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A Robust and Sensitive Method For the Analysis of 510 Pesticides Using LC/MS/MS

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Introduction

Pesticides play a significant role in agriculture by mitigating insects, rodents, fungi, and weeds to improve crop and food production. Regulatory organizations like the World Health Organization (WHO), the Food and Agricultural Organization (FAO), the U.S. Environmental Protection Agency (EPA), and the European Union (EU) have developed and established internationally accepted maximum residue limits (MRL) to protect food consumers from adverse effects of pesticides. This points to the demand and need for highly sensitive analysis methods of multiresidue pesticides in food matrices.[1] In this poster, we describe a comprehensive LC/MS/MS workflow that was developed, and performance verified in multiple laboratories to deliver reliable analysis of over 500 pesticide residues in different types of food commodity groups to help streamline routine pesticide analysis, and therefore accelerates lab throughput and productivity.[2,3]

Experimental

Pesticide Standards

The pesticide standards, including 10 custom standard mix, were from Agilent. An intermediate standard mix comprised of 510 targets at a concentration of 1,000 µg/L was prepared in ACN from stock standard solutions.

Sample Preparation

A fast and easy sample preparation protocol based on QuEChERS extraction followed by dSPE (Dispersive Solid Phase Extraction) or Captiva EMR-Lipid cleanup, as illustrated in Figure 1, was optimized and used for five representative food commodity groups: tomato and onion (high water content), wheat (high starch content), olive oil (high oil content), honey (high sugar content), and black pepper (difficult commodities).

Experimental

Instrumentation

An Agilent 1290 Infinity II LC system coupled to a 6470 Triple Quadrupole LC/MS was used. The LC/TQ system conditions and parameters are listed in Tables below.

Table 1. LC conditions

Column	Agilent Zorbax Eclipse Plus C18, 2.1 mm x 150 mm, 1.8 µm at 40 °C		
Injection volume	2 µL		
Mobile phase A	5 mM ammonium formate in water with 0.1 % formic acid at 0.4 mL/min		
Mobile phase B	5 mM ammonium formate in methanol with 0.1 % formic acid at 0.4 mL/min		
	Time/min	%A	%B
Gradient program	0	95	5
	3	70	30
	17	0	100
	20	0	100

Table 2. TQ parameters

Ionization mode	Positive / Negative ESI with Agilent Jet Stream
Scan type	Dynamic MRM
Cycle time	750 ms
MS1/MS2 resolution	Unit /Wide
Gas temperature	200 °C
Gas flow	9 L/min
Nebulizer	35 psi
Sheath gas temperature	400 °C
Sheath gas flow	12 L/min
Nozzle votage	0 V
Capillary voltage	2500 (+) / 3000 (-) V

Matrix-Matched Calibration Standard

Matrix-matched calibration standards were prepared by spiking the intermediate standard solution into each matrix blank to make the seven calibration concentration levels of 1, 2, 5, 10, 25, 50, and 100 µg/L.

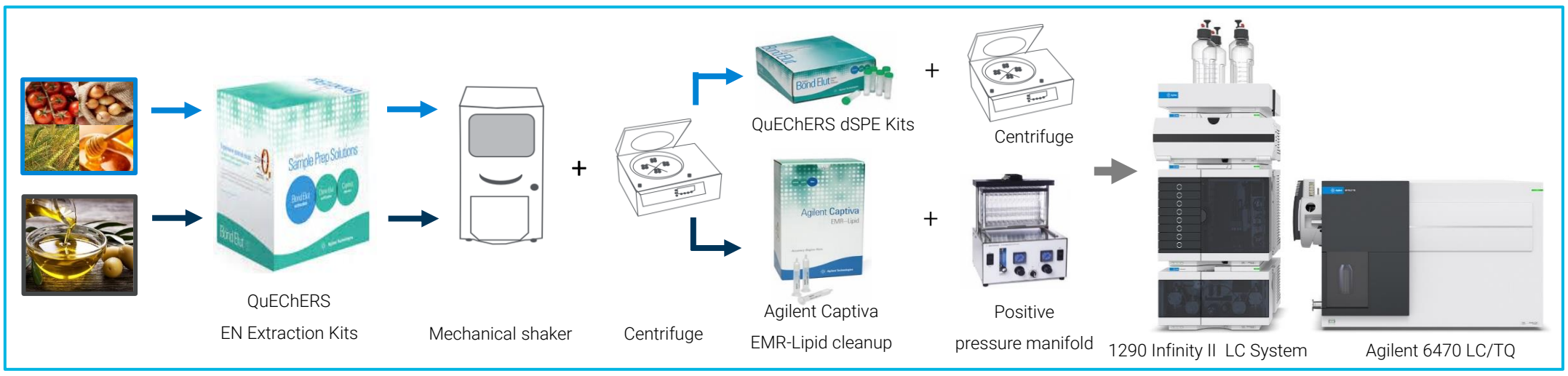

Figure 1. Sample preparation procedure

Development of LC/TQ Method

A major part of this work was the development of dynamic MRM transitions for 510 pesticide compounds. For each compound, MRM transitions were optimized using automated MassHunter Optimizer software by flow injection. Figure 2 shows a representative MRM chromatogram for all 510 pesticides in honey matrix extract. The symmetric sharp peaks demonstrate the efficient chromatographic separation of targets within the RT window, and effective sugar removal by Dispersive SPE cleanup for honey matrix samples.

