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Multiresidue Pesticides Analysis in Food Matrices Using an Enhanced Triple Quadrupole LC/MS System

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Introduction

Pesticides are integral for protecting crops, but there is concern in the current market of organic and non-organic labeled products – where food authenticity or contamination can affect the quality of organic products. Typical Maximum Residue Limits (MRLs) are on the order of $\mu\text{g}/\text{kg}$ food (parts-per-billion) and thus require very sensitive instrumentation to detect compounds. This is especially true for food products that have many endogenous components that cause heavy matrix effects, such as black tea.

An LC-MS/MS screening method for the detection and quantification of 244 pesticides in heavy & diverse food matrixes was developed:

- Organic loose-leaf black tea and whole organic oranges were obtained from a local grocery store.
- Extracts were prepared following Agilent's QuEChERS extract and EN dispersive SPE protocols.
- Agilent's comprehensive pesticide mixture (p/n 5190-0551) was spiked into extracts of organic black tea and whole orange, respectively.
- Samples were analyzed with a dynamic Multiple Reaction Monitoring (dMRM) method using the 1290 Infinity II LC system coupled to the 6470B triple quadrupole LC/MS (LC/TQ).

The 6470B Triple Quadrupole LC/MS contains hardware improvements on several aspects:

- VacShield technology allowing vent-free ion source maintenance to increase instrument uptime
- Faster electronics with improved settling time parameterization providing chromatographic peak reproducibility at very low dwell times.

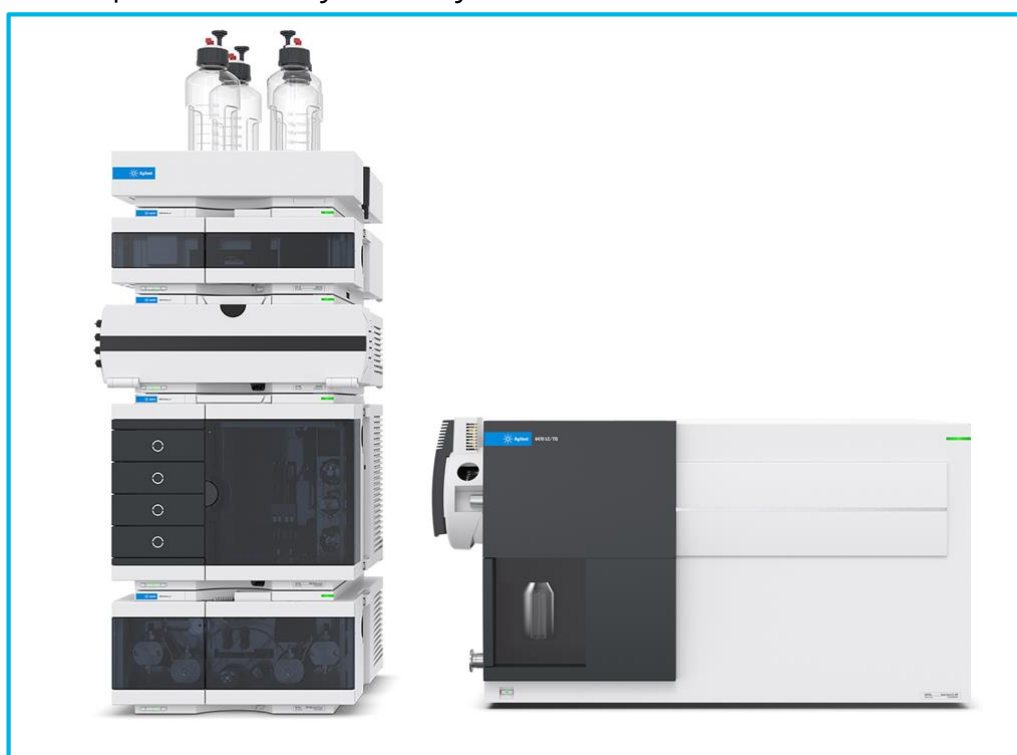


Figure 1. 6470B Triple Quadrupole LC/MS with 1290 Infinity II LC system

Experimental

Instrumentation

- 1290 Infinity II High Speed Pump (G7120A)
- 1290 Infinity II Multisampler with Sample Cooler (G7167B, #100)
- 1290 Infinity II Multicolumn Thermostat (G7116B)
- 6470B Triple Quadrupole LC/MS (G6470B) w/ Jet Steam electrospray ionization source (G1958-65638)

