# DEVELOPING A ROBUST LC-MS/MS METHOD FOR THE DETERMINATION OF ANIONIC POLAR PESTICIDES IN A RANGE OF FOODSTUFFS WITHOUT DERIVATIZATION

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## INTRODUCTION

Interest in the determination of highly polar, anionic pesticides in foodstuffs has increased noticeably in the last 5 years, this is the result of concerns regarding the potential safety of glyphosate. As a consequence of this the demand for surveillance has increased. Due to 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. 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. A number of developments have been made recently, which can provide improvements in chromatographic retention and separation and avoid the need for a number of different single-residue methods using different chromatographic conditions and avoiding derivatization or ion-pairing.

This poster highlights a modern, alternative chromatographic approach, which provides excellent retention, separation and detection for a range of polar anionic pesticides, using Waters' new Anionic Polar Pesticide column on a standard UPLC-MS/MS platform and discusses key steps taken to ensure robust and reliable LC-MS/MS methods were developed. [1] With a desire to maximize efficiencies and ability to extract multiple polar analytes using a single method, this approach looks at extending the analytical scope from the traditional glyphosate, glusfosinate and AMPA target list. In developing these methods, consideration was given to the main renowned challenges:

1. **Retention**: Highly polar, low molecular weight compounds can create challenges for reversed phase $C_{18}$ columns. Good analytical practice calls for all analytes to elute after the column's void volume.
2. **Separation**: Focussing on an extended scope of analytes, including metabolites, increases the importance for baseline chromatographic separation, to avoid false detections of incurred residues.
3. **Matrix complexity**: Applying generic analyte extraction methods, crude food extracts are typically generated, which can cause increased matrix load on the LC-MS/MS system, resulting in unwanted matrix effects.
4. **Detection**: Required limits of detection vary depending on food commodity, compound and defined residue definition (*eg*: compound specific or summed MRL), where reliable detection should be achievable routinely and within accepted guidelines for good analytical practices.

## METHODS

All samples were purchased from local retail outlets, homogenized and extracted using a version of the EURL Quick Polar Pesticides (QuPPe) extraction method. [2] Applying the QuPPe extraction, the resultant food extracts are in acidified acetonitrile. Similar, previously published, [3] generic aqueous extractions were also investigated and applied to this LC-MS/MS method, on the Xevo TQ-S micro with acceptable results.

In developing this LC-MS/MS method, the stationary phase of the analytical column of DEA chemistry was selected. Consisting 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 2.1 x 100 mm, 5 µm column (P/N: 186009287) was used.

n order to achieve robust methodologies to overcome the renowned challenges, without sample derivatization, a couple of LC methods were identified, based on the key drivers for analysis. These two methods are summarised and presented here, as Method A and Method B, demonstrating the column's overall performance for these highly polar, anionic compounds.

Full sample extraction and method details are available. For more information, scan the QR code below or visit **www.waters.com/polarpesticides**.

Briefly, LC methods A and B are summarized as follows:

**Method A: With buffer**

| | |
|---|---|
| **Mobile phase A** | 50 mM ammonium formate with 0.9% formic acid |
| **Mobile phase B** | 0.9% formic acid in acetonitrile |

**Method B: Without buffer**

| | |
|---|---|
| **Mobile phase A** | 0.9% formic acid in LCMS water |
| **Mobile phase B** | 0.9% formic acid in acetonitrile |


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## RESULTS AND DISCUSSION

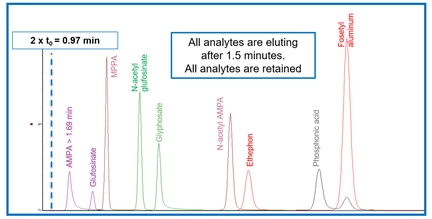



**Figure 1**. The SANTE guidelines state that 2 x the column void volume of retention is required. AMPA, the first analyte to elute is shown with 3.5 x the $t_0$ or 'dead volume' of the column, with a 0.5 ml/min flow rate.

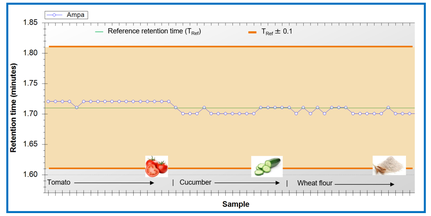



**Figure 2**. Retention time stability within matrix should not shift > 0.1 min during a run. Excellent stability was shown for all target compounds across commodities, with the example shown for AMPA in tomato, cucumber and wheat flour. This data is not internal standard corrected.

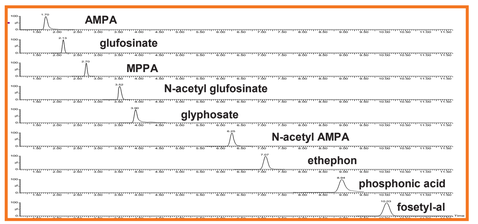



**Figure 3**. Example of chromatography showing elution order and separation using formic acid. All representative compounds give excellent peak shapes and crucial separations are achieved, such as critical pairs of AMPA, phosphonic acid and fosetyl aluminium.

