DEVELOPMENT AND VALIDATION OF MULTI-RESIDUE PESTICIDES METHOD FOR ROUTINE ANALYSIS OF FOOD SAMPLES USING UPLC-MS/MS

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INTRODUCTION

Multi-residue pesticides methods can pose beside cost, time and labor other challenges associated with global trade and regulatory issues in different countries when it comes to pesticides use and misuse, regulatory limits, or pesticide residue definitions. It is strategy of choice for laboratories performing routine surveillance monitoring. Here we demonstrate that the performance of the UPLC-MS/MS method in terms of sensitivity, LOQ, robustness, precision in food matrices (vegetables, rice), 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.

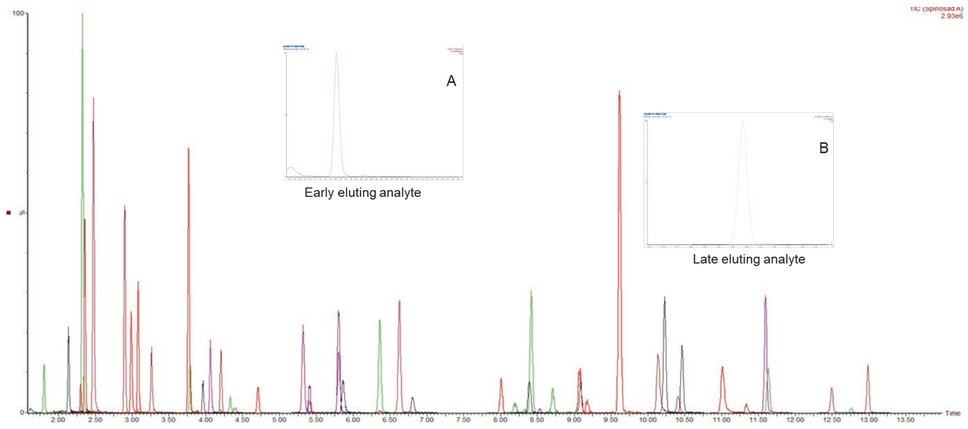

***Figure 1.**: Typical chromatographic peak shapes for a selection of representative analytes across the elution profile in cucumber matrix matched standard at 0.01 mg/kg). Insets show zoom of A) early and B) late eluting compounds*

METHODS

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.

For the purpose of this work, a representative selection of over 40 analytes were used to demonstrate the performance of the UPLC-MS/MS method against the validation criteria. The selection was based on a) spanning the physiochemical diversity; b) including those defined in the coordinated multiannual plan 2017/660; and c) a selection of pesticides reported as border rejections in 2019 under the European Rapid Alert System for Food and Feed. In addition, the identification criteria for tandem quadrupole systems operated in MRM acquisition mode was met throughout.

Extraction of the samples consisted of CEN QuEChERS method using 10 ml of acetonitrile for 10 g of vegetables and 5 g in case of brown rice, which was additionally rehydrated with 10 ml of water. After centrifuge, an aliquot of the acetonitrile supernatant was transferred to a 15 ml dSPE tube containing 1200 mg $MgSO_4$ and 400 mg PSA and shaken for 30 seconds. The dSPE tube was then centrifuged and final extract was diluted in ratio 1:10 into mobile phase A.

UPLC	
System:	ACQUITY UPLC I-Class PLUS
Column:	ACQUITY UPLC HSS T3
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
Injection volume	3 µl
Column temp.	45 °C
Run time	19 min

MS instrument	
System:	Xevo TQ-S cronos
Ionization:	Electrospray +/-
Capillary voltage	+0.4/-0.5 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

RESULTS AND DISCUSSION

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 averaged calibration standards. 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 3 –6 seconds were obtained across the elution profile.

