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Determination of Cationic Polar Pesticides and Plant Growth Regulators Using UPLC-MS/MS with the ACQUITY UPLC BEH Amide Column

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Abstract

The purpose of this work is to demonstrate a single extract LC-MS/MS method for the determination of highly polar cationic pesticide residues and plant growth regulators in several food commodities that meet or exceed the MRL detection requirements in the European Commission pesticides database. The method performance study was completed on an ACQUITY UPLC I-Class System with a Xevo TQ-S micro using an ACQUITY UPLC BEH Amide Column after extraction following the QuPPe method. A method validation study was carried out on 4 representative commodities; namely apple, cucumber, flour, and potatoes. The method performance was assessed using 2 spike levels, 0.01 and 0.05 mg/kg for all analytes except maleic hydrazide which was spiked at 0.5 and 1.5 mg/kg with 5 replicates at each level. Difenzoquat and aminocyclopyrachlor were the only analytes not internally standardized. 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 lower than 3%.

Benefits

- Provides a 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
- Provides sufficient sensitivity to determine residues at concentrations as low as 0.01 mg/kg in crude extracts without cleanup

Introduction

There are various methods available to analyze food for pesticide residues focusing on multi-residue methods such as QuEChERS. The extraction and determination of polar, however, still remain a considerable

challenge. The QuPPe (Quick Polar Pesticides) method[1] has been developed by EURL-SRM (European Union Reference Laboratory – Single Residue Method) which allows for the simultaneous extraction of many highly polar pesticides, their metabolites, and plant growth regulators. The QuPPe method 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.

For the cationic polar pesticides in this study there are a variety of MRLs (see Table 1 for a selection) which range from default MRLs of 0.01 mg/kg to 7 mg/kg for chlormequat.[2] In addition, there are components that are not currently part of official EU MRL residue definitions such as melamine (metabolite of cyromazine), aminocyclopyrachlor, ETU, and PTU. The monitoring of their concentrations in food is still of interest for safety reasons.

Compound		MRL (mg/kg)			
		Apple	Potato	Wheat	Cucumber
Difenzoquat	Herbicide	0.01	0.01	0.01	0.01
Propamocarb	Fungicide	0.01	0.3	0.01	5.0
Cyromazine	Growth regulator	0.05	0.05	0.05	2.0
Nereistoxin	Insecticide	0.01	0.01	0.01	0.01
Mepiquat	Growth regulator	0.02	0.02	3.0	0.02
Chlormequat	Growth regulator	0.01	0.01	7.0	0.01
Amitrole	Herbicide	0.01	0.01	0.01	0.01
Trimethylsulfonium	Glyphosate counterion	0.05	0.05	5.0	0.05
Daminozide	Growth regulator	0.06	0.06	0.06	0.06
Maleic hydrazide	Growth regulator	0.2	60	0.2	0.2

Table 1. Current MRLs[2,3] in the four representative matrices for the compounds included in the EU pesticides database.

In this application note, example performance data is provided from Waters ACQUITY UPLC I-Class System and Xevo TQ-S micro on four commodities which represent high water content and high starch, low water content sample types. Organic wheat flour, cucumber, apple, and potato were extracted following the QuPPe method, to assess various performance factors of the UPLC-MS/MS method such as calibration linearity, retention time stability, method precision, trueness, and analyte identification.

Experimental

Sample Description

Organic apple, cucumber, and potato were purchased from a retail outlet and finely homogenized in the laboratory and stored at 4 °C in a fridge until analysis. Organic wheat flour was purchased and 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.

Method Conditions

Homogenized organic apple, cucumber, potato, and wheat flour were extracted using the QuPPe method as shown in Figure 1. For wheat flour additional steps were carried out, freezing for 2 hours at -20 °C and reduced sample mass was used (as outlined in the QuPPe method). The supernatant from the QuPPe extracts were then filtered using a 0.45 µm PTFE filter before analysis by LC-MS/MS.

Recovery spikes were carried out for all 4 commodities with 5 replicates at 0.01 mg/kg (0.5 mg/kg for maleic hydrazide) and 5 replicates at a higher level of 0.05 mg/kg (1.5 mg/kg for maleic hydrazide). Matrix matched standards were prepared in the respective blank extracts and were spiked after filtering. Calibration ranges for apples, cucumber, and potatoes were 0.002 to 0.2 mg/kg for all analytes except maleic hydrazide which had a calibration range of 0.1 to 2 mg/kg. For flour the calibration range values were 0.004 to 0.4 mg/kg for all analytes except for maleic hydrazide at 0.2 to 4 mg/kg. Quantification of spiked samples was by matrix matched bracketed calibration. The MRMs listed in Table 2 were used in this application for quantification and confirmation of residues.

