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Nota applicativa

Development and Validation of a Routine Multi-Residue Method for the Quantitative Determination of Pesticide Residues in Fruits, Vegetables, and Rice Using UPLC-MS/MS

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Abstract

This application note describes the performance of a multi-residue method for LC amenable pesticides in food commodities belonging to commodity groups 1 and 5 defined under SANTE/11813/2017 by UPLC-MS/MS on an ACQUITY UPLC I-Class PLUS System coupled to the Xevo TQ-S cronos Tandem Quadrupole Mass Spectrometer using generic MS parameters obtained from the Quanpedia database.

Benefits

- Reliable, quantitative, multi-residue method for the routine analysis of pesticides in food commodities with simplified QuEChERS (DisQuE dispersive sample preparation) sample preparation for compliance with regulatory limits and method performance guidelines.
- Demonstrate robust performance in complex matrices maximizing instrument uptime.

Introduction

This application note describes the development and validation of a robust, quantitative method for the routine determination of a wide range of LC-amenable pesticides following QuEChERS (DisQuE dispersive sample preparation) procedure and LC-MS/MS using a Waters ACQUITY UPLC I-Class PLUS System coupled to the Xevo TQ-S cronos Tandem Quadrupole Mass Spectrometer.

In addition to the efficiency benefits (cost, time, and labor), multi-residue pesticide methods can address challenges associated with global trade and regulatory issues in different countries when it comes to pesticide use and misuse, regulatory limits, or pesticide residue definitions. It is the strategy of choice for laboratories performing routine surveillance monitoring.

The Xevo TQ-S cronos instrument has been developed as a reliable system for routine quantitative analysis, incorporating sample cone design elements that were previously utilized in the extremely popular ACQUITY QDa Mass Detector. As part of the reverse cone design, the narrowest restriction is in the center of the cone, whereas the entrance to the cone is comparatively wide. Thus, ensuring that sample matrix and mobile-phase buffer salts will not aggregate and block the orifice. This design helps increase the up-time of the instrument between cone cleans and provides reliable sensitivity in food matrices.

Here we demonstrate that the performance of the Xevo TQ-S cronos System, in terms of sensitivity, LOQ, robustness, precision in complex matrices, acquisition speed (fast scanning rates), polarity switching, and dynamic range is suitable for the simultaneous quantitative determination of many pesticides required to check compliance with EU MRLs.

Experimental

Sample preparation

Samples of organic cucumber, tomato, red pepper, green pepper, and brown rice belonging to commodity groups 1 (high water content) and 5 (high starch) of the SANTE guidelines, respectively, were sourced from a retail outlet and screened to ensure they were free of target pesticide residues. The selection of commodities was based on the products to be sampled in the European Union's coordinated multiannual control plan 2017/660.[1]

Step 1. CEN QuEChERS extraction

High aqueous samples - Ten grams of comminuted sample [cucumber, tomato, capsicum] and 10 mL of acetonitrile were placed into a 50 mL centrifuge tube, vortex mixed for 20 seconds, and vigorously shaken for one minute.

High starch samples - Five grams of ground cereal grain [brown rice] and 10 mL of ultrapure water were placed into a 50 mL centrifuge tube and left for 10 minutes for reconstitution. Ten milliliters of acetonitrile were added, followed by vortex mixing for 20 seconds, and shaking for one minute.

The contents of DisQuE pouch for CEN QuEChERS (p/n: 186006813) was added to each centrifuge tube and the contents shaken for one minute. The extraction mixture was centrifuged at 5000 rpm (4200 g) for five minutes at ambient temperature. An aliquot of the supernatant was removed for subsequent cleanup.

Step 2. dSPE cleanup for base/neutral multi-residue LC amenable pesticides

The QuEChERS supernatant (between 1–6 mL) was then transferred to a 15 mL dSPE tube [p/n: 186008072] containing 1200 mg $MgSO_4$ and 400 mg PSA and shaken for 30 seconds. The dSPE tube was then centrifugated at ≥5000 rpm (4200 g) for five minutes at ambient temperature.

Step 3. Dilute the extract with mobile phase A

A 1:10 dilution for the acetonitrile extract was performed, e.g. 100 µL of the acetonitrile extract from Step 2 and combined with 900 µL mobile phase A (5 mM ammonium formate in water).

Matrix matched calibration standards were prepared via spiking blank matrix post extraction as follow: Take 100 µL of the acetonitrile extract, add 875 µL mobile phase A (5 mM ammonium formate in water) and 25 µL 204 pesticide spiking solution at 40 ng/mL to give solution concentration of 1 ng/mL (equivalent to 0.01 mg/kg in matrix).

