

Multiresidue Pesticide Analysis in Black Tea and Orange Matrix Using the Agilent 6470B Triple Quadrupole LC/MS System

Authors

Kyle Covert and Linfeng Wu
Agilent Technologies, Inc.

Abstract

This application note describes an LC/MS/MS screening method for the detection of 244 pesticide residues in food matrices using an Agilent 6470B triple quadrupole LC/MS system. Organic oranges and black tea were chosen to evaluate the detection of pesticides in food matrices because of their complexity. For the orange matrix, all but one of the targeted pesticides were detected at or below the default MRL of 10 µg/kg, as specified by the European Commission.¹ For the heavy black tea matrix, 239 pesticides were detected at or below 10 µg/kg. Matrix effects on analyte response were also studied, showing that 50% of the pesticides in the orange matrix and 40% in the black tea matrix were recovered within the SANTE guidelines (80 to 120%).² These results demonstrate that the 6470B triple quadrupole LC/MS can achieve the high sensitivity requirements to accurately and precisely quantify pesticides in heavy food matrices.

Introduction

Pesticides are integral for protecting crops and are necessary in most growing environments to obtain high yields of products. There is concern over the presence and amount of pesticides in the products we purchase. This is especially concerning in the current market of organic and nonorganic labeled products—where food authenticity or contamination can affect the quality of organic products. Regulations are put in place by regulatory authorities (e.g., the US-EPA^{1,3} and the European Commission⁴) that restrict the use and amount of pesticides allowed so that food is safe for consumers. A maximum residue limit (MRL) is the highest level of a pesticide residue legally tolerated in or on food or feed when pesticides are applied correctly (good agricultural practice). Typical MRLs are on the order of $\mu\text{g}/\text{kg}$ food (parts per billion (ppb)) and therefore 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.

This application note describes an LC/MS/MS screening method for the detection and quantification of 244 pesticides in heavy and diverse food matrices. The Agilent comprehensive pesticide mixture was spiked into extracts of organic black tea and whole orange, respectively. Extracts were prepared following the Agilent QuEChERS extract and EN dispersive SPE protocols described below. Samples were analyzed with a dynamic multiple reaction monitoring (dMRM) method using the Agilent 1290 Infinity II LC

coupled to the 6470B triple quadrupole LC/MS. The 6470B triple quadrupole LC/MS contains hardware improvements of several aspects, such as VacShield technology allowing vent-free ion source maintenance to increase instrument uptime, and faster electronics with improved settling time parameterization, providing chromatographic peak reproducibility at very low dwell times.

Experimental

Reagents and standards

The Agilent comprehensive pesticide mixture (part number 5190-0551) was used. The eight submixes of the comprehensive pesticide mixture were combined and further diluted with acetonitrile (ACN) to a final pesticide working solution concentration of $10 \mu\text{g}/\text{g}$. This solution was spiked into the QuEChERS extracts for the preparation of the calibration samples. For method detection limit (MDL) and lower limit of quantitation (LLOQ) determination in sample matrices, seven calibration samples with concentrations ranging from 0.5 to 100 ppb were prepared in the sample extract of black tea and organic orange, respectively.

All reagents and solvents were HPLC or LC/MS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA). Ultrapure water was produced with a Milli-Q Integral system equipped with a LC-Pak Polisher and a $0.22 \mu\text{m}$ point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid and ammonium formate were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA).

Sample preparation

Organic loose-leaf black tea and whole organic oranges were obtained from a local grocery store. Two grams of tea samples were wetted with 8 mL of water and incubated for 2 hours at room temperature. Well blended homogenized orange fruit samples were prepared with a ceramic homogenizer (part number 5982-9312), and 10 g was weighed out for extraction. The 2 g of solid tea and 10 g of solid orange were extracted with 10 mL of acetonitrile for 1 minute with vigorous shaking. One pouch of Agilent EN extraction salts (part number 5982-6650) was added to each mixture, then shaken for 1 minute followed by centrifugation at $3,000 \times g$ for 5 minutes. Six mL of tea supernatant was added into the Agilent QuEChERS dispersive SPE for high pigment EN (part number 5982-5356). Similarly, 6 mL of orange supernatant was added into the Agilent QuEChERS dispersive SPE for pigmented fruits and vegetables EN (part number 5982-5256). Both were shaken for 1 minute and centrifuged at $3,000 \times g$ for 5 minutes. The resulting supernatant was collected and passed through a $0.45 \mu\text{m}$ syringe filter. The previously prepared pesticide working solution was spiked into black tea and orange extract at $100 \text{ ng}/\text{g}$ (ppb) of the dry weight of the original solid food matrices. Further dilution with the appropriate matrix was done to achieve pesticide calibration samples at seven levels, 0.5, 1, 5, 10, 20, 50, and 100 ppb, and were prepared immediately before injection with six replicate injections per level. Pesticides were also spiked into pure acetonitrile for signal recovery comparison at $10 \text{ ng}/\text{g}$ in both matrices.