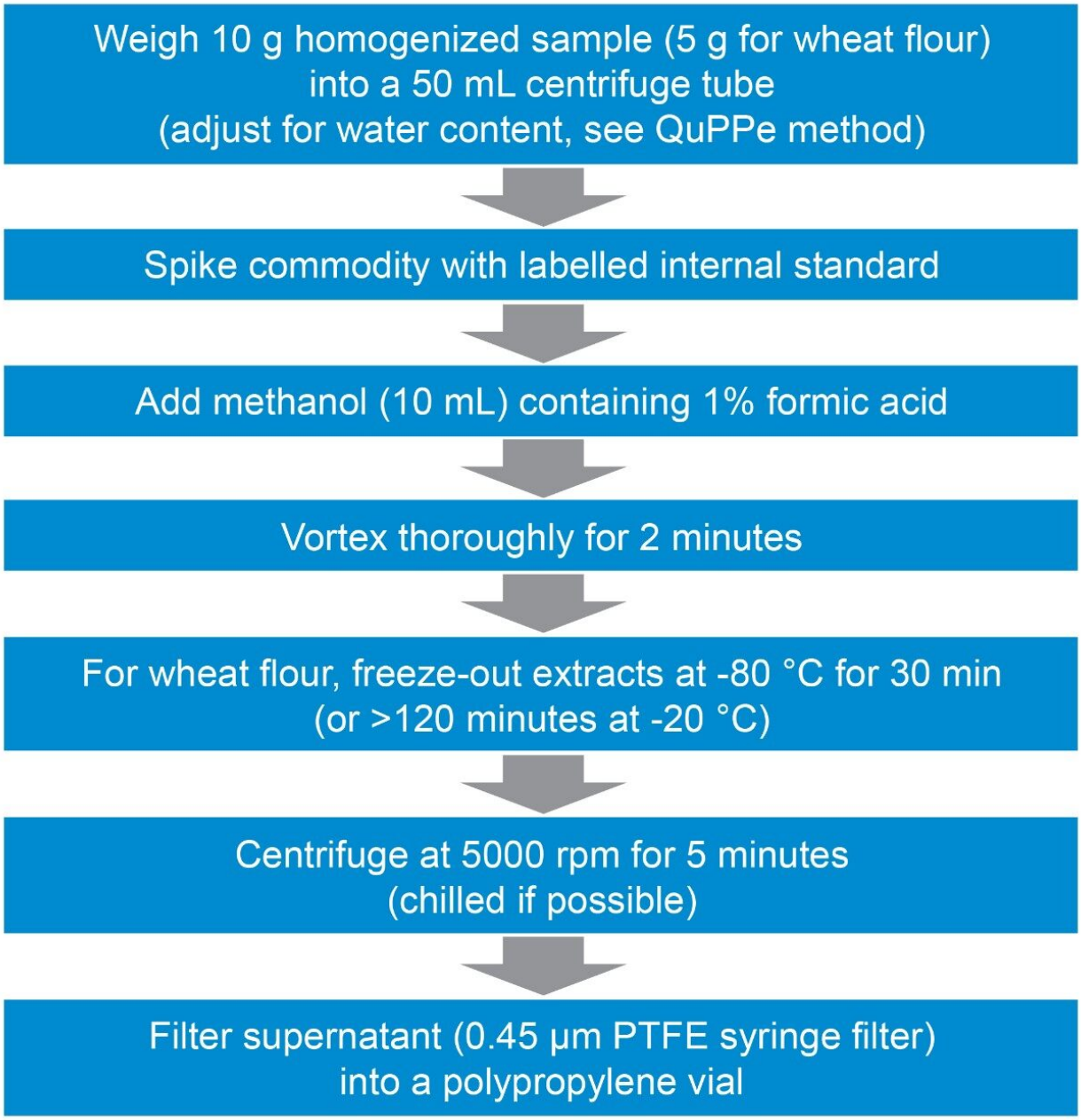

Figure 1. QuPPe sample extraction workflows for apple, cucumber, potato, and wheat flour.