The details of 204 pesticide spiking solution including the compound names and molecular formulae can be found in the LC Multiresidue Pesticide Standards Kit Care and Use Manual (p/n: 720005342EN).[2]

UPLC-MS/MS

System:	ACQUITY UPLC I-Class PLUS System
Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 × 100 mm (p/n: 1860003539)
Mobile phase A:	5 mM ammonium formate in water + 0.1% formic acid
Mobile phase B:	5 mM ammonium formate in 50:50 MeCN: MeOH + 0.1% formic acid
Flow rate:	0.5 mL/min
Injection volume:	3 µL
Column temp.:	45 °C
Sample temp.:	10 °C
Run time:	19 min

Time	Flow rate	% A	% B	Curve
Initial	0.5	99.0	1.0	Initial
0.5	0.5	99.0	1.0	6
3.50	0.5	60.0	40.0	6
12.50	0.5	15.0	85.0	6
12.60	0.5	1.0	99.0	6
15.00	0.5	1.0	99.0	6
15.10	0.5	99.0	1.0	6
19.00	0.5	99.0	1.0	6

MS instrument:	Xevo TQ-S cronos
Ionization:	Electrospray
Polarity:	+/-
Capillary voltage:	+0.4/-0.54 kV
Desolvation temp.:	600 °C
Desolvation gas flow:	1000 L/Hr
Source temp.:	150 °C
Cone gas flow:	0 L/Hr
Collision gas (argon) flow:	0.14 mL/min

The data were acquired using MassLynx 4.2 Software and processed using TargetLynx XS Application

Manager. The UPLC method, MRM transitions, and compound-specific MS parameters (cone voltage and collision energy) for the 204 pesticides were taken from the relevant Quanpedia Database to automatically create the acquisition and processing methods. For comparison purposes, a selection of representative pesticides spanning the physiochemical diversity within the multi-residue suite were manually tuned on the Xevo TQ-S cronos. In all cases, the manually optimized parameters were in close agreement to the Quanpedia Database values.

The source conditions (capillary voltage and ESI probe position) were optimized to favor mid-mass range compounds via infusion of a standard solution of 10 representative pesticides in both ESI^- and ESI^+ polarities. A mid-mass-range, mid-polarity compound (picoxystrobin) from the multi-reside suite was used for ESI probe optimization purposes. The auto-dwell functionality in the MS experimental method was used for all 204 compounds and set based on the width of the narrowest chromatographic peak (c. 3 seconds), achieving between 12–25 points across all peaks.

Results and Discussion

The LC-MS/MS multi-residue method performance was assessed in accordance with the relevant guidelines in SANTE/11813/2017 for quantitative methods.[3] The scope of the method includes commodity group 1 (high water content vegetable and fruits) and commodity group 5 (high starch and/or protein content and low waters and fat content). For the purposes of this application note, a representative selection of over 40 analytes were used to demonstrate the performance of the LC-MS/MS method against the validation parameter criteria. These representative analytes were selected based on (a) spanning the physiochemical diversity; (b) including those defined in the coordinated multiannual control plan 2017/6601; and (c) a selection of pesticides reported as border rejections in 2019 under the European Rapid Alert System for Food Feed.[4] In addition, the identification criteria for tandem quadrupole systems operated in MRM acquisition mode was met throughout. For all representative analytes in matrix extracts, a minimum of two product ions were acquired for each analyte, detected with S/N ≥3, showing fully overlapping extracted ion chromatograms and achieving ion ratios within ±30% of those of the averaged calibration standards. Retention time for the first eluting analyte (cyromazine) is greater than two times the time corresponding to the void volume of the analyte column. The retention time of the analytes in matrix was found to be within ±0.1 minute of the matrix matched standards. Figure 1 shows typical chromatography for the representative analytes spiked into cucumber extract at 10 ng/mL (0.01 mg/kg), injected in 90% aqueous and 10% acetonitrile. Gaussian peaks, with widths of between 3–6 seconds were obtained across the elution profile.