MassHunter Acquisition (ver. 10.1) and MassHunter Quantitative Analysis (ver. 10.1) software was used for data acquisition and analysis respectively.

1290 Infinity II UHPLC System

Column	ZORBAX RRHD Eclipse Plus C18, 3.0 x 100 mm, 1.8 μm at 40 °C (p/n 959758-302)	
Inj. Vol.	2 μL	
Sampler temperature	4 °C	
Needle wash	10 second wash in flush port (75:25 methanol/H ₂ O)	
Mobile phase	A) 5 mM ammonium formate + 0.1% formic acid in H ₂ O B) 5 mM ammonium formate + 0.1% formic acid in methanol	
Flow rate	0.400 mL/min	
Gradient program	Time	B (%)
	0.00	5
	0.50	5
	2.00	40
	13.00	98
	14.50	98
	14.60	5
Post time	2 minutes	

Table 1. 1290 Infinity II LC Method

6470B Triple Quadrupole Mass Spectrometer

Ion source	Agilent Jet Stream (AJS) source
Polarity	Positive and Negative
Gas temperature	225 °C
Drying gas	11 L/min
Nebulizer gas	30 psi
Sheath gas	350 °C
Sheath gas flow	12 L/min
Capillary voltage	3500 \pm V
Nozzle voltage	500 \pm V
Scan type	Dynamic MRM (dMRM)
Q1/Q2 Resolution	Unit (0.7 amu)
Delta EMV	\pm 200 V
Cell accel. voltage	3–7 V
Cycle time	500 ms

Table 2. 6470B Triple Quadrupole LC/MS Method

Dynamic MRM Method with Fast Separation

The multiresidue pesticide screening method developed for the previous Agilent LC/TQ instrument model (G6470A) was directly applied in the 6470B Triple Quadrupole LC/MS system (G6470B). The MassHunter Software Dynamic MRM Update Options was used to automatically adjust retention times that may have shifted due to LC or column changes.

Figure 2 shows the overlapped MRM chromatogram of 244 pesticides spiked in orange extract at a concentration of 1 ng/g. All compounds MRM transitions are baseline separated within a 14.5-minute LC gradient. Most pesticides were quantifiable at 10% of the default MRL (1/10 of MRL), which is the highest level of a pesticide residue that is legally tolerated in or on food or feed when pesticides are applied correctly (Good Agricultural Practice).