LC Conditions

LC system:	Waters ACQUITY UPLC I-Class with fixed-loop Sample Manager

Detection:	Xevo TQ-S micro
Vials:	Waters Polypropylene 12 x 32 mm Snap Neck Vials, with Cap and Preslit PTFE/Silicone Septum, 700 µL (p/n 186005222)
Column(s):	ACQUITY UPLC BEH Amide, 1.7 µm, 2.1 × 100 mm (p/n 186004801)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	0.5 µL (partial loop needle overfill)
Flow rate:	0.5 mL/min
Mobile phase A:	20 mM ammonium formate (pH 2.95, adjusted with LC-MS grade formic acid)
Mobile phase B:	Acetonitrile

Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.5	3	97	6
0.50	0.5	3	97	6
4.00	0.5	30	70	4
5.00	0.5	40	60	6

Time (min)	Flow (mL/min)	%A	%B	Curve
6.00	0.5	40	60	6
6.10	0.5	3	97	6
10.00	0.5	3	97	6

MS Conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI+/ESI- for maleic hydrazide
Acquisition range:	MRM
Capillary voltage:	1.00 kV
Desolvation temperature:	600 °C
Desolvation gas flow:	1000 L/Hr
Source temperature:	150 °C
Cone gas flow:	150 L/Hr

MRM Transitions

Compound	Retention time (min)	MRM transition	Dwell time (sec)	Cone voltage (V)	Collision energy (eV)
Aminocyclopyrachlor	2.67	**214.1>168.0**	0.009	30	16
		214.1>196.0	0.009		13
Amitrole	1.95	**85.0>43.0**	0.014	30	15
		85.0>57.0	0.014		
Amitrole-^{15}N	1.95	86.0>43.0	0.014	30	15
Chlormequat	2.31	**122.1>58.1**	0.009	20	22
		122.1>63.1	0.009		18
Chlormequat-D_4	2.31	126.1>58.1	0.009	20	22
Cyromazine	2.27	**167.2>85.2**	0.009	30	20
		167.2>68.1	0.009		28
Cyromazine-D_4	2.28	171.1>86.1	0.009	30	20
Daminozide	1.86	**161.2>143.2**	0.014	30	10
		161.2>61.2	0.014		
Daminozide-D_6	1.86	167.2>149.2	0.014	30	10
Difenzoquat	1.92	**249.2>130.1**	0.014	30	40
		249.2>77.0	0.014		45
Ethylenethiourea (ETU)	0.69	**103.0>44.0**	0.014	30	13
		103.0>86.0	0.014		25
Ethylenethiourea-D_4 (ETU-D_4)	0.69	107.1>48.0	0.014	30	15
Propylenethiourea (PTU)	0.64	**117.1>58.1**	0.014	30	13
		117.1>60.1	0.014		20
N,N-(1,2-Propylene) thiourea-methyl-D_3	0.64	120.1>61.1	0.014	30	20
Maleic hydrazide	2.12	**111.0>82.0**	0.009	25	15
		111.0>83.0	0.009		12
Maleic hydrazide-D_2	2.12	113.2>85.1	0.009	25	12
Melamine	2.92	**127.0>60.0**	0.009	30	18
		127.0>85.0	0.009		16
Melamine-$^{15}N_3$	2.92	130.0>87.0	0.009	30	16
Mepiquat	2.44	**114.2>98.2**	0.009	30	20
		114.2>58.2	0.009		20
Mepiquat-D_{16}	2.44	130.3>110.2	0.009	30	24
Nereistoxin	0.95	**150.0>105.0**	0.014	30	15
		150.0>61.0	0.014		30
Nereistoxin-D_6	1.11	156.0>105.0	0.014	30	15
Propamocarb	2.40	**189.1>102.0**	0.009	30	16
		189.1>74.0	0.009		25
Propamocarb-D_7	2.40	196.1>103.0	0.009	30	16
Trimethylsulfonium	2.68	**77.1>62.1**	0.009	30	16
		77.1>47.1	0.009		10
Trimethylsulfonium-D_9	2.68	86.1>68.1	0.009	30	10

Table 2. MRM transitions of the analytes and respective isotopically labelled internal standards, optimum dwell time was set automatically using the auto-dwell function (quantitative transitions in bold).