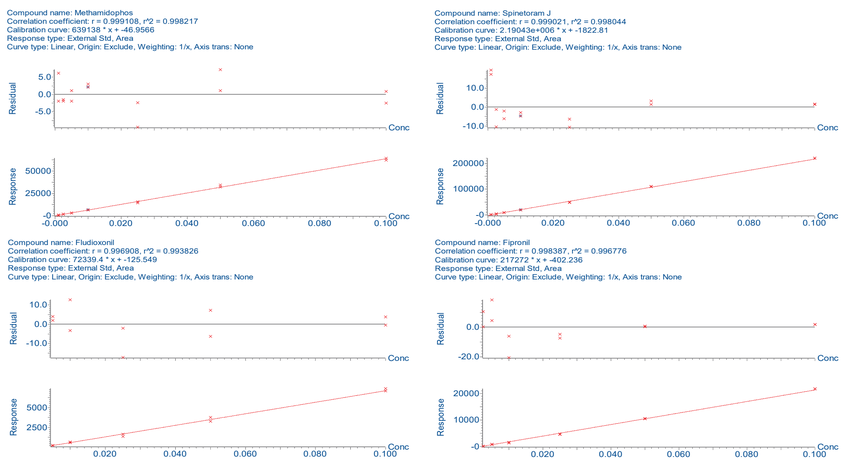

***Figure 2.**: Linearity for compounds ionizing in ESI+ (Methamidophos and Spinetoram) and ESI- (Fludioxonil and Fipronil)*

Sensitivity and linearity

Matrix matched standards were prepared in both cucumber and brown rice extract at seven concentrations in range between 0.001 and 0.1 mg/kg. The linear range for the representative compounds was found to be 0.99 or greater with a deviation of back calculated concentration from the true concentration (TargetLynx % residuals) of < 20 %. Examples for compounds ionizing in ESI+ and ESI– and shown in **Figure 2**.

The method LOQ for the representative analytes in both cucumber and rice was estimated using the inter-day (three consecutive days) repeatability data and defined as the lowest matrx-matched standard achieving relative standard deviation (RSD) of ≤ 20 %. 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 ≤ 0.5x default MRL. Chromatograms for both MRM, quantifiers and qualifiers, are shown for 10 selected compounds at 0.01 mg/kg (1 default MRL) in cucumber extract in **Figure 3.**

