically requires either specialized liquid chromatography equipment or the use of the highest-performance, tandem quadrupole systems to meet the sensitivity requirements of this analysis. While these approaches allow for direct analysis, they do lead to additional laboratory costs and larger system footprints.

In previous work,[3] the method for underivatized determination of anionic polar pesticides has been presented on a Xevo TQ-XS Mass Spectrometer employing Waters™ Anionic Polar pesticide, 5 µm, 2.1x100 mm Column (p/n: 186009287) in HILIC mode, with excellent performance achieved. The aim of this technology brief is to evaluate the performance of the compact, refreshed Xevo TQ-S micro for key representative compounds to determine whether the solution can be used to meet the default MRL for key representative compounds, when evaluated against the SANTE guidelines.[4]

THE SOLUTION

To achieve the required retention and separation for this analysis, an underivatized HILIC-based method was used. The column stationary phase consisted 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 provides chromatographic characteristics well suited to the retention and separation of polar anionic compounds.

A panel of eight pesticides (AMPA, glyphosate, n-acetyl glufosinate, glufosinate, n-acetyl AMPA, ethephon, fosetyl aluminum, and phosphonic acid) were analyzed in different food commodities using electrospray negative ionization mode. All food commodities were extracted following the QuPPe methodology.

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 analytical column provided excellent retention of all compounds. Example chromatography for the analysis of the representative pesticides spiked into cucumber at 0.010 mg/kg can be seen in Figure 1. This method also provided excellent retention-time stability, in accordance with the SANTE guidelines tolerance of ±0.1 minute, across a selection of relevant commodities, as shown in Figure 2.

Excellent linearity (R^2 >0.99, residuals <20%) was found for calibration curves of all analytes in the absence of isotopically labelled standards. An example of matrix-matched, bracketed curves for AMPA and glyphosate in cucumber and tomato are shown in Figures 3a and 3b, where the concentration ranged from 2.5 ng/mL to 100 ng/mL in a vial (0.005 mg/kg–0.200 mg/kg matrix matched).

To evaluate the performance of the TQ-S micro for the routine analysis of anionic polar pesticides, the panel of eight representative compounds was spiked into tomato and cucumber at the targeted LOQ of 0.010 mg/kg as well as 2x LOQ and 5x LOQ, each at n = 6.

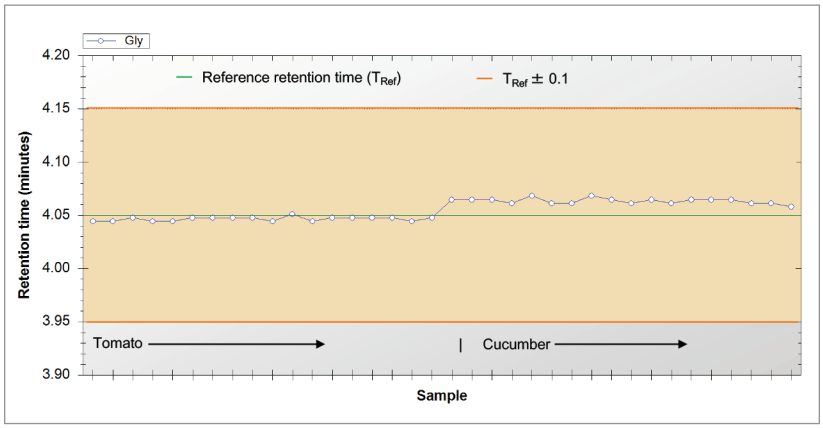

Figure 2. Retention time stability of glyphosate plotted in TrendPlot for the two commodities, each at n = 30, for samples spiked at various levels.

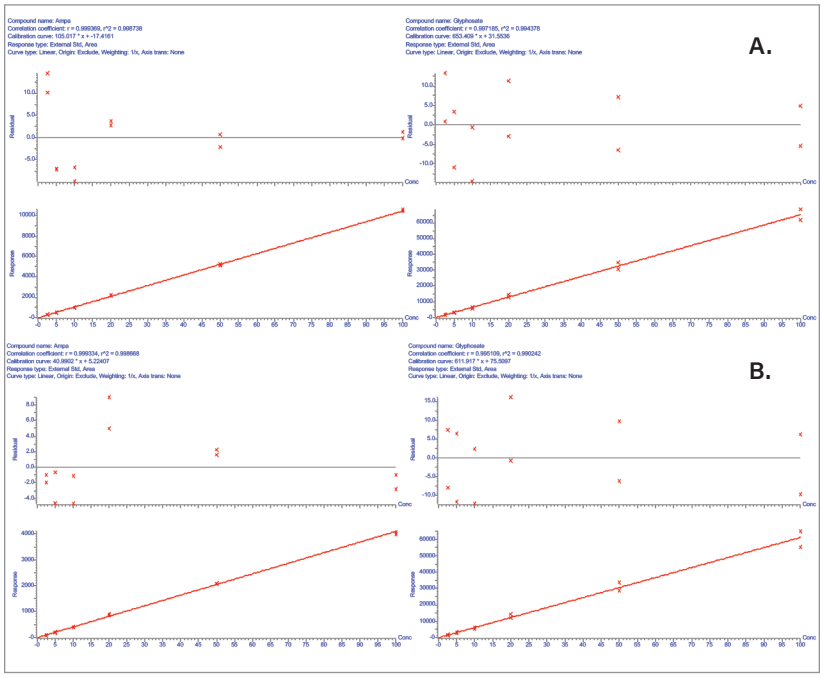

Figure 3. Example of matrix matched, bracketed calibration curves of AMPA and glyphosate in tomato (a) and cucumber (b) at 0.005 mg/kg–0.200 mg/kg.