Data Management

Informatics:	MassLynx v4.2

Results and Discussion

The MRMs listed in Table 2 highlight the optimized transitions used for quantification and confirmation of the cationic polar pesticides in this application. Auto dwell was used to calculate the dwell times. Using this feature, at least 12 points across the peak were acquired at the bottom calibration standard. The transition used for quantification is denoted in Table 2 in bold. For certain analyte/commodity combinations this was not always the most abundant transition, but was used to ensure consistency across the batches.

Method performance was assessed over 4 validation batches which covered the following commodities with a range of different properties; apples, cucumbers, flour, and potatoes. Each of these batches contained 5 spiked recoveries at two levels, 0.01 and 0.05 mg/kg (except maleic hydrazide where the spike levels were 0.5 and 1.5 mg/kg). Figure 2 demonstrates a typical chromatogram at the 0.02 mg/kg (0.5 mg/kg for maleic hydrazide) in a flour matrix matched calibration standard that was routinely achieved using this method during the method validation work. This level of sensitivity was achieved using a low injection volume of 0.5 µL which helps to mitigate matrix effects.

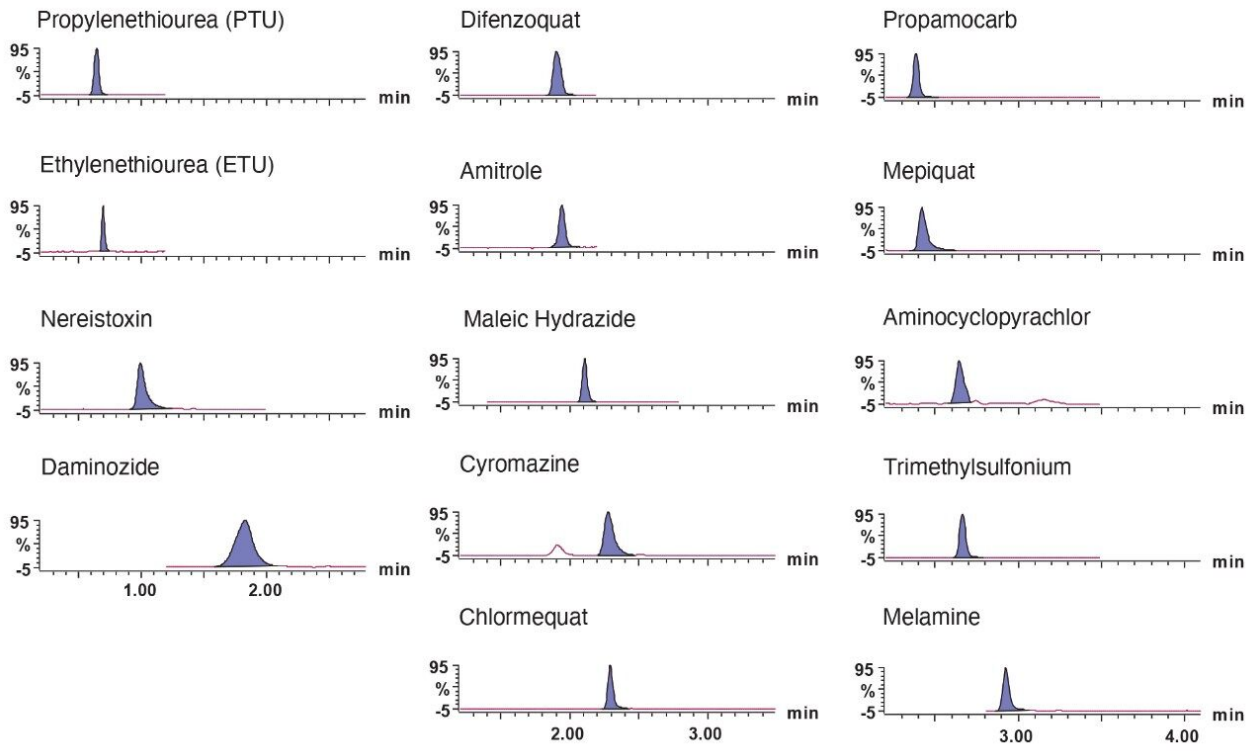

Figure 2. Example chromatograms for a 0.02 mg/kg (0.5 mg/kg maleic hydrazide) wheat flour matrix matched calibration standard.

