Achieving method reliability in the routine determination of anionic polar pesticides in food

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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 C18 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 solution, which provides excellent retention, separation and detection for a range of polar anionic pesticides, using the Anionic polar pesticide column (186009287) 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, glufosinate and AMPA target list. In developing these methods, consideration was given to the main renowned challenges: **Retention**: Highly polar, low molecular weight compounds can create challenges for reversed phase C18 columns. Good analytical practice calls for all analytes to elute after the column's void volume. **Separation**: Focusing on an extended scope of analytes, including metabolites, increases the importance for baseline chromatographic separation, to avoid false detections of incurred residues. **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. **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.

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 methanol. Similar, previously published, [3] generic aqueous extractions were also investigated and applied to this LC-MS/MS method with acceptable results.

In 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 summarized 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 here:

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

RESULTS AND DISCUSSION

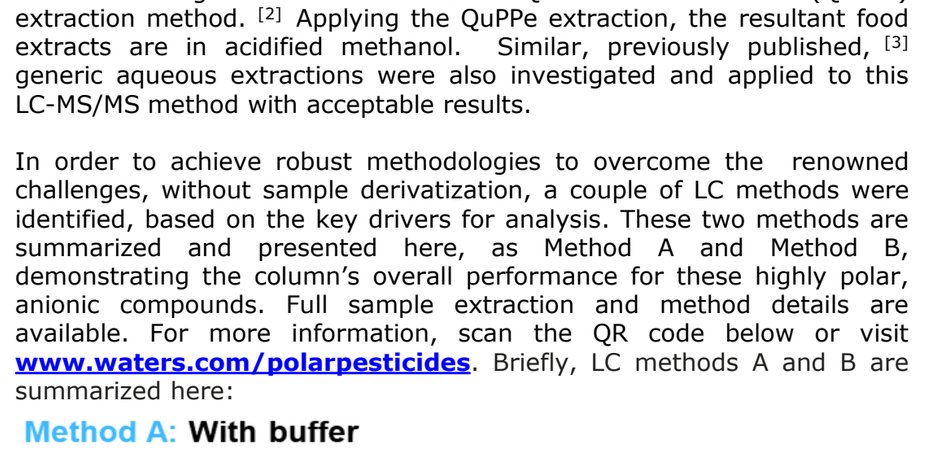
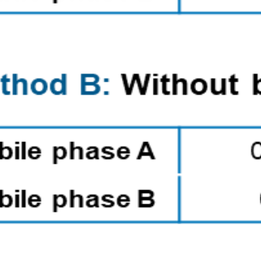
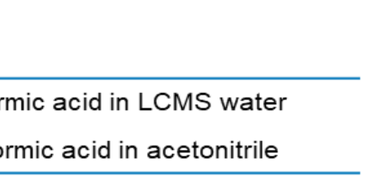


Figure 1. The SANTE guidelines state that 2 x the column void volume of retention is required. AMPA, the first analyte to elute is shown with 3.5 x the t0 or 'dead volume' of the column, with a 0.5 ml/min flow rate.





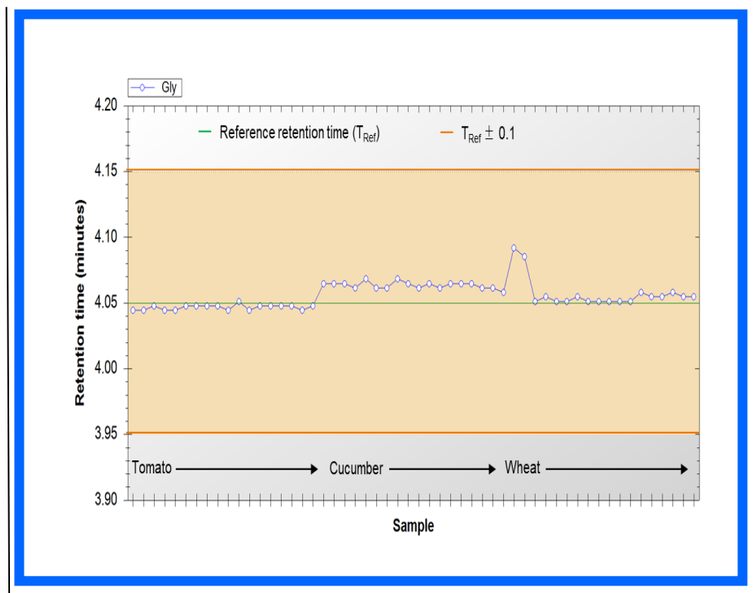


Figure 2. Retention time stability within matrix should not shift > 0.1 min during a run. Excellent stability was shown for all target compounds, with the example shown for glyphosate in tomato, cucumber and wheat flour.