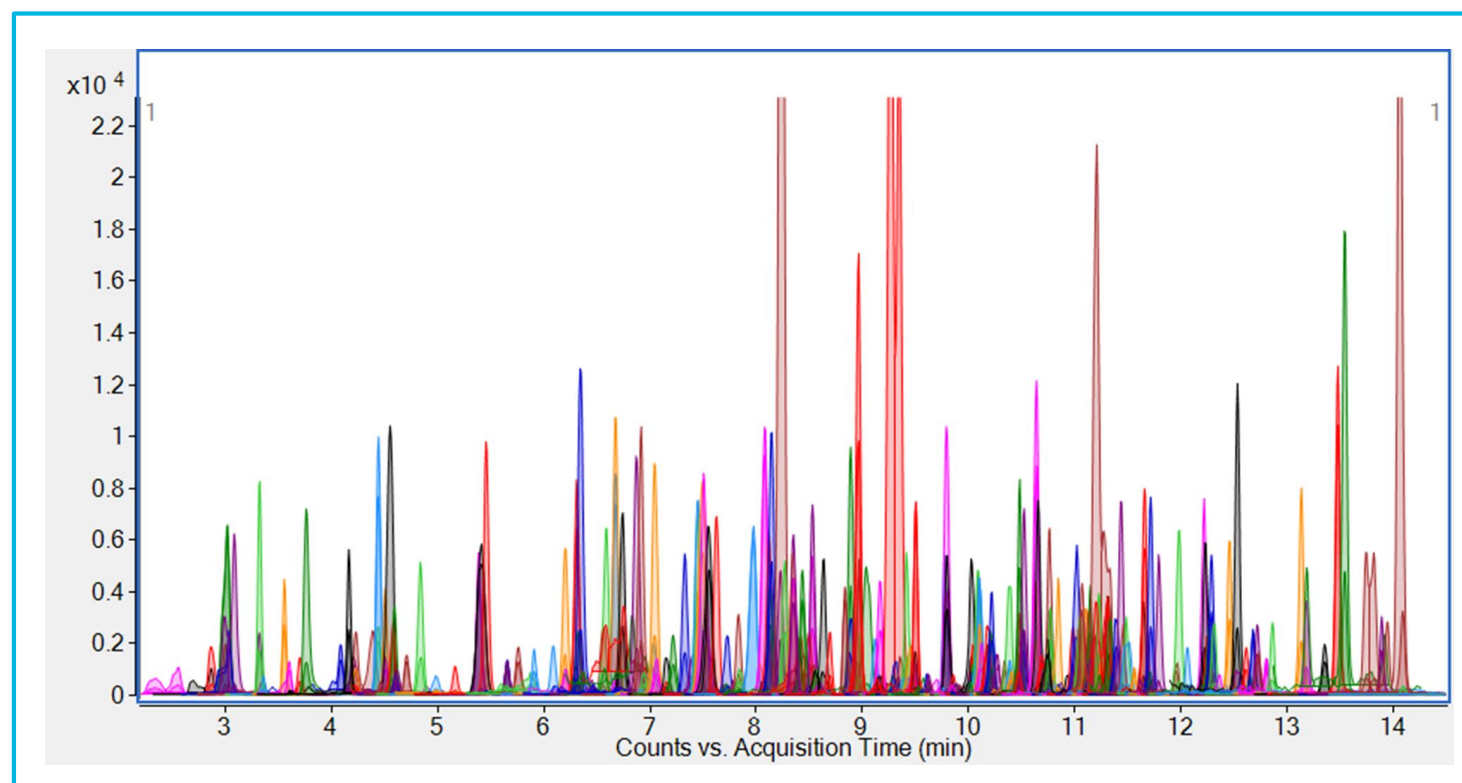


Figure 2. Overlapped MRM chromatograms of 244 pesticides spiked into orange at 1 ng/g