As can be seen from Figure 2, ETU and PTU elute before 2 column void volumes, but upon investigation both showed a consistent Gaussian peak shape across all validation batches with good retention time stability. The effects of matrix on response of early eluting compounds was investigated for ETU and PTU. When matrix effects were calculated for PTU they ranged between 102–145% and for ETU 95–191% without internal standardization. For all analytes that labelled internal standards were available for, the use of these corrected for matrix effects and analyte recovery. For difenzoquat and aminocyclopyrachlor, no labelled internal standards were available and matrix effects ranged between 98 to 107% and 132 to 216% respectively. The results of the 4 validation batches are presented in Figure 3. Melamine was detected at significant levels in the cucumber blank material and the results have been excluded for this residue/commodity combination in the validation data.

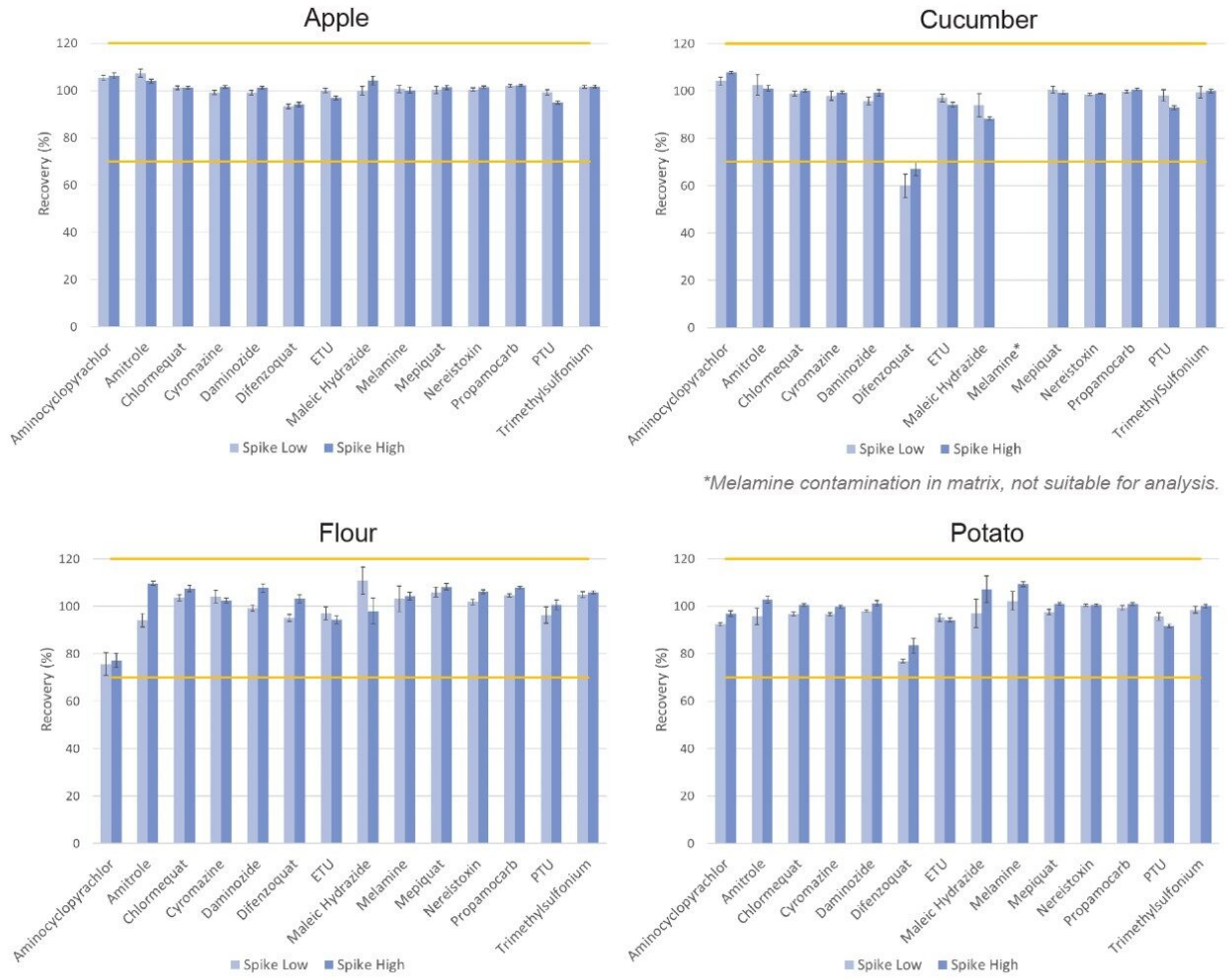

Figure 3. Validation batch data for apple, cucumber, flour, and potato at each spike level (n=5). The orange bars represent recovery criteria from SANTE[4] and the error bars represent RSD(%) at each level.