Equipment

Separation was performed using the Agilent 1290 Infinity II LC System consisting of the following modules:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Multisampler with Sample Cooler (G7167B, #100)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)

The LC system was coupled to the Agilent 6470B triple quadrupole LC/MS (G6470B) equipped with the Agilent Jet Stream Technology ion source (G1958-65638). Agilent MassHunter Acquisition (ver. 10.1) and MassHunter Quantitative Analysis (ver. 10.1) software was used for data acquisition and analysis, respectively.

Methods

The LC/MS parameters are provided in Table 1. MRM parameters, such as polarity, precursor, and product ions, as well as collision energies, were copied from well established methods. Source conditions were optimized for selected poor-responding analytes. Data acquisition was carried out in fast polarity switching dMRM mode. A 2 μ L amount of the final extract was injected into the LC/MS system. For calibration curve regression, linear fitting with origin ignored and 1/x weighting was used.

Table 1. LC/MS parameters.

Agilent 1290 Infinity II LC System															
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 3.0 \times 100 mm, 1.8 μ m (p/n 959758-302)														
Column Temperature	40 $^{\circ}$ C														
Injection Volume	2 μ L														
Autosampler Temperature	4 $^{\circ}$ 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	<table border="1"> <thead> <tr> <th>Time</th> <th>B (%)</th> </tr> </thead> <tbody> <tr> <td>0.00</td> <td>5</td> </tr> <tr> <td>0.50</td> <td>5</td> </tr> <tr> <td>2.00</td> <td>40</td> </tr> <tr> <td>13.00</td> <td>98</td> </tr> <tr> <td>14.50</td> <td>98</td> </tr> <tr> <td>14.60</td> <td>5</td> </tr> </tbody> </table>	Time	B (%)	0.00	5	0.50	5	2.00	40	13.00	98	14.50	98	14.60	5
Time	B (%)														
0.00	5														
0.50	5														
2.00	40														
13.00	98														
14.50	98														
14.60	5														
Post Time	2 minutes														

Agilent 6470B Triple Quadrupole LC/MS	
Ion Source	Agilent Jet Stream (AJS)
Polarity	Positive and negative
Gas Temperature	225 $^{\circ}$ C
Drying Gas (Nitrogen)	11 L/min
Nebulizer Gas	30 psi
Sheath Gas	350 $^{\circ}$ 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 Acceleration Voltage	3 to 7 V
Cycle Time	500 ms

Results and discussion

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). Since the LC column

and the LC system were not the same previously, which caused retention times to slightly shift, the MassHunter software dynamic MRM update option was used to automatically adjust retention times for all pesticides. Figure 1 shows the overlapped MRM chromatogram of 244 pesticides spiked into orange extract at a concentration of 1 ng/g with the Y-axis zoomed-in for the low-responding

pesticides. All compounds' MRM chromatograms are baseline-separated within a 14.5-minute LC gradient. Most pesticides were quantifiable at 10% of the default MRL.