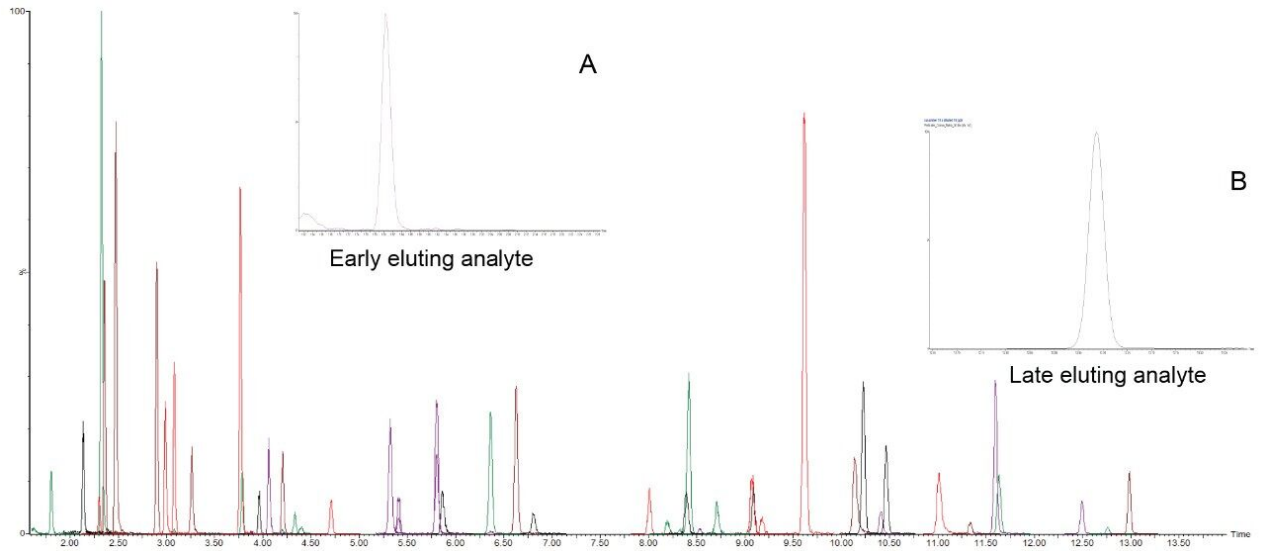

Figure 1. Typical chromatographic peak shapes for a selection of representative analytes across the elution profile in cucumber matrix matched standard at 1 ng/mL (equivalent to 0.01 mg/kg). Insets show zoom of (A) early and (B) late eluting compounds.

Matrix Effects

The effect of the matrix on LC-MS response was investigated in the five commodities included in the scope of the validation. Matrix matched standards were prepared at a concentration of 0.5 ng/mL (equivalent to 0.5x "default" 0.01 mg/kg MRL typically used for multi-residue pesticide analysis) and the peak areas obtained from the quantitative ion transition were compared to those obtained from a solvent standard at the same concentration and expressed as a percentage difference. The results (Figure 2) reveal that both ion enhancement and suppression effects are evident depending on the matrix/analyte combination. For the majority of compounds assessed (>80%) the deviation is within ±20% of the solvent standard response. Larger % deviations (in the range of 20–28%) are observed for the higher mass ions (emmamectin *m/z* 886 and spinetoram J *m/z* 748) for this reason, the use of matrix matched calibration standards is recommended for accurate quantification within this multi-residue method.

