ROUTINE DETERMINATION OF HIGHLY POLAR CATIONIC PESTICIDES AND PLANT GROWTH REGULATORS BY LC-MS/MS

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INTRODUCTION

There are various methods available to analyze food for pesticide residues which focus on multi-residue methods such as QuEChERS. However, the extraction and determination of polar cationic pesticides remains a considerable challenge still, and direct methods of analysis are limited and often require the use of derivatisation, ion pairing or specialised inlets.

The QuPPe (Quick Polar Pesticides) method has been developed by EURL-SRM (European Union Reference Laboratory - Single Residue Method) and allows for the simultaneous extraction of many highly polar pesticides, their metabolites and plant growth regulators. It focuses on using LC-MS/MS instruments offering high sensitivity in part to deal with matrix effects as there is no current generic clean-up that effectively deals with all matrix types.

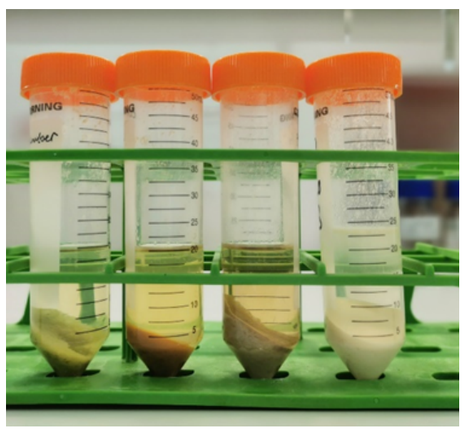
METHOD

Sample preparation, extraction and analysis:

Organic apple, cucumber, potato and wheat flour samples were purchased from a retail outlet and finely homogenised in the laboratory and stored at 4 ºC until analysis. Flour was stored at room temperature.

A certified QC sample (T09127QC) from FAPAS was purchased. The sample contained a mix of polar pesticides (including chlormequat and mepiquat) with assigned values and acceptance limits for the compounds included.

A calibration range of 0.002 to 0.2 mg/kg (0.1 to 2 mg/kg for maleic hydrazide) was set for apples, cucumber and potatoes. The values for wheat flour were 0.004 to 0.4 mg/kg (0.2 to 4 mg/kg for maleic hydrazide). Quantification of spiked samples was calculated by matrix matched bracketed calibration.



Matrix matched standards were prepared in respective blank matrix extracts and spiked after filtering.

Recovery samples were spiked in matrix at the equivalent of 0.01 and 0.05 mg/kg (0.5 and 1.5 mg/kg for maleic hydrazide) prior to extraction.

Figure 2. Each extract immediately before filtration *(order from left to right: cucumber, apple, potato and wheat flour).*