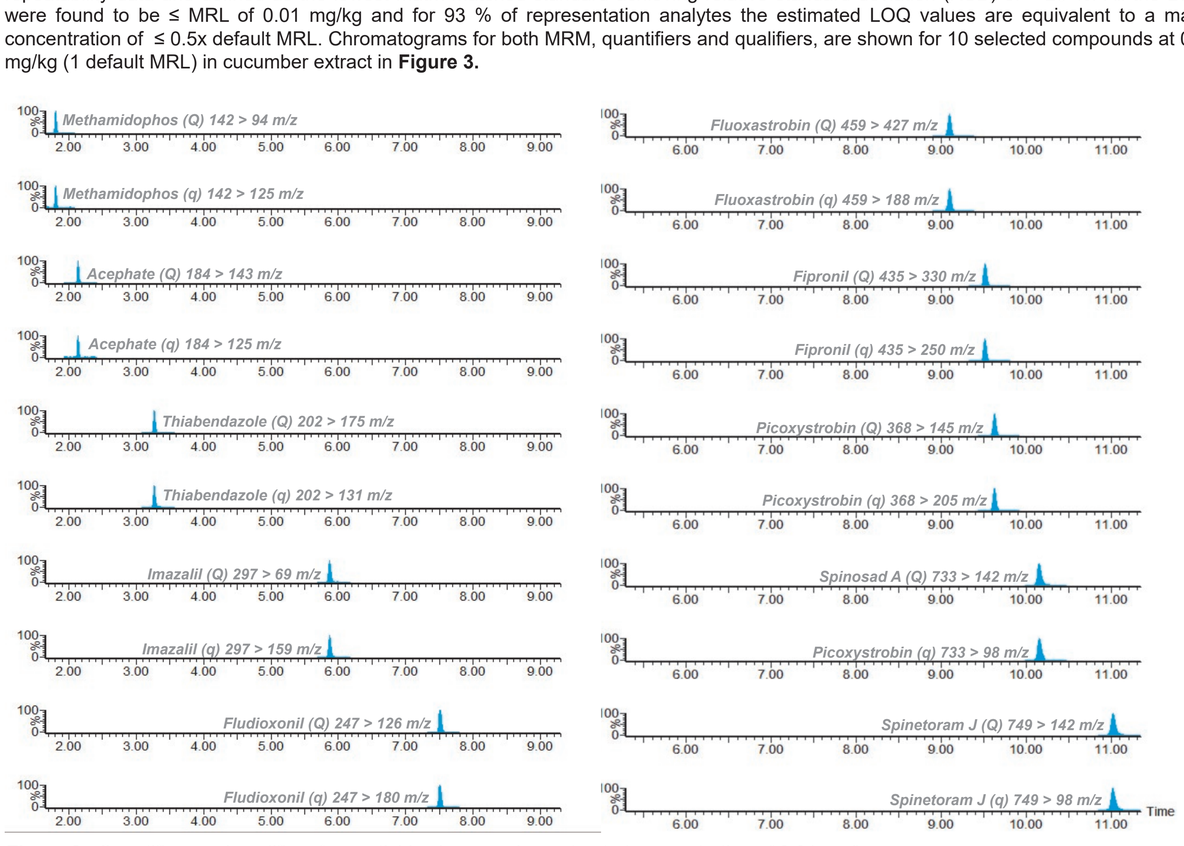

***Figure 3.**: Quantifiers and qualifiers for pesticides in cucumber extract at concentration of 0.01 mg/kg*

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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 standard was prepared at concentration equivalent to 0.5x default MRL 0.01 mg/kg and the peak areas were compared to those obtained from a solvent standard at the same concentration. The results (**Figure 4.**) shows that for the majority of the compounds assessed (>80 %) the deviation is within 20% of the solvent standard response.

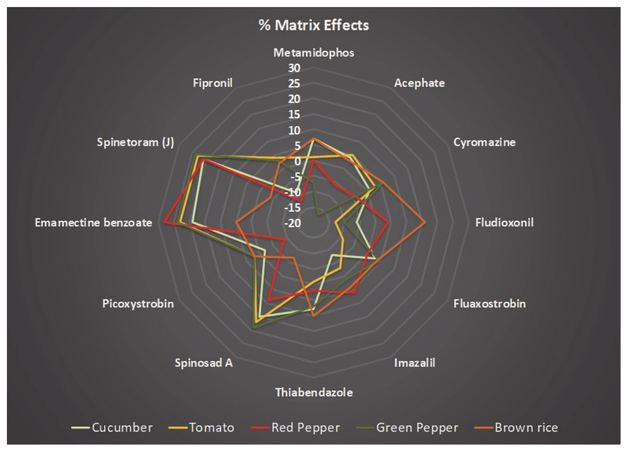
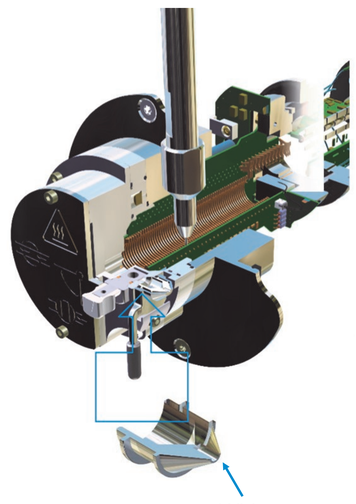


***Figure 4.**: Combined representation of the mean % matrix effects in matrix match standards of cucumber, red pepper, green pepper, tomato and brown rice*

Precision and robustness

Repeatability was assessed by repeated injection (n = 6) of cucumber and brown rice extract spiked at three concentrations 0.005, 0.01 and 0.1 mg/kg. Experiment was performed on three different days giving replicate measurements (n=18) per matrix/spiking concentration level. **Figure 5.** shows results for rice, where the within-lab reproducibility of the LC-MS/MS method was ≤ 20 % at the default MRL equivalent concentration.

Robustness of the system, following repeated injections of a matrix matched standard at 1 ng/ml (equivalent to the 1MRL in matrix), was investigated in cucumber. Over 200 consecutive 3 µl injections were performed with no user intervention. This represents 67 hours and total matrix load of over 60 mg. A control chart showing the peak area for the quantitative ion transitions of four analytes are shown in **Figure 6.** Robustness of the system is enhanced by using the reverse design of the MS source cone. The outer orifice is wider (1.5 mm) then the conventional cone and inner orifice is narrower (0.25 mm).