The results of the validation batches indicate that ETU and PTU gave acceptable performance with the use of the labelled internal standards. Aminocyclopyrachlor exceeded validation requirements without the need for a labelled internal standard. The low recoveries of difenzoquat were investigated and found to be an issue depending on which type of filters were used prior to LC-MS/MS analysis. Where PTFE filters (0.45 µm) were used, the validation results exceeded the requirements in the SANTE[4] method validation criteria. All other analytes met or exceeded method validation criteria.

Matrix matched calibration was used throughout the method validation study and examples of calibration graphs achieved are demonstrated in Figure 4. For all analytes, each calibration graph in the validation study displayed coefficients of determination of 0.99 or higher, with residuals all <20%. These values exceed the requirements for calibration as set out in SANTE guidelines.

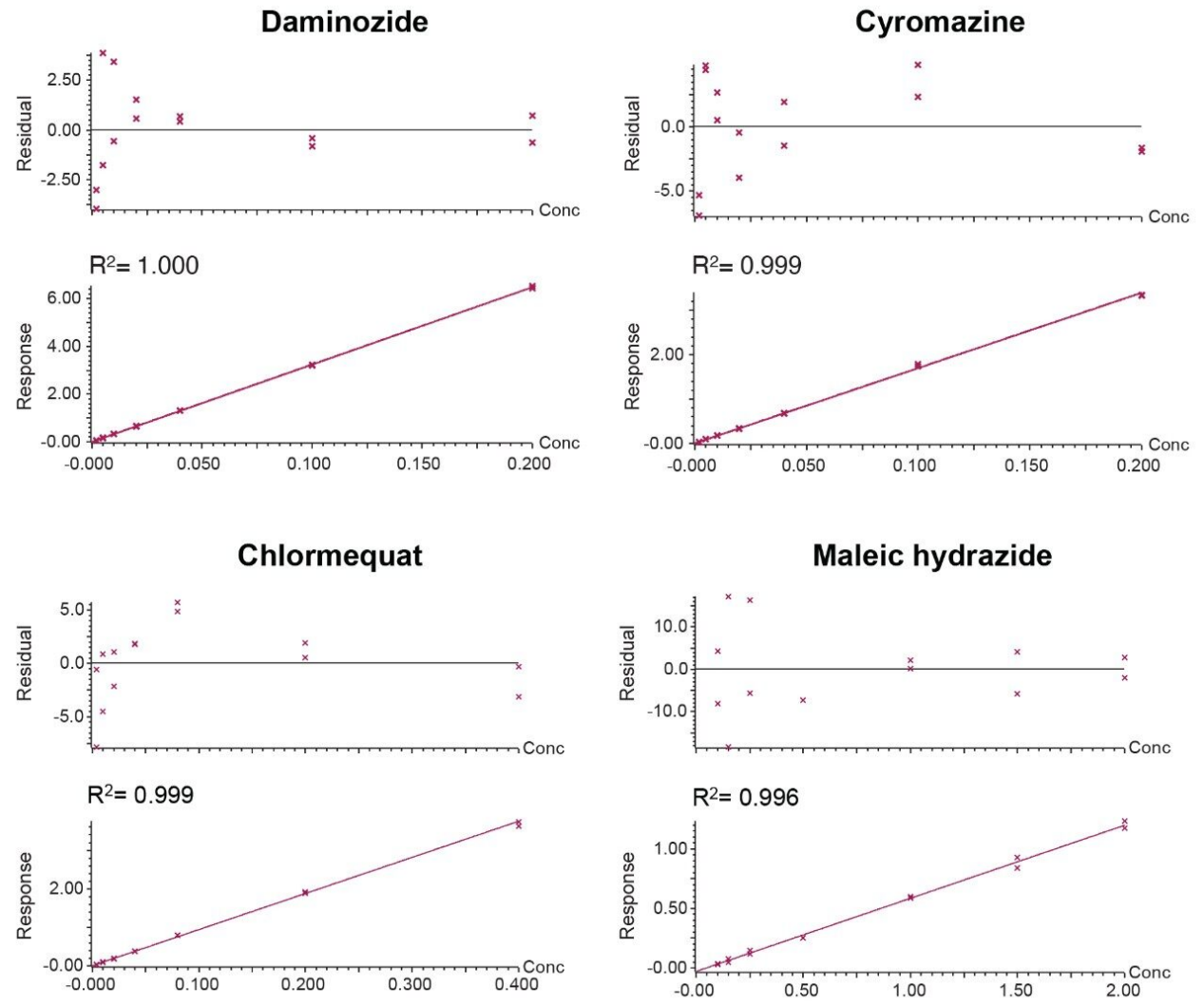