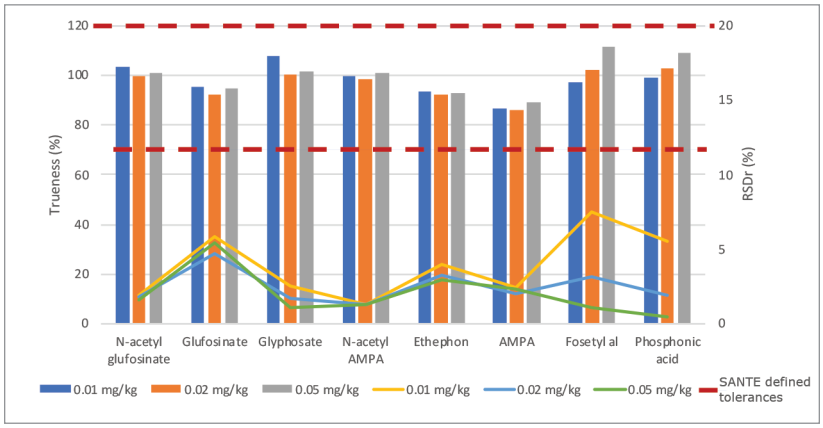

Figure 4. Percent trueness data at 0.010 mg/kg, 0.020 mg/kg, and 0.050 mg/kg spiking levels in tomato are plotted in the bar chart with the respective %RSDr plotted as lines on the secondary y-axis. The tolerances permitted by 11813/2017/SANTE are plotted in red for trueness (within 70 and 120%) and repeatability (< 20%).

Table 1. Method validation results are summarized for the tomato matrix against the criteria set out in the SANTE guidelines.

Compound	Retention time (±0.1 min)	% Trueness (70–120%)	% Precision (RSDr ≤20%)	Linearity (Residuals ≤ ±20%)	Ion Ratio (±30%)	LOQ 0.010 mg/kg
AMPA	✓	✓	✓	✓	✓	✓
Glufosinate	✓	✓	✓	✓	✓	✓
N-acetyl glufosinate	✓	✓	✓	✓	✓	✓
Glyphosate	✓	✓	✓	✓	✓	✓
N-acetyl AMPA	✓	✓	✓	✓	✓	✓
Ethephon	✓	✓	✓	✓	✓	✓
Phosphonic acid	✓	✓	✓	✓	✓	✓
Fosetyl aluminum	✓	✓	✓	✓	✓	✓

The spiked samples were quantified against a matrix matched calibration curve, as described above, to assess the capability of the Xevo TQ-S micro to reach the target LOQ level as well as the trueness (%) and precision (%RSDr) of the method. Figure 4 shows the trueness (%) data for the compounds at the spiking levels in tomato, where all compounds were within the range of 70–120% and the target LOQ was achieved for all compounds tested. The repeatability (%RSDr) of the method are also plotted in Figure 4, where all compounds at each level were <20% RSDr.

The overall results for tomato, compared against the analytical criteria defined in the 11813/2017/SANTE guidelines, are summarized in Table 1.

SUMMARY

The analysis of highly polar pesticides on a small footprint, routine-level tandem quadrupole has been demonstrated in this technology brief. The panel of eight compounds were spiked into various food matrices and excellent retention, retention time stability, and separation were achieved on a novel HILIC column. The Xevo TQ-S micro provided excellent performance in terms of sensitivity, linearity, and calibration range for the target compounds. The trueness and precision of the LC-MS/MS method determined at three levels was found to be acceptable for all compounds. Overall the performance data indicated that the configuration of the ACQUITY™ UPLC™ I-Class System coupled with Xevo TQ-S micro Mass Spectrometer, when used in combination with the Waters polar pesticides column, is suitable for checking MRL/tolerance compliance in a routine laboratory for these target compounds.

References

1. European Food Safety Authority (2017), EFSA statement addressing stakeholder concerns relating to EU assessment of glyphosate.
2. European Commission (2016) EU Pesticide Database [Online] http://ec.europa.eu/food/plant/pesticides/eu-pesticidesdatabase/public/?event=pesticide.residue.selection&language=EN.
3. Improved Chromatographic Retention and Resolution for the Analysis of Anionic Polar Pesticides and Plant Growth Regulators in Food Commodities, Waters Application Note, 720006505EN (2019).
4. European Union (2018), Document No. SANTE 11813/2017. Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed.

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