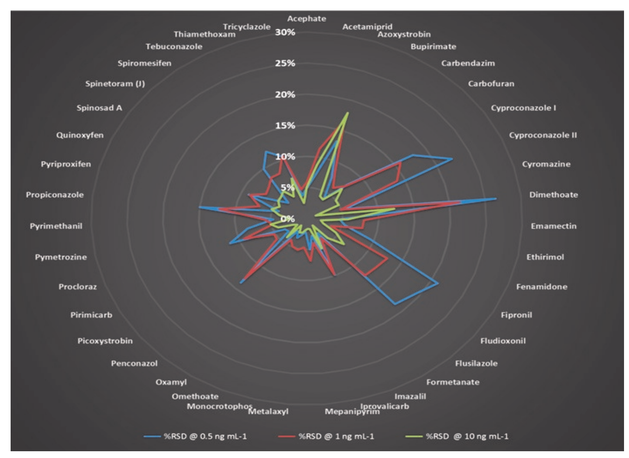


***Figure 5.**: RSDwr inter-day reproducibility (n=18) for a selection of representative pesticides spiked into brown rice at 5, 10 &100 ng/ml (equivalent to 0.005, 0.01 & 0.1 mg/kg)*

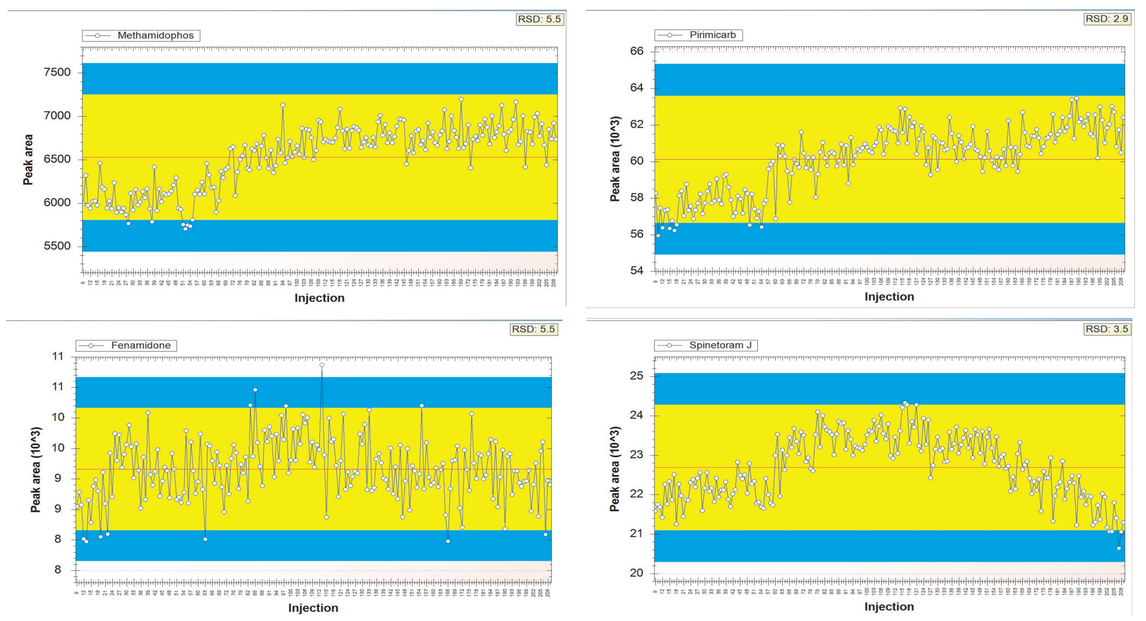


***Figure 6.**: Repeated injections (>200) of cucumber extract plotted for Methamidophos, Pirimicarb, Fenamidone and Spinetoram at 1 ng/ml (equivalent to 0.01 mg/kg of matrix). Yellow represents 2x standard deviation and blue 3x standard deviation warning line*

CONCLUSION

- **Demonstrated performance of a multi-residue method for LC amenable pesticides in food by UPLC-MS/MS using generic MS parameters (including polarity switching)**
- **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**
- **The system is shown to be reliable in routine operation with minimal requirements for user intervention during extended periods of analysis**

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