Figure 4. Bracketed matrix-matched calibration curves for daminozide in apple and cyromazine in cucumber at 0.002 to 0.2 mg/kg, chlormequat in wheat flour at 0.004 to 0.4 mg/kg, and maleic hydrazide in potato at 0.1 to 2 mg/kg.

The results were assessed in accordance with SANTE guidelines for identification and all analytes in the recovery samples confirmed by both ion ratio and retention time. Figure 5 displays the retention times for all analytes across the 4 method validation batches and demonstrates retention time stability during the validation studies. Additional retention time stability tests were conducted on the wheat flour sample where 200 injections of a 0.02 mg/kg matrix calibration standard were run without operator intervention. Figure 6 demonstrates the 1st and 200th injection of selected analytes. The retention times were found to be stable and there was no significant change in the observed peak shape.

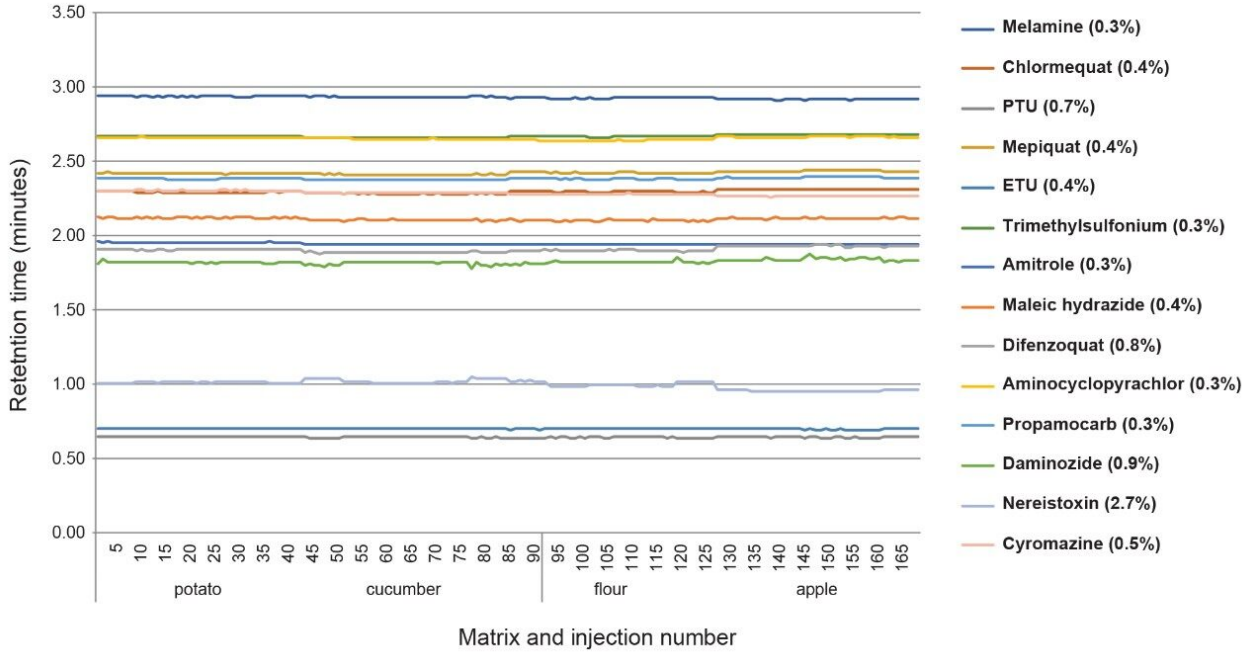

Figure 5. Retention time stability across four separate batches and commodities (RSD% values across whole set given in brackets).