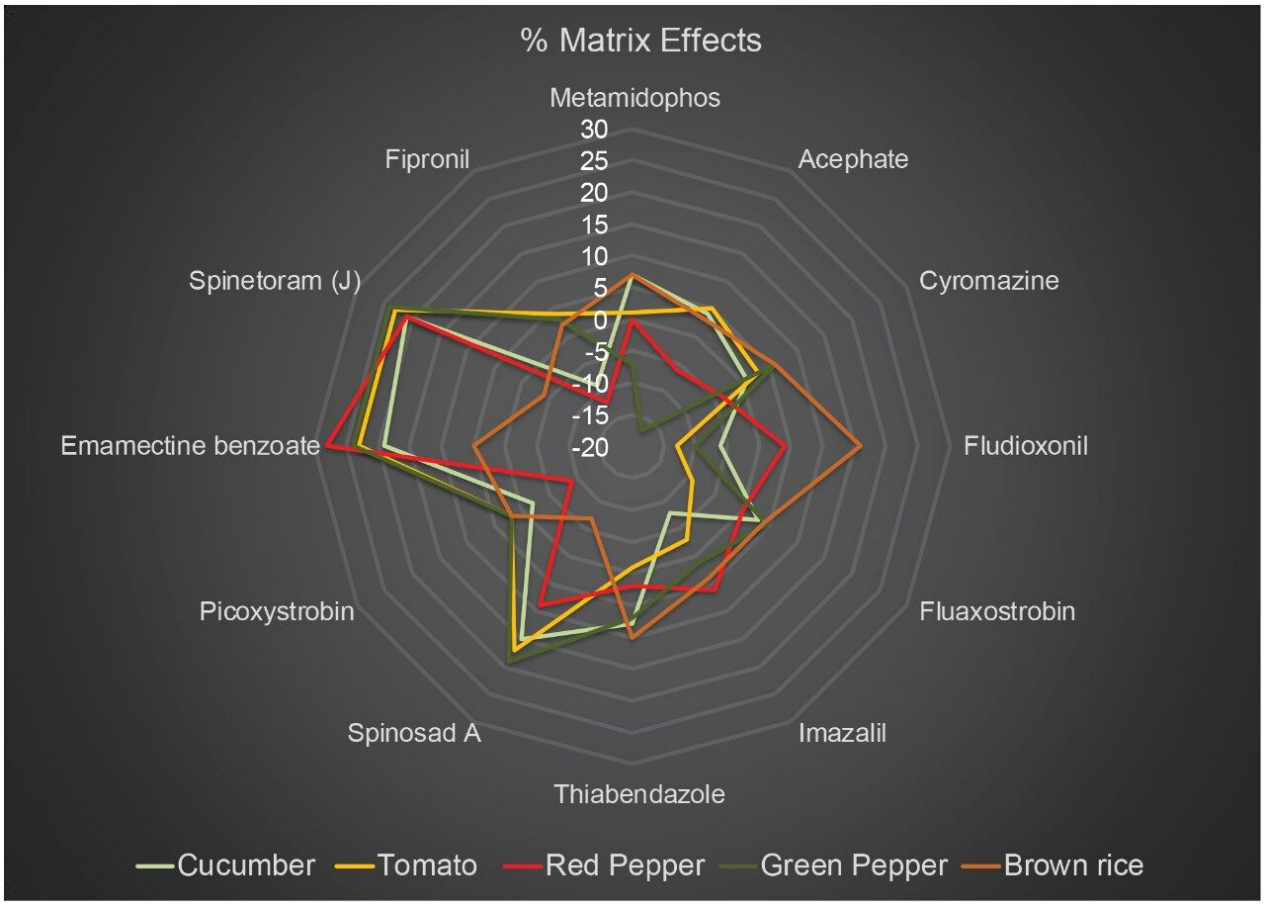

Figure 2. Combined representation of the mean % matrix effects in matrix match standards of cucumber, red pepper, green pepper, tomato, and brown rice (n=3) spiked at 0.5 ng/mL (equivalent to 0.005 mg/kg).

Sensitivity and Linearity

Matrix matched standards (MMS) were prepared in both cucumber and brown rice extract at seven concentrations (0.1, 0.25, 0.5, 1.0, 2.5, 5.0, and 10.0 ng/mL) equivalent to residue concentrations in matrix spanning the range between 0.001 to 0.1 mg/kg to determine the linear working range of the method.

The linear range for the representative compounds achieving an R^2 value of 0.99 (or greater) with a deviation of back calculated concentration from the true concentration (TargetLynx %residuals) of <20% is shown in Table 1. Example MMS curves in cucumber extract, and the extracted ion chromatograms for two transitions for a representative ESI^+ compound (acephate) and ESIcompound (fipronil) spiked at the "default" MRL equivalent concentration are shown in Figures 3A and 3B, respectively.