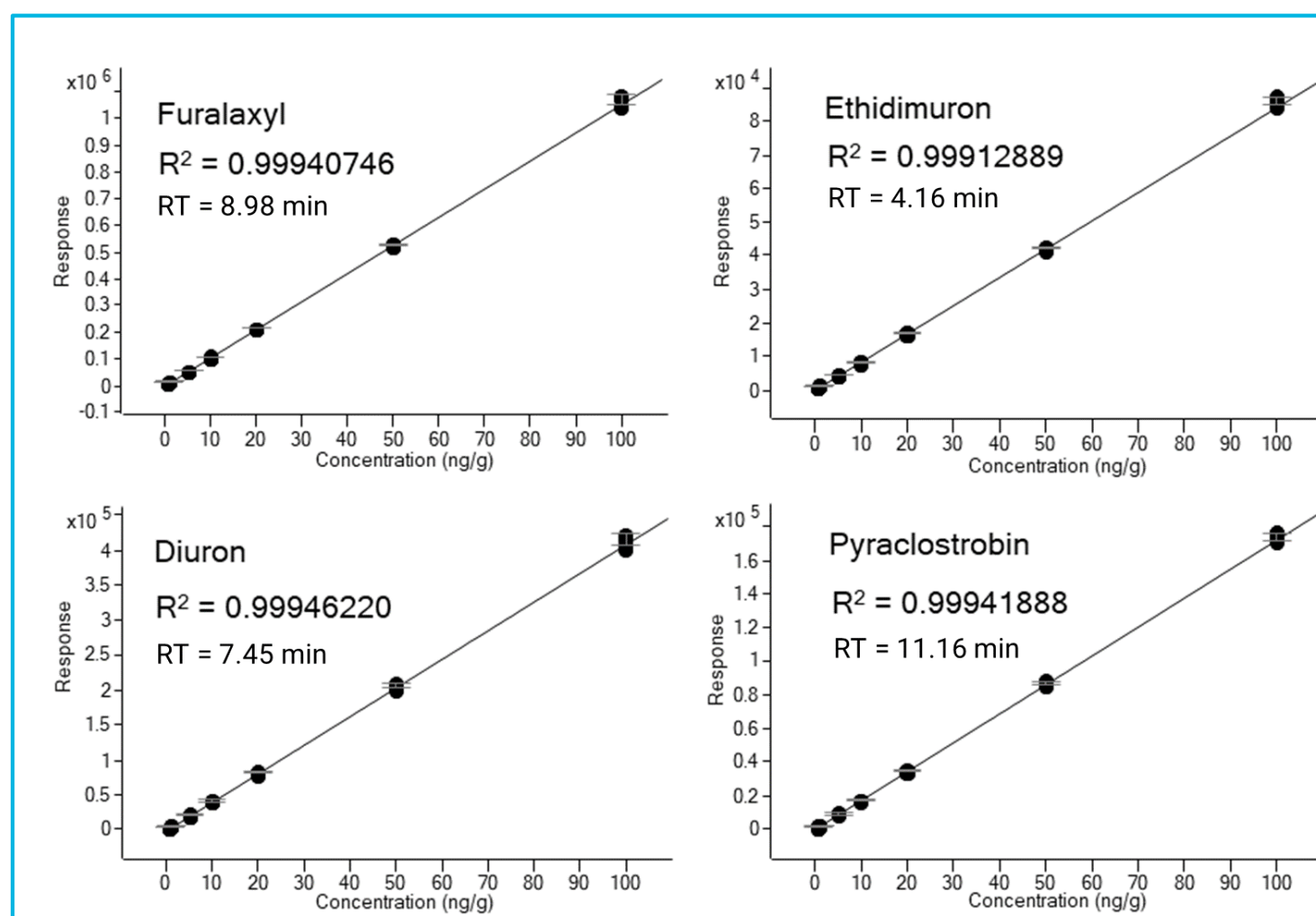


Figure 3. Calibration curves of Furalaxyl, Ethidimuron, Diuron, and Pyraclostrobin pesticide residues spiked into black tea

Standards Curve Analysis

The precision and accuracy of multi-residue pesticide measurements were evaluated in both black tea and orange matrix by injecting a matrix-matched calibration curve at 7 concentrations ranging from 0.5 to 100 ng/g with replicates (n=6).

The results show:

- Excellent precision with relative standard deviation (RSD) < 20% and average accuracy (calculated concentration/expected concentration) within 80-120% at and above LLOQ
- Correlation coefficients (R^2) for calibration curves were higher than 0.99 for all 244 pesticides in the orange extract
- 230 out 244 pesticides in the black tea extract show $R^2 > 0.99$
- Calibration curves for four selected representative pesticides in black tea matrix are shown in Figure 3

6470B Triple Quadrupole LC/MS Demonstrates Great Sensitivity even in Heavy Matrices

In this study, calibration curves were used to determine LLOQ of each pesticide in the matrix, defined as the lowest level with an accuracy within 80-120% and RSD < 20% for peak areas from all 6 replicates. Figure 4 shows the LLOQs distribution for pesticide compounds in both black tea and orange matrices.