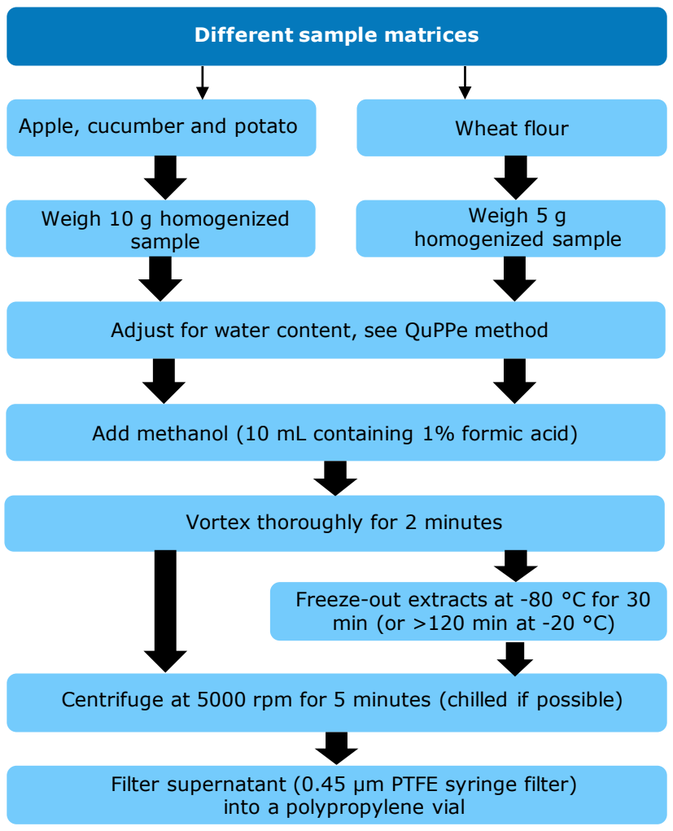


Figure 1. QuPPe sample extraction workflows for tested matrices.

Instrumental conditions:

UPLC System:	ACQUITY UPLC® I-Class-FL
Column:	ACQUITY UPLC® BEH Amide, 1.7 µm; 2.1 x 50 mm (p/n 186002350)
Mobile Phase A:	20 mM ammonium formate (pH2.95)
Mobile Phase B:	Acetonitrile
Column Temp:	40˚C
Sample Temp:	10˚C
Injection Volume:	0.5 µL

MS System:	Xevo TQ-S micro
Ionisation Mode:	ESI+ (ESI- for maleic hydrazide)
Acquisition:	MRM with at least 2 transitions per compound (primary transition reported)
Capillary Voltage:	1.00 kV
Desolvation Temp:	600˚C
Desolvation Gas Flow:	1000 L/Hr
Source Temp:	150˚C

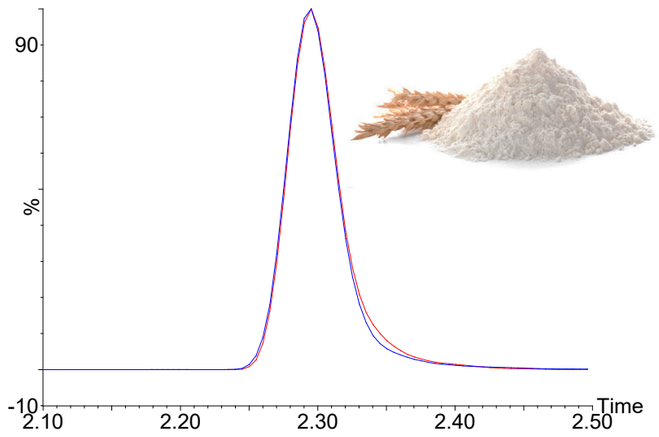
Full MRM parameters detailed in application note #720007201 at www.waters.com

RESULTS AND DISCUSSION

Method performance factors such as calibration linearity, retention time stability, method precision, trueness and analyte identification were assessed over 4 validation batches in which the commodities represented a range of different properties such as high water content, high starch and low water content. Method evaluation was based on SANTE/12682/2019 guidelines.

Figure 3 demonstrates a typical chromatogram of chlormequat, a common growth regulator in wheat, at 0.02 mg/kg in a flour matrix matched calibration standard. Sensitivity was achieved using a low injection volume of 0.5 µL which helps to mitigate matrix effects. HILIC columns have a reputation for unsteady retention, however a high level of stability was achieved when over 200 injections of flour matrix were made onto the column with a max shift of 0.01 minutes observed and RSDs under 0.3% for all analytes.

Figure 3. Chromatogram of the 1st and 200th injection (blue and red traces, respectively) of chlormequat in wheat flour matrix at 0.02 mg/kg.



In line with SANTE guidelines, the residuals for all analytes were within 20%. All compounds achieved linear regression using 1/X weighting with R^2 values ≥0.995. Figure 5 shows the calibration curve of cyromazine, an insecticide commonly found on cucumbers.