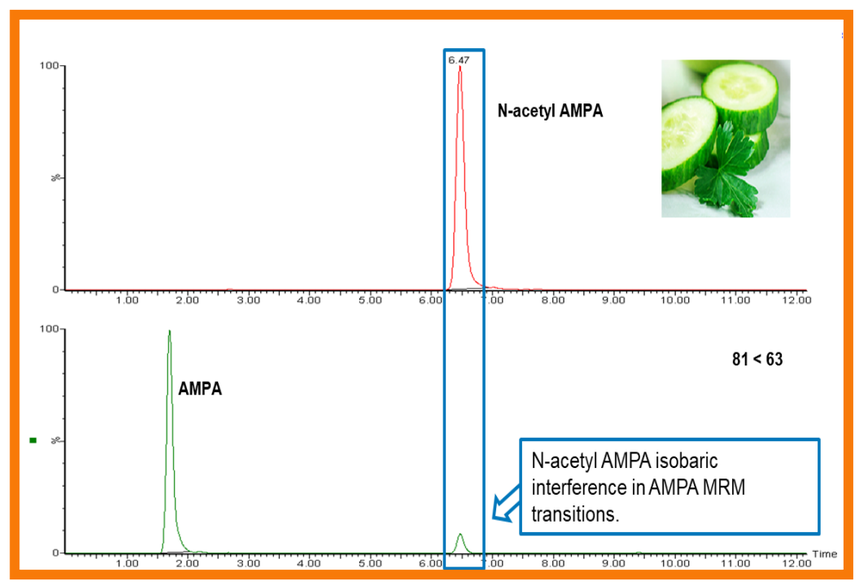


Figure 3. Due to the potential of n-acetyl AMPA being formed into AMPA, baseline separation of the critical pair is essential to avoid false detections from isobaric interferences. Similar separation is required for phosphonic acid and fosetyl aluminium from AMPA, which are additional isobaric specifies.

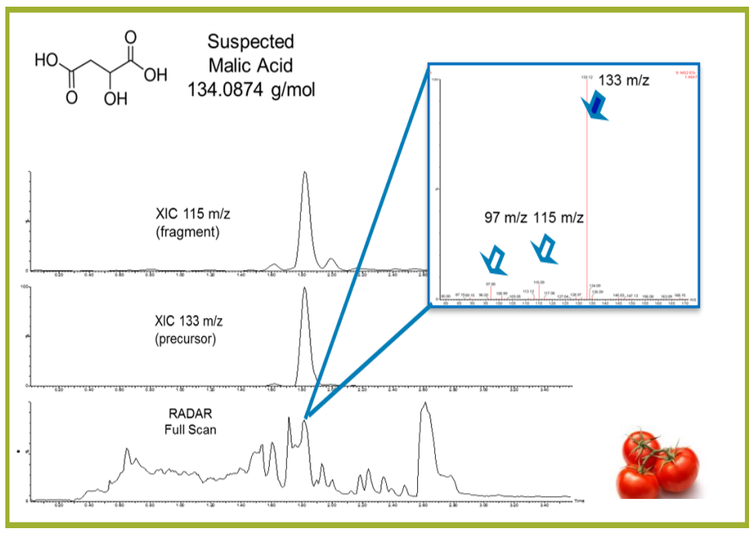


Figure 4. RADAR scan of a blank QuPPe extract of tomato, highlights the complexity of crude QuPPe extracts of food commodities and potential for ion suppression, due to matrix effects.

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.