The new 6470B Triple Quadrupole LC/MS system allows quantitation of most targeted pesticides in black tea and orange below the default MRL of 10 µg/kg specified by the European Commission¹:

- 239 out of 244 pesticides in the black tea and 243 out of 244 pesticides in the orange have an LLOQ equal to or below 10 ng/g, respectively
- For compounds with specific tolerances by the US-EPA², all of them were quantified below or at their MRL

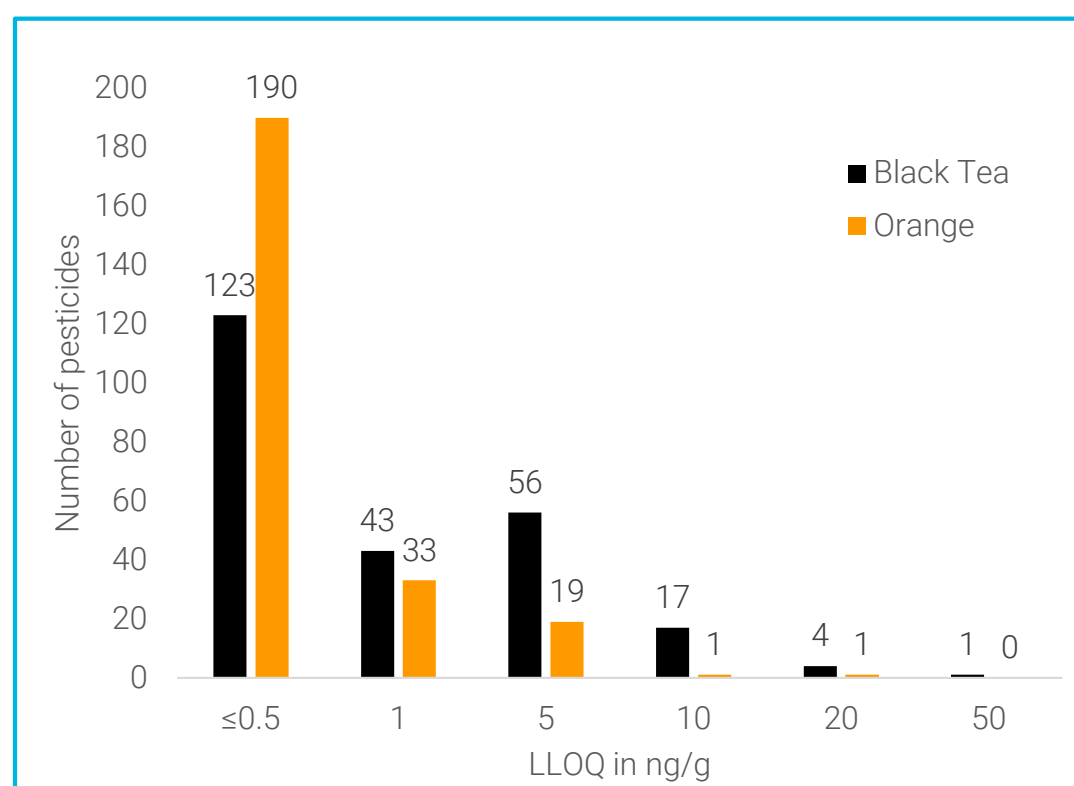


Figure 4. LLOQs for pesticides spiked into black tea and orange

Conclusions

The excellent quantification performance of 244 pesticide residues in black tea and orange has been demonstrated on an improved LC/MS platform, including the ultra-high-performance Agilent 1290 Infinity II LC system coupled to the 6470B Triple Quadrupole LC/MS with the high sensitivity Jet Stream Technology Ion Source (AJS).