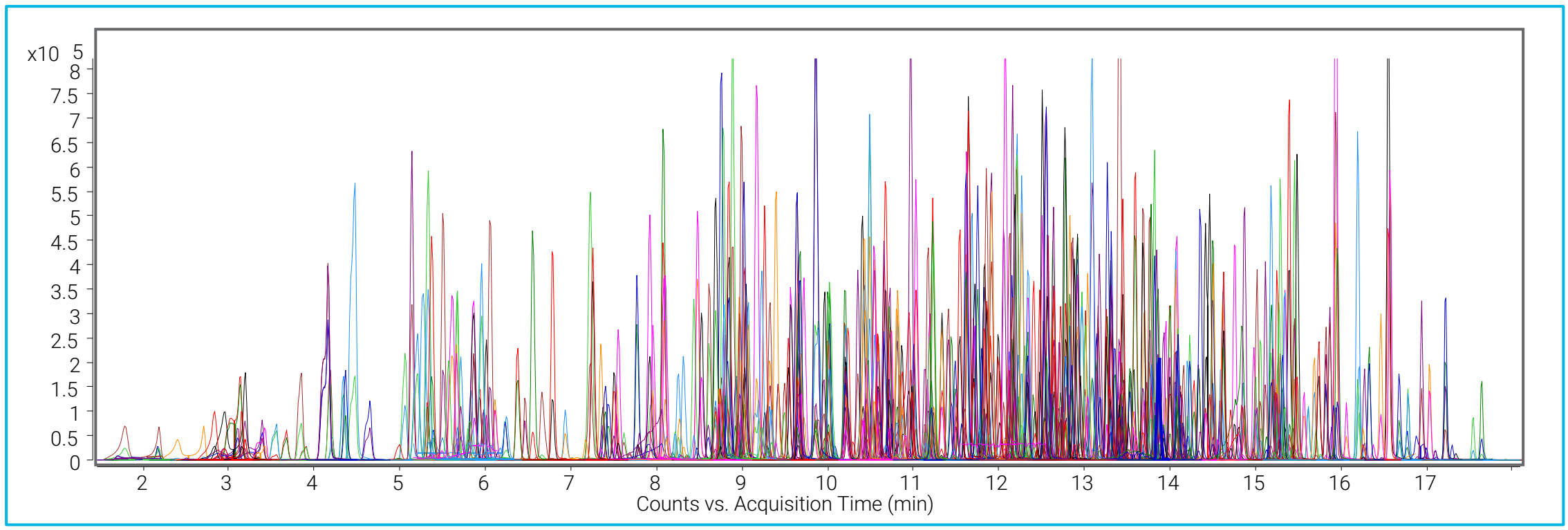

Figure 2. Representative MRM chromatogram of 510 pesticides at 10 µg/L in honey extract.

Matrix effect evaluation

Matrix effect (ME) in terms of suppression or enhancement of the MS detection system response was assessed by the ratio of target response in matrix-matched standards to that in corresponding solvent standards. Based on SANTE guidelines,[1] 80-120% ME is considered as insignificant ME. In this study, more than 20% of 510 targets showed significant ME in tomato, wheat, honey and olive oil, while more than 70% of compounds demonstrated significant ion suppression in onion and black pepper. Therefore, matrix-matched calibration standards were used in this study to compensate the ME caused by different types of food matrices.

Linearity and sensitivity

Matrix-matched calibrations for each matrix were observed to achieve R^2 ≥0.99 for over 90% of compounds. The instrument method provided LOD ≤10 µg/L for over 99% of 510 targets in tomato, onion, wheat, honey and olive oil. Figure 3 shows MRM chromatogram overlays of triplicate injections of acetamiprid at 1 µg/L in all matrix extract. This demonstrates high sensitivity of Agilent 6470 LC/TQ mass spectrometer and sample prep consistency across different commodity groups. The analytical workflow LOQ was obtained from pre-spiked QC. ≥82% of targets achieved LOQ ≤10 µg/kg in tomato, onion, wheat, honey and olive oil, which met the MRL requirement from EU regulation. These results were duplicated at our Singapore and Waldbronn facilities, demonstrating the transferability of the overall method. As for black pepper, around 70% of pesticides achieved LOQ ≤10 µg/kg due to significant ion suppression. Further study is ongoing to optimize and improve the extraction efficiency of multiresidue pesticides in black pepper.