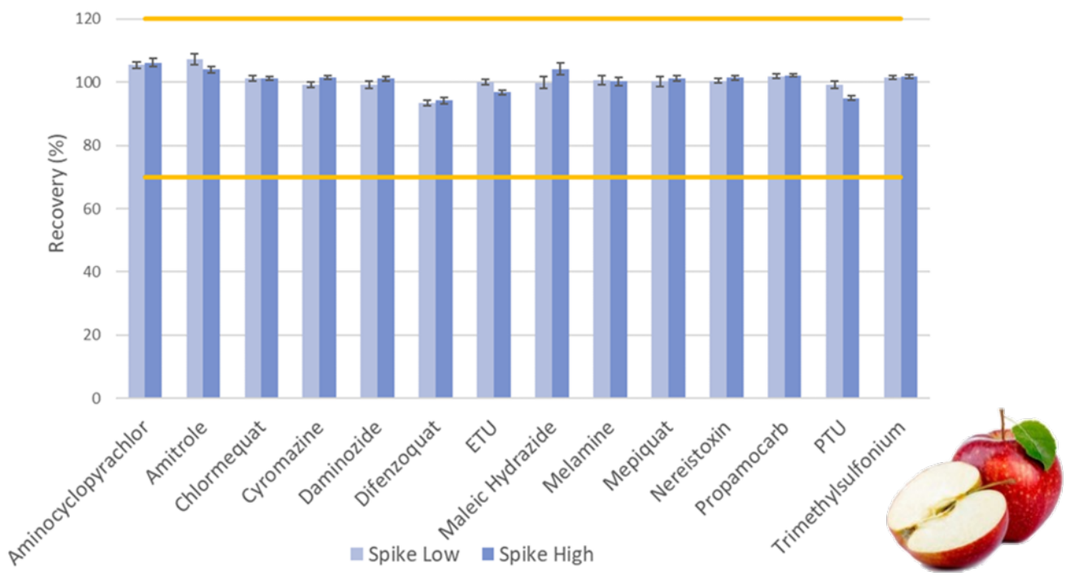


Figure 4. Recovery for the analytes tested in apple at each spike level (n=5). The orange bars represent SANTE recovery criteria, and the error bars represent RSD.

Method performance for trueness was 92 to 108% across all commodities with the exception of difenzoquat in cucumber (60 – 67%) where it was identified that PVDF filters were not suitable to use for this analysis. RSDs were all at or below 12%.

A FAPAS QC flour sample was extracted on two occasions in triplicate one month apart and all results were within 20% of the assigned value and within the range necessary to achieve an acceptable z-score. All calibration graphs had residuals below 20% and R^2 values of 0.99 or higher. Retention time stability across all the method validation study batches for all analytes was less than 3%.

The short method run time of 10 minutes and utilizing the QuPPe extraction method allows a high sample throughput for the analysis of the cationic polar pesticides in various food commodities investigated.

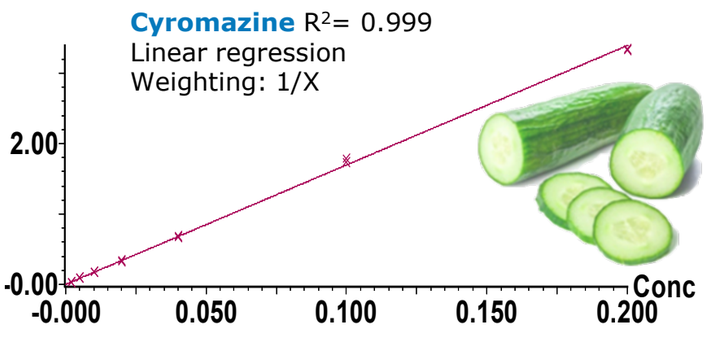


Figure 5. Matrix matched calibration curve of cyromazine in cucumber ranging 0.002 to 0.2 mg/kg.

CONCLUSIONS

- Single extraction (QuPPe) and LC-MS/MS method suitable for the determination of various highly polar cationic pesticides and plant growth regulators in cereals, fruit, and vegetable commodities to facilitate monitoring of MRL/tolerance compliance.
- Offers sufficient chromatographic retention, selectivity, peak shape, and stability to comply with SANTE guidelines.
- Sufficient LOQ demonstrated to determine residues at concentrations as low as 0.01 mg/kg.

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