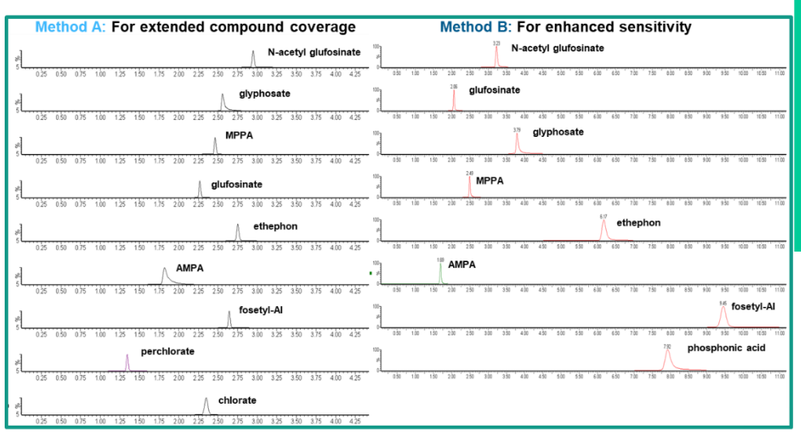


Figure 6. By ensuring the challenges of retention, separation and matrix complexity are addressed, detection of these challenging compounds is simplified and an optimised method to meet your needs can be delivered using the polar pesticides column.

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.

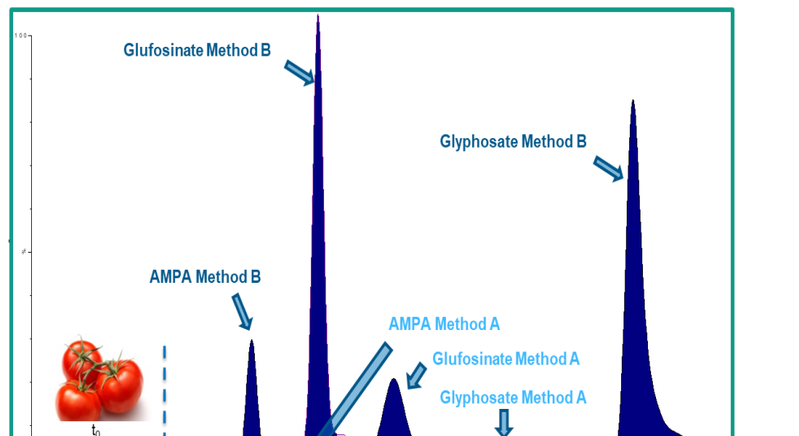


Figure 5. Comparing both methods for the three key analytes, retention, separation and detection are uncompromised. Tomato extract at 0.01 mg/kg is shown where excellent chromatographic stability and peak shape are achieved for both methods.

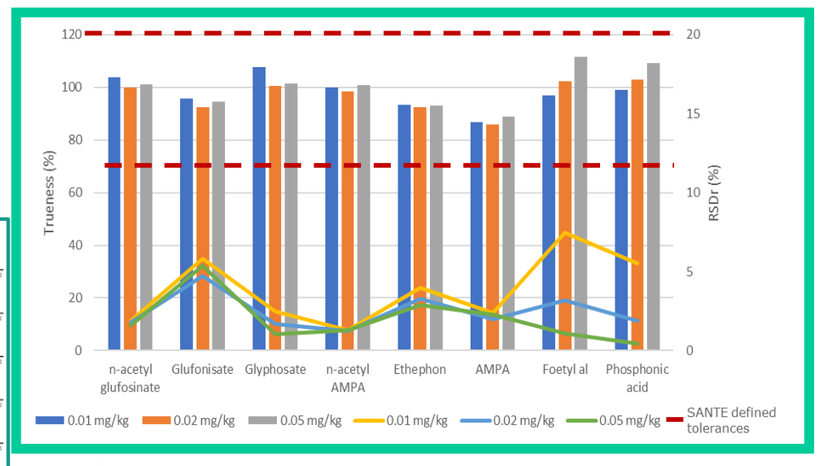


Figure 7. When developing a reliable method for the underivatized determination of anionic polar pesticides, we focused on optimising the analytical column parameters and chromatographic conditions, which has simplified analyte detection and method performance.
Method detection, in terms of accuracy and precision are shown for replicate samples (n=15) of tomato, cucumber and wheat flour, using method B. Taking sample weight into consideration, samples were spiked at 0.01 mg/kg and 2 x and 5 x, where all accuracy was within the 70 to 120 % range and %RSD < 5%.

CONCLUSIONS

- **Simple, reliable method for the determination of anionic highly polar pesticides has been developed for routine operation on standard UPLC-MS/MS.**
- **Methodology has focused around retaining, resolving and quantifying these physiochemically challenging compounds, enabling reliable and sensitive detection, far exceeding the current MRLs.**
- **Small does not have to limit capabilities– delivering purpose driven performance, the determination of these small molecular weight, highly polar, anionic pesticides is now routine.**
- **For more information, please speak to one of the authors.**

REFERENCES

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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.