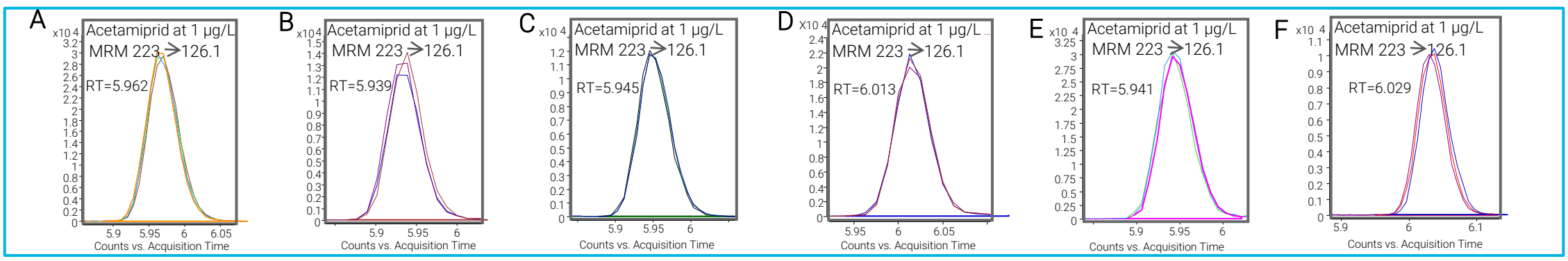

Figure 3. MRM chromatogram overlay of acetamiprid for triplicate injections at 1 µg/L in tomato (A), onion (B), wheat (C), honey (D), black pepper (E) and olive oil (F) matrix extract.

Results and Discussion

Method precision and recovery

Method precision was demonstrated using intralaboratory study (RSD_r) and interlaboratory study (RSD_{iR}) based on technical replicates of prespiked QC at 10 µg/kg in different matrices. RSD_r % was calculated based on the recoveries of six replicates of prespiked QCs within a batch (intralaboratory). RSD_{iR} % was calculated based on the recoveries of 12 replicates of QCs across two batches, prepared by different personnel in different labs, and run on two different LC/TQ instruments. Over 90% targets in all matrices provided ≤20% in RSD_r, demonstrating good method consistency.

Variation of retention time (RT) for all targets within the batch was also monitored to evaluate the precision of the chromatographic method. RT tolerance of all targets for each matrix was within ±0.1 minutes. Figure 4 shows TIC overlay of triplicate injections of 510 pesticides at 10 µg/L in olive oil matrix extract, which confirms the reproducibility of the elution profile and of the MS detection.