Compound name	Retention time (min)	Polarity	MRM *m/z*	Linear calibration range (mg kg^{-1})	Slope	Cucumber LOQ (mg kg^{-1})	Brown rice LOQ (mg kg^{-1})
Acephate	2.1	ESI+	183.9>142.9 (183.9>125.0)	0.001=0.1	1296.3	<0.005	<0.005
Acetamiprid	4.0	ESI+	223.0>126.0 (223.0>56.1)	0.001=0.1	537.6	<0.005	<0.005
Azoxystrobin	8.0	ESI+	404.0>329.0 (404.0>372.0)	0.001=0.1	769.6	<0.005	<0.005
Bupirimate	9.1	ESI+	317.0>108 (317.0>166.0)	0.001=0.1	999.2	<0.005	<0.005
Carbendazim	3.0	ESI+	192.1>160.0 (192.1>132.1)	0.001=0.1	2853.5	<0.005	<0.005
Carbofuran	5.4	ESI+	222.1>165.1 (222.1>123.0)	0.001=0.1	470.0	<0.005	<0.005
Cypronidil	8.7	ESI+	226.0>93 (226.0>108.0)	0.001=0.1	548.8	<0.005	<0.005
Cyproconazole I	8.0	ESI+	292.2>70.2 (292.2>125.0)	0.005=0.1	219.3	0.005	0.005
Cyromazine	1.8	ESI+	167.0>68.1 (167.0>55.04)	0.0025=0.1	619.3	<0.005	<0.005
Dimethoate	3.9	ESI+	230.1>125.0 (230.1>199.0)	0.005=0.1	44.3	**<0.010**	**<0.010**
Emamectine benzoate	11.7	ESI+	886.6>158.0 (886.6>126.0)	0.0025=0.1	1771.4	<0.005	<0.005
Ethirimol	4.1	ESI+	210.1>140.0 (210.1>98.0)	0.0025=0.1	995.6	<0.005	<0.005
Fenamidone	8.0	ESI+	312.1>92.0 (312.>236.1)	0.0025=0.1	910.8	<0.005	0.005
Fenazaquin	12.5	ESI+	307.2>57.2 (307.2>161.0)	0.001=0.1	3484.5	<0.005	<0.005
Fipronil	9.5	ESI-	434.8>329.9 (434.8>249.8)	0.0025=0.1	218.1	<0.005	<0.005
Fluaxostrobin	9.1	ESI+	459.0>427.0 (459.0>188.0)	0.001=0.1	902.8	<0.005	<0.005
Fludioxonil	7.5	ESI-	247.0>180.0 (247.0>126.0)	0.005=0.1	78.9	0.005	0.005
Flusilazole	9.0	ESI+	316.0>247.0 (316.0>165.0)	0.005=0.1	143.2	0.005	0.005
Formetanate	2.3	ESI+	222.0>165.0 (222.0>46.0)	0.001=0.1	4828.8	<0.005	<0.005
Imazalil	5.9	ESI+	297.0>69.0 (297.0>159.0)	0.001=0.1	724.0	<0.005	<0.005
Iprovalicarb I/II	8.4	ESI+	321.1>119.1 (321.1>203.1)	0.001=0.1	3405.8	<0.005	<0.005
Mepanipyrim	8.3	ESI+	224.1>106 (224.1>77.0)	0.001=0.1	903.8	<0.005	<0.005
Metalaxyl	6.7	ESI+	280.1>220.1 (280.1>192.1)	0.001=0.1	2115.9	<0.005	<0.005
Methamidophos	1.8	ESI+	142.0>93.9 (142.0>124.9)	0.001=0.1	672.3	<0.005	<0.005
Monocrotophos	3.1	ESI+	224.1>127.1 (224.1>109.0)	0.005=0.1	2537.1	<0.005	<0.005
Omethoate	2.4	ESI+	214.1>183.1 (214.1>125.1)	0.001=0.1	1993.6	<0.005	<0.005
Oxamyl	2.9	ESI+	237.0>72 (237.0>90.0)	0.001=0.1	3491.5	<0.005	<0.005
Penconazol	9.2	ESI+	284.0>70.1 (284.0>159.0)	0.005=0.1	485.4	0.005	0.005
Picoxystrobin	9.6	ESI+	368.0>205.1 (368.0>145.1)	0.001=0.1	4385.0	<0.005	<0.005
Pirimicarb	4.9	ESI+	239.1>72 (239.1>182.1)	0.001=0.1	6007.4	<0.005	<0.005
Procloraz	9.7	ESI+	375.8>307.9 (375.8>70.1)	0.0025=0.1	492.3	<0.005	<0.005
Pymetrozine	2.3	ESI+	218.0>105 (218.0>79.0)	0.0025=0.1	492.4	<0.005	<0.005
Pyridaben	13.0	ESI+	365.1>147.1 (365.1>309.1)	0.001=0.1	892.9	<0.005	<0.005
Pyrimethanil	6.8	ESI+	200.0>82 (200.0>107.0)	0.0025=0.1	295.9	<0.005	<0.005
Propiconazole	9.6	ESI+	342>69.0 (342.0>159.0)	0.005=0.1	298.6	0.005	0.005
Pyriproxifen	11.6	ESI+	322.1>96.0 (322.1>227.1)	0.001=0.1	4050.1	<0.005	<0.005
Quinoxyfen	11.4	ESI+	308.0>197.0 (308.0>161.9)	0.005=0.1	254.3	<0.005	<0.005
Spinosad A	10.2	ESI+	732.6>142.0 (732.6>98.1)	0.001=0.1	2440.1	<0.005	<0.005
Spinetoram (J)	11.0	ESI+	748.5>142.1 (748.5>98.1)	0.001=0.1	2210.8	<0.005	<0.005
Spiromesifen	12.8	ESI+	371.1>273.1 (371.1>255.1)	0.005=0.1	111.1	**<0.010**	**<0.010**
Tebuconazole	9.2	ESI+	308.0>70.1 (308.0>125.0)	0.005=0.1	517.6	0.005	0.005
Thiabendazole	3.3	ESI+	202.0>175.0 (202.0>131.0)	0.001=0.1	552.8	<0.005	<0.005
Thiamethoxam	3.3	ESI+	292.0>211.2 (292.0>132.0)	0.001=0.1	387.0	<0.005	<0.005
Tricyclazole	4.2	ESI+	190.0>163.0 (190.0>136.0)	0.001=0.1	912.7	<0.005	<0.005