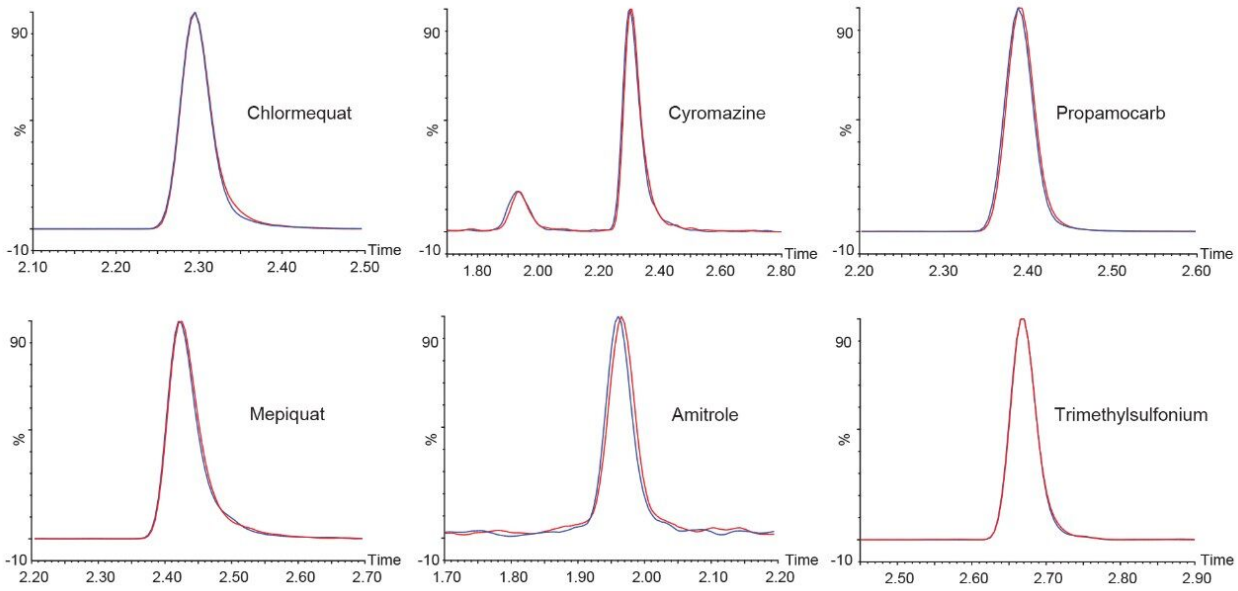

Figure 6. Example chromatography of the 1st and 200th injection (blue and red traces, respectively) of an organic wheat flour matrix standard, 0.02 mg/kg (5 ng/mL in vial concentration). These were a set of continuous injections without any intervention by the operator. Retention time RSDs for all compounds across the whole run were within 0.3%.

The method was tested with a FAPAS QC sample (T09127, wheat flour) containing chlormequat and mepiquat where the sample was extracted on two occasions in triplicate one month apart. The results obtained from this work are shown in Table 3. All the replicates fall within the range for the QC material with the average concentration within 20% of the assigned value for both analytes and are within range for an acceptable z-score. The within laboratory repeatability (RSD) was <2% for the 6 replicates. Both analytes were confirmed by retention time and by ion ratio in all replicates as per SANTE guidelines.[5]

	Assigned value (µg/kg)	Range for |z| ≤2 (µg/kg)	Calculated concentration (µg/kg)	Average calculated concentration (µg/kg)	Difference from assigned (%)	RSD (%)
Chlormequat	210	125–295	180	177	-15.6	1.8
			173			
			179			
			182			
			179			
			181			
Mepiquat	100	56–144	81.1	80.5	-19.5	1.7
			78.6			
			81.7			
			79.3			
			82.1			
			81.0			

Table 3. Results from analysis of FAPAS T09127QC (wheat flour).

Conclusion

The method validation study results demonstrate a robust analytical method for the determination of cationic polar pesticides using the established QuPPe extraction protocol with analysis using an ACQUITY UPLC BEH Amide Column fitted in an ACQUITY UPLC I-Class PLUS System coupled to a Xevo TQ-S micro. The results for most compounds exceed the requirements for method validation in the SANTE guidelines. The trueness and precision of this LC-MS/MS method determined at two matrix QC levels with 5 replicate injections was found to be acceptable for all compounds. Retention time stability and robustness was proven over the course of the study with RSDs for all compounds under 2% across all method performance batches. In most cases, limits of quantification surpass the MRL requirements. 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.

Scientists must validate the method in their own laboratories and demonstrate that the performance is fit for purpose and meets the needs of the relevant analytical control assurance system.

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