Recoveries in Food Matrices

In order to evaluate matrix effects (ion suppression and enhancement), recoveries were calculated by comparing the response of pesticides in the matrix, against those in neat solvent at the default MRL of 10 µg/kg, as shown in Figure 5.

- In the orange matrix, about 50% of the compounds achieved a recovery within SANTE guidelines³ of 80–120%
- In the black tea matrix, about 40% of compounds were recovered within the guideline.³
- The black tea matrix showed more matrix effects than the orange matrix
- A matrix-matched calibration curve is generally recommended for samples with heavy matrix effects

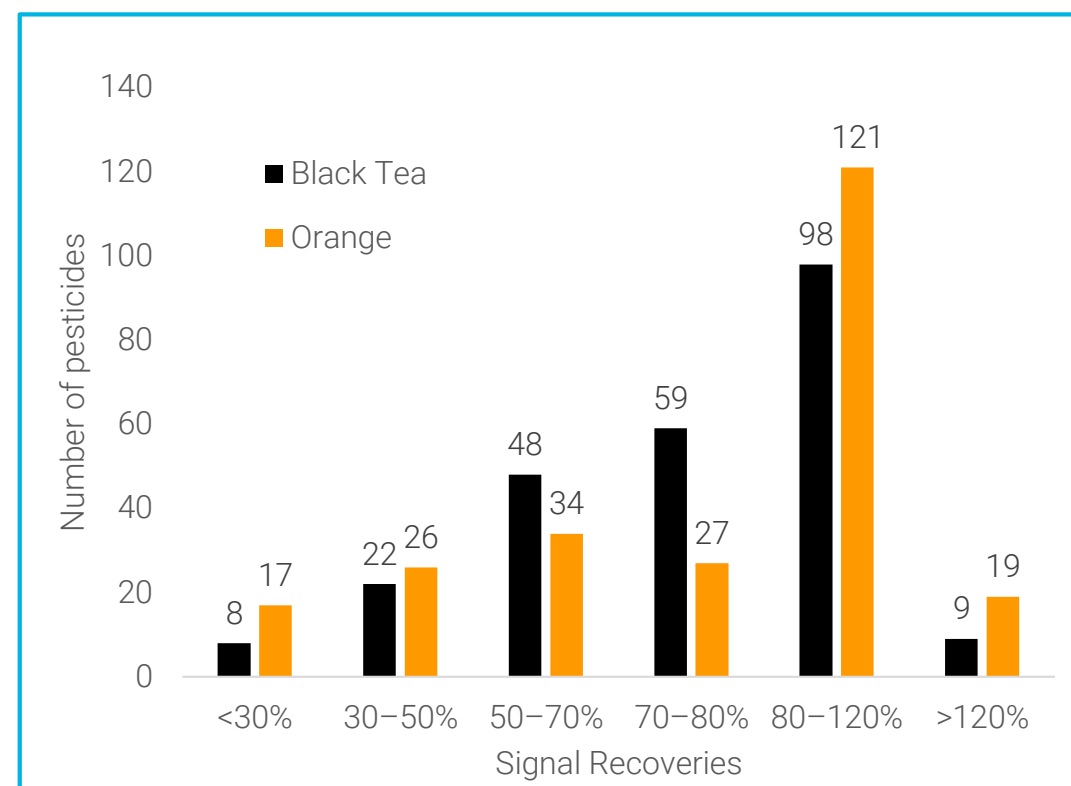


Figure 5. Histogram of recoveries for pesticides spiked into black tea and orange at the default MRL of 10 µg/kg

References

1. Regulation (EC) No 396/2005. European Commission. Retrieved 04/09/20
2. Title 40 U.S. Code of Federal Regulations – Part 180. US-EPA. Retrieved 04/09/20
3. SANTE/12682/2019 European Commission. Retrieved 04/20/20.