Table 1. LC-MS/MS parameters, linear dynamic range (LDR) and estimated LOQ for the representative pesticides in cucumber and brown rice extract.

**SANTE guidelines LOQ is the lowest spike level meeting the method performance criteria for trueness and precision (%RSD <20 from repeatability data).*
LDR (0.1–10 ng/mL), % residual deviation <20%, and R^2 (>0.99).

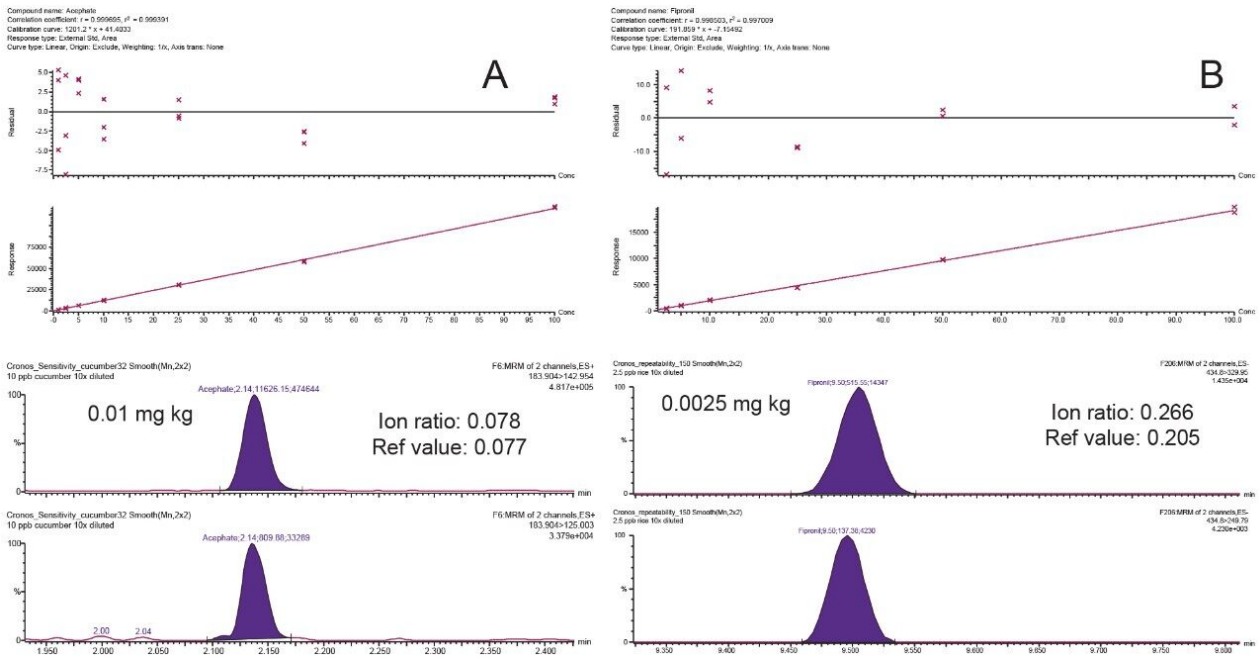

Figure 3. Linearity in extract spiked between 1–100 ng/mL (0.001- 0.1 mg/kg): (A) ESI^+ compound acephate in cucumber, and (B) ESI^- compound fipronil in brown rice. Extracted ion chromatograms for two transitions.

The method trueness, defined as the mean recovery using the DisQuE extraction protocol have been previously determined and found to be within the acceptable range of 70–120% in a wide range of commodities and was not investigated as part of the scope of this study.[5]

The method LOQ for the representative analytes in both cucumber and brown rice was estimated using the inter-day (three consecutive days) repeatability data and defined as the lowest matrix-matched standard achieving a relative standard deviation (RSD) of ≤20% (n=18). All the LOQs were found to be ≤MRL of 0.01 mg/kg and for 93% of representation analytes the estimated LOQ values are equivalent to a matrix concentration of ≤0.5× default MRL. Where possible, the S/N at the LOQ concentration was calculated and found to be >10:1 in all cases.