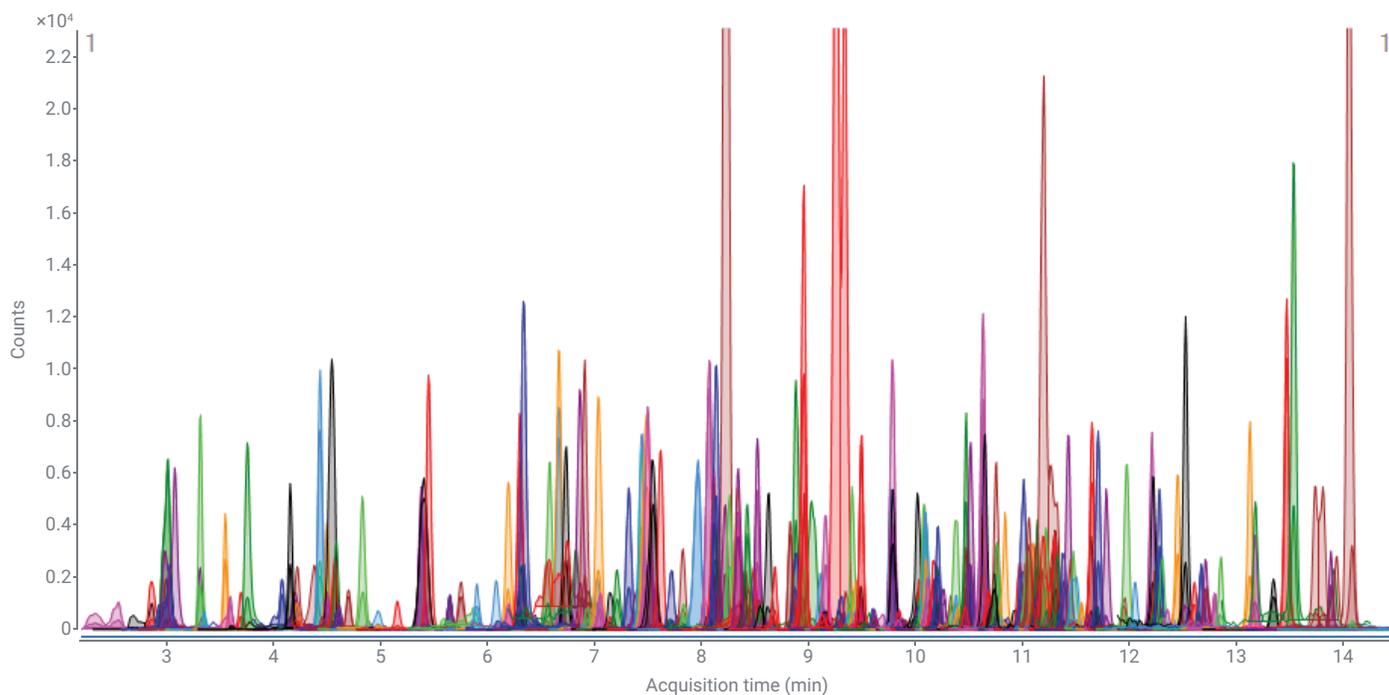


Figure 1. Overlapped MRM chromatograms of 244 pesticides spiked into orange at a concentration of 1 ng/g with the Y-axis zoomed in for the low-responding pesticides.

Standard curve analysis

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

with relative standard deviation (RSD) <20% at and above LLOQ as well as average accuracy (calculated concentration/expected concentration within 80 to 120% at and above LLOQ) were obtained. Correlation coefficients (R^2) for calibration curves were higher than 0.99 for all 244 pesticides in

the orange extract, and for 230 out of 244 pesticides in the black tea extract. The low R^2 (<0.99) values for the 14 pesticides in black tea reflect the severe matrix effects. Calibration curves for four selected representative pesticides in black tea matrix are shown in Figure 2.