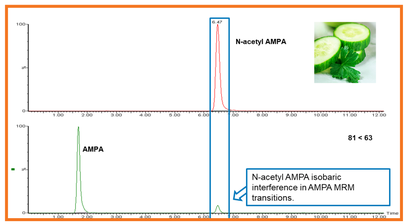
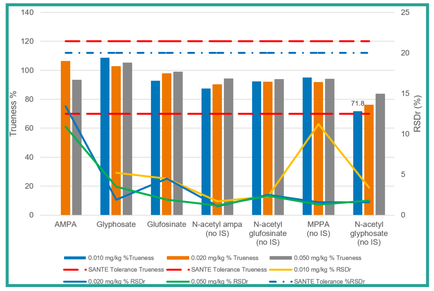


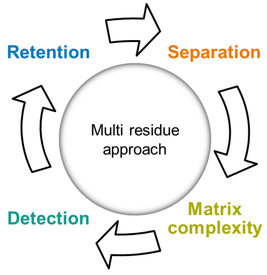

**Figure 4**. Due to the potential of n-acetyl AMPA being formed into AMPA, baseline separation of the critical pair is essential to avoid false detections from isobaric interferences. Similar separation is required for phosphonic acid and fosetyl aluminium from AMPA, as shown in Fig 3.

**Figure 10**. When developing a reliable method for the underivatized determination of anionic polar pesticides, we focused on optimising the analytical column parameters and chromatographic conditions, which has simplified analyte detection and method performance.

Method detection, in terms of accuracy and precision are shown for replicate samples (n=6) of wheat flour, using method B. Taking sample weight into consideration, samples were spiked at 0.01 (5 ppb in vial), 0.02 (10 ppb in vial) and 0.05 (25 ppb in vial) mg/kg, where all accuracy was within the 70 to 120 % range and %RSD < 15%.

Although not shown here, all matrix matched calibration curves were linear ($R^2$> 0.995; back calculated concentrations/ residuals <20 %) over suitable concentration ranges (0.002 to 0.2 mg/kg). In this analysis, only the response for glyphosate, glufosinate and AMPA were corrected with internal standard.



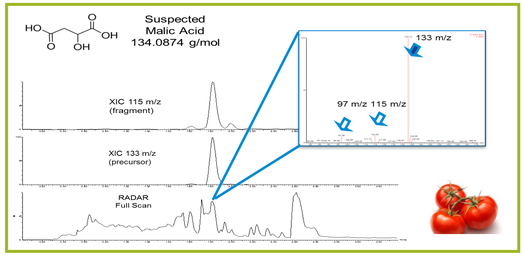




**Figure 5**. RADAR scan of a blank QuPPe extract of tomato, highlights the complexity of crude QuPPe extracts of food commodities and potential for ion suppression, due to matrix effects.

By combining data under a RADAR acquired peak at an elution time, full spectral information is obtained, allowing for ions for extraction (XIC) to be identified.

The ability to use RADAR to monitor matrices allows for the collection of full scan information, which is useful if considering a clean-up step during method development.

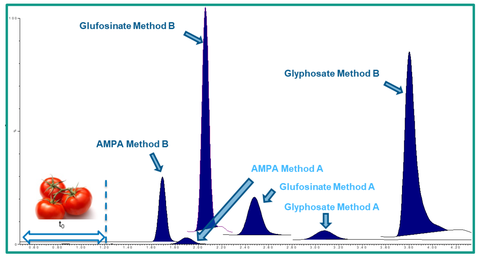



**Figure 9**. Comparing both methods for the three key analytes, retention, separation and detection are uncompromised. Tomato extract at 0.01 mg/kg is shown where excellent chromatographic stability and peak shape are achieved for both methods.

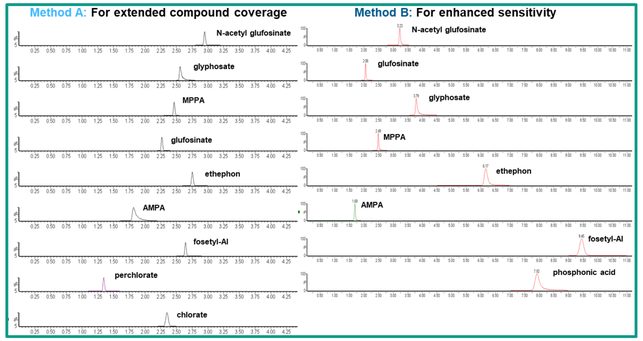



**Figure 7**. By ensuring the challenges of retention, separation and matrix complexity are addressed, detection of these challenging compounds is simplified and an optimised method to meet your needs can be delivered using the DEA chemistry.

Running **Method A** (buffered formic acid mobile phase), chlorate and perchlorate can be included, allowing for at least 13 compounds in a single injection.

**Method B** (formic acid based mobile phase) has been developed for improved sensitivity, if required.

Both methods provide the benefits and enhanced performance in terms of retention, separation and matrix complexity, as previously discussed, while excellent reliability and detection is readily achieved in low ppb, far exceeding the current MRLs.

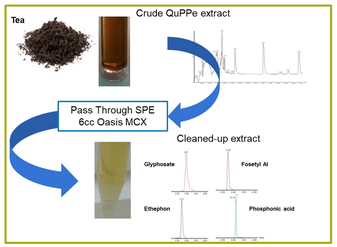



**Figure 6**. The crude tea extract showed significant matrix effects, suppressing the response of key analytes. Visibly cleaner extracts were obtained following simple cleanup, where hydrophobic pigments and lipids were removed, reducing ion suppression and improving analyte detection.

**References**

1. 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
2. European Commission (2019) *QuPPe Method* [Online]. http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf
3. Chamkasem, N.; Harmon, T. (2016). *Anal Bioanal Chem.* 408(18),4995–5004.