Testing laboratories often set the reporting limit (RL) for multi-residue methods at 0.5× MRL across the scope of target pesticides. The observed sensitivity suggests that detection and quantification for many

pesticides at concentrations equivalent to, or lower than 0.5× default MRL in matrix is possible.

Specificity

Reagent blanks and matrix blanks for cucumber, tomato, red/green pepper, and brown rice were analyzed and the response (peak area) in both the quantifier and qualifier MRM transitions was investigated for the representative compounds. No significant interferences (≤30% of RL) in either of the quantifier or qualifier transitions were observed and the relevant retention times.

Measurement Precision

The measurement repeatability was investigated by repeated injection (n=6) of cucumber and brown rice extract spiked at three concentrations, 0.5, 1, and 10 ng/mL equivalent to matrix concentrations of 0.005, 0.01, and 0.1 mg/kg, respectively. The repeatability experiment was performed on three different days giving replicate measurements (n=18) per matrix/spiking concentration level. The results in Figure 4 for cucumber (A) and for brown rice (B) show that the within-lab reproducibility of the LC-MS/MS method was ≤20% at the default MRL equivalent concentration.

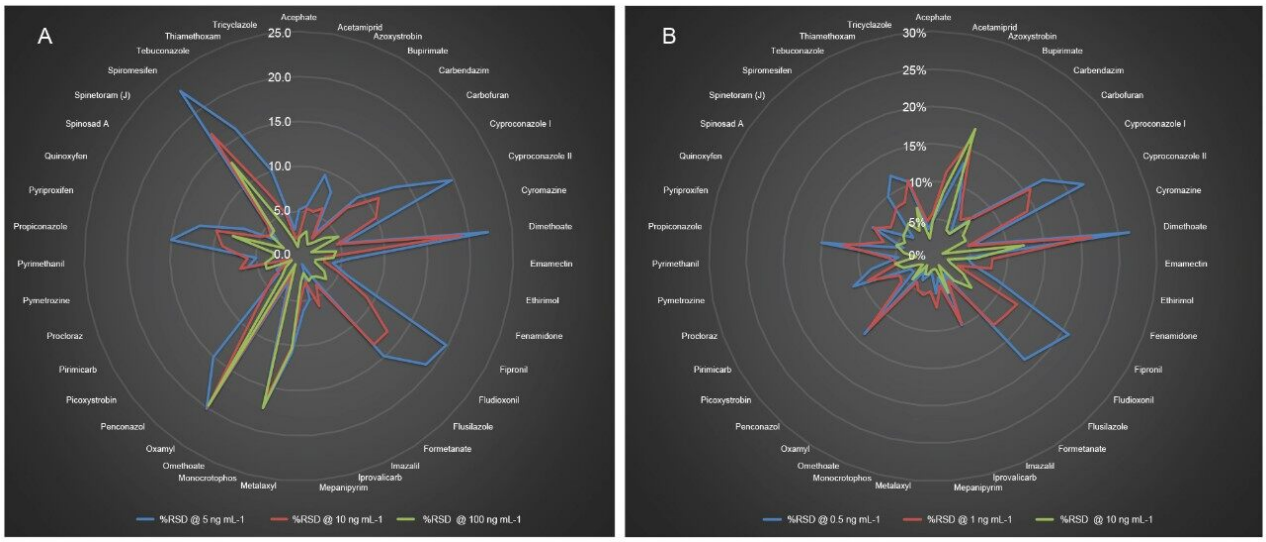

Figure 4. RSDwr inter-day reproducibility (n=18) for a selection of representative pesticides spiked into (A) cucumber extract and (B) brown rice at 5, 10, and 100 ng/mL (equivalent to 0.005, 0.01, and 0.1 mg/kg).

Measurement Robustness

The robustness of the instrument, following repeated injections of a matrix matched standard at 1 ng/mL (equivalent to the MRL concentration in matrix), was investigated in cucumber. Over 200 consecutive 3 µL

injections using the 19-minute LC gradient were performed with no user intervention. This represents a continuous running time equal to 67 hours (2.8 days) and total matrix load of over 60 mg following the 207 injections. A control chart showing the peak area for the quantitative ion transitions of four analytes (oxamyl, metalaxyl, monocrotophos, and spinosad A) were plotted in TrendPlot. Figure 5 shows the peak area within the control limits (±3 standard deviations of the running mean) and shows overall %RSDs of ≤3.