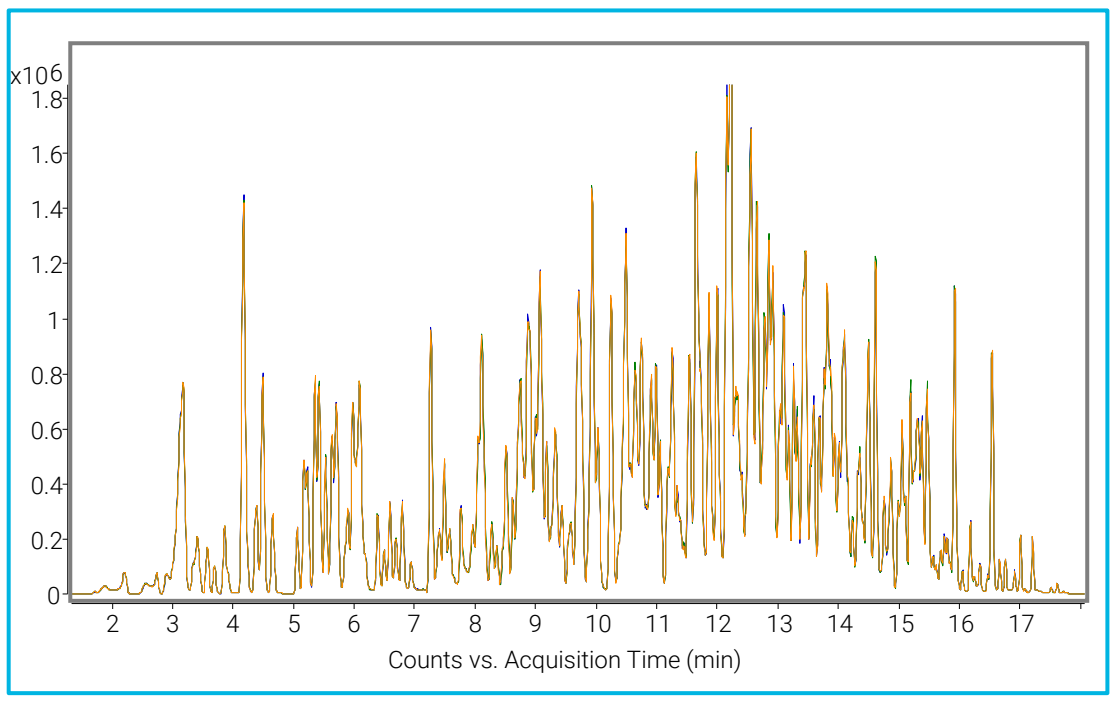

Figure 4. TIC overlay of triplicate injections of 510 pesticides at 10 µg/L in olive Oil matrix extract.

Targets recovery is a critical parameter for the quantitative analytical workflow method evaluation. Prespiked QCs at 10 µg/kg was used to evaluate the targets recovery in all matrices. Recovery was calculated based on the ratio of analyte response in prespiked QCs (n = 6) to that in corresponding level of matrix-matched standard. According to SANTE/12682/2019, acceptable average recoveries should be within the range of 40 to 120% if they are consistent (RSD_r ≤20%). Based on these criteria, the average recovery results of 82% targets met the acceptance criteria in all matrices except black pepper. Furthermore, 70 to 120% recovery was achieved for ≥65% of targets in tomato, onion, wheat, olive oil and honey as well.

Robustness assessment

In this study, robustness was evaluated by two days' continuous injection of olive oil extract spiked with pesticides at 50 µg/L. Nine compounds were selected as shown in Figure 5 to represent different pesticide classes. Over two days' continuous running, the analyte responses were observed in good consistency with RSD <3.5%. This demonstrates that the use of dMRM mode can produce consistent responses for day-to-day operation.

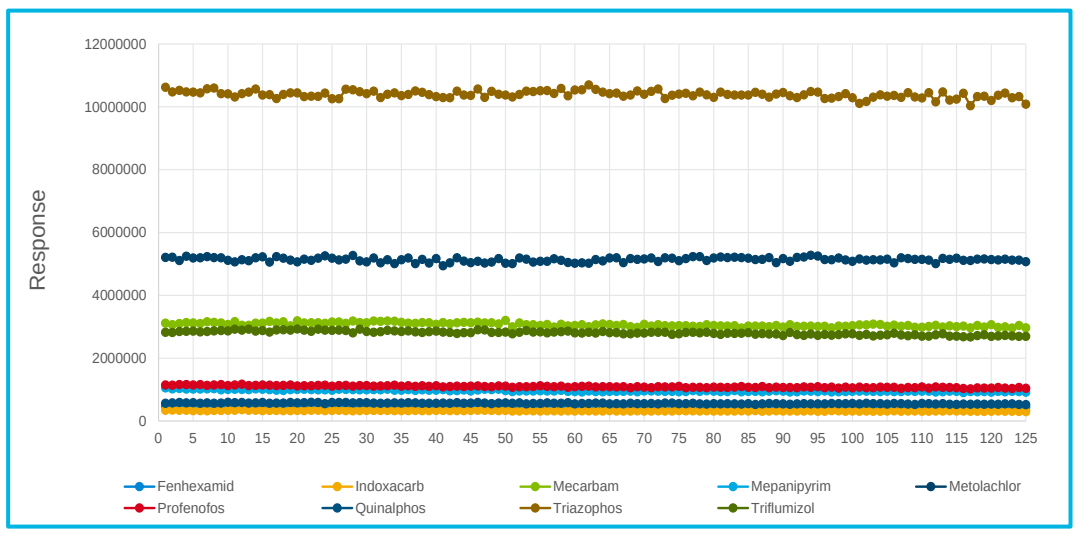

Figure 5. Response of pesticides at 50 µg/L for two days' continuous injections in olive oil extract.

Conclusions

The dMRM method was created and developed based on Agilent Pesticide tMRM Database including over 750 pesticides that can be saved to any name for customization. The method was verified across two laboratories based in Singapore and Germany and achieved 40 to 120% recovery for the majority of compounds, with RSD_r and RSD_{iR} within the limit of 20% per to SANTE guidelines. This sensitive and reliable workflow demonstrates the applicability of quantitation for over 500 pesticides in various food matrices.

References

1. European Commission. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed, SANTE/12682/2019, 2019.
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3. Analysis of 510 Pesticide Residues in Honey and Onion on an Agilent 6470 Triple Quadrupole LC/MS System, Agilent application note, 5994-3573EN, 2021.

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