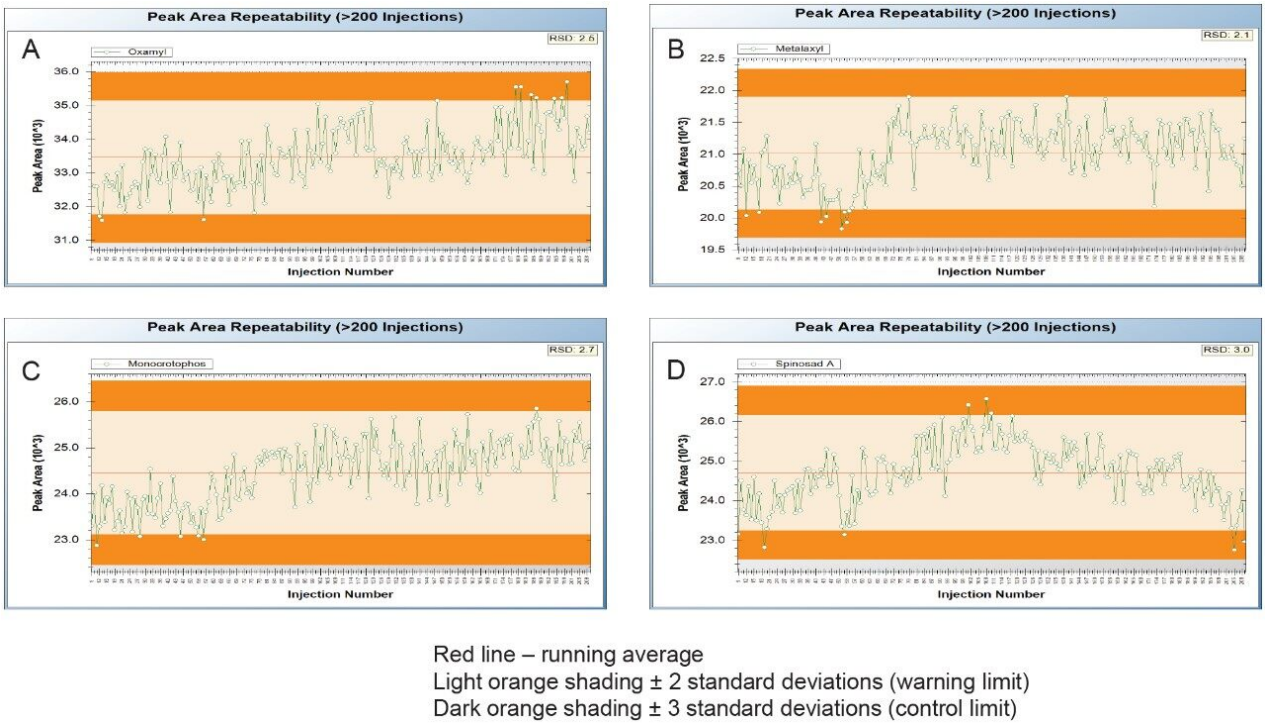

Figure 5. (A) Oxamyl, (B) metalaxyl, (C) monocroptophos, (D) spinosad A quantitative ion (ES^+ 237.0>72.0) peak area repeatability following >200 consecutive injections in cucumber extract spiked with 204 pesticide mix at 1 ng/mL (equivalent to 0.01 mg/kg). Representing over 72 hours continuous analysis time without operator intervention.

Conclusion

This application note describes the performance of a multi-residue method for LC amenable pesticides in food commodities belonging to commodity groups 1 and 5 defined under SANTE/11813/2017 by UPLC-MS/MS on an ACQUITY UPLC I-Class PLUS System coupled to the Xevo TQ-S cronos Tandem Quadrupole Mass Spectrometer using generic MS parameters (including polarity switching) obtained from the

Quanpedia database. The results of our internal validation indicate that the method performance meets regulatory guidelines for official control and due diligence testing of pesticides. Calibration characteristics, linearity, sensitivity, and within-lab reproducibility were all shown to be suitable for use with CEN QuEChERS for checking compliance with EU MRLs for pesticide residues. Furthermore, the system is shown to be reliable in routine operation with minimal requirements for user intervention during extended periods of analysis.

Footnote

Analysts must validate the method in their own laboratories and demonstrate that the method's performance is fit-for-purpose and meets the needs of the relevant analytical control assurance systems.

References

1. Regulation (EU) 2017/660 concerning a coordinated multiannual control programme of the Union for 2018, 2019 and 2020 to ensure compliance with maximum residue levels of pesticides and to assess the consumer exposure to pesticide residues in and on food of plant and animal origin. (2017) *Official Journal of the European Union*, L94/12–24.
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