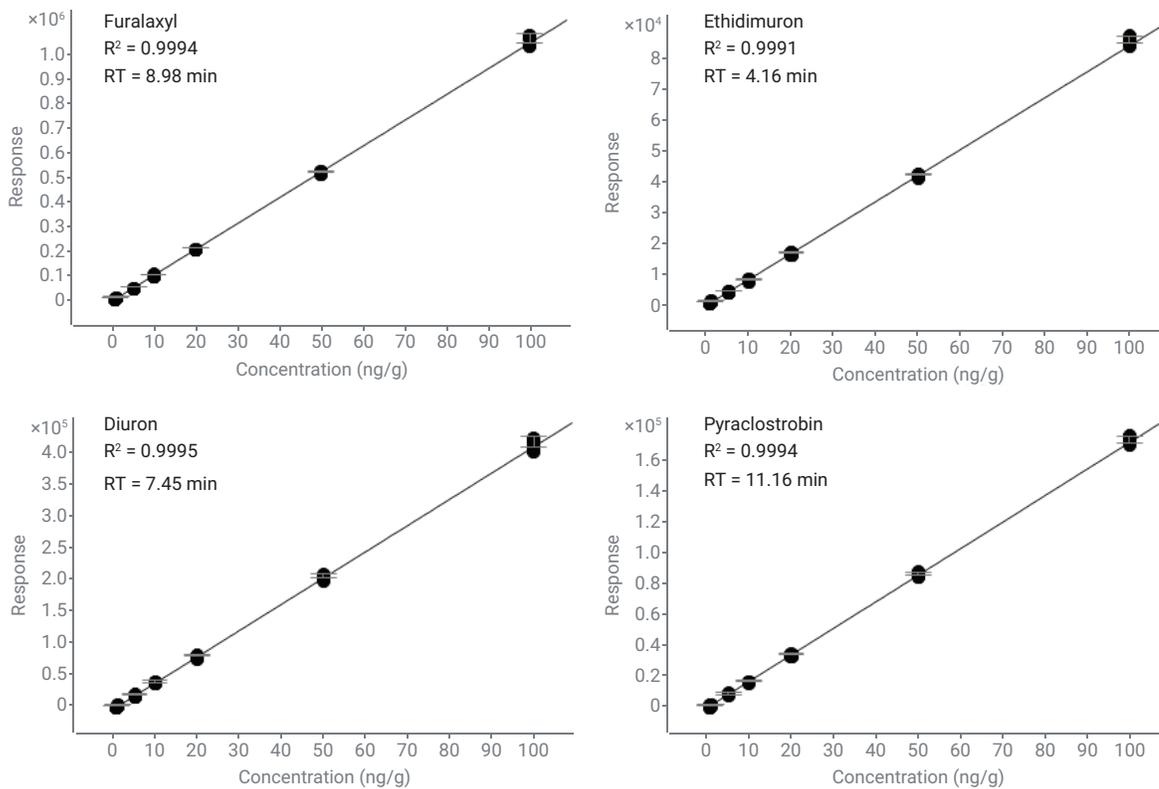


Figure 2. Calibration curves of furalaxyl, ethidimuron, diuron, and pyraclostrobin pesticide residues spiked into black tea.

In this study, the 6470B triple quadrupole LC/MS can detect all the pesticides (signal-to-noise >3) in the orange matrix below 10 µg/kg for screening protocols. For the black tea matrix, all but one compound (bosclid) were detected at or below 10 µg/kg. The calibration curves were used to determine LLOQ of each pesticide in the matrix, defined as the lowest level with accuracy within 80 to 120% and RSD <20% for peak areas from all six replicates. Figure 3 shows the LLOQs for pesticide compounds in both black tea and orange matrices. The improved 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 Commission⁴: 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 set by the US-EPA,³ all were quantified below or at their MRL.

Recoveries in food matrices

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 4. In the orange matrix, approximately 50% of the compounds achieved a recovery within SANTE guidelines of 80 to 120%, while approximately 40% of compounds were recovered within the guideline in the black tea matrix.² As expected, the black tea matrix showed more matrix effects than the orange matrix. Therefore, a matrix-matched calibration curve is generally recommended for samples with heavy matrix effects. According to analysis on the matrix blanks, 12 pesticides were present in the original black tea or orange samples even though they were purchased as “organic” products from a local supermarket.

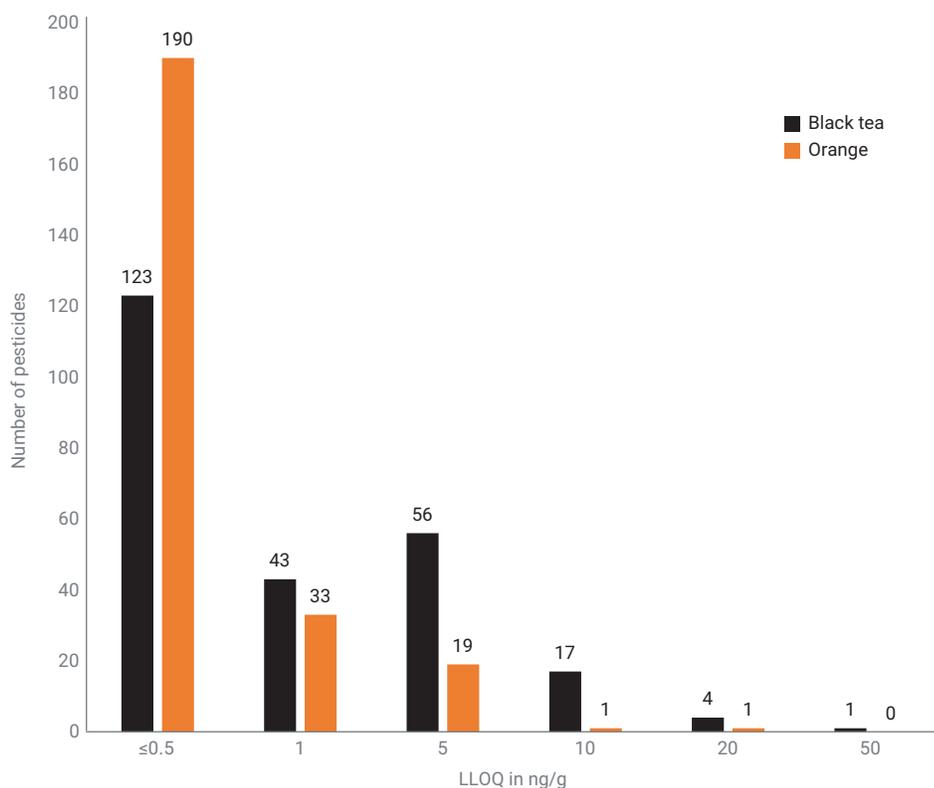


Figure 3. LLOQs for pesticides spiked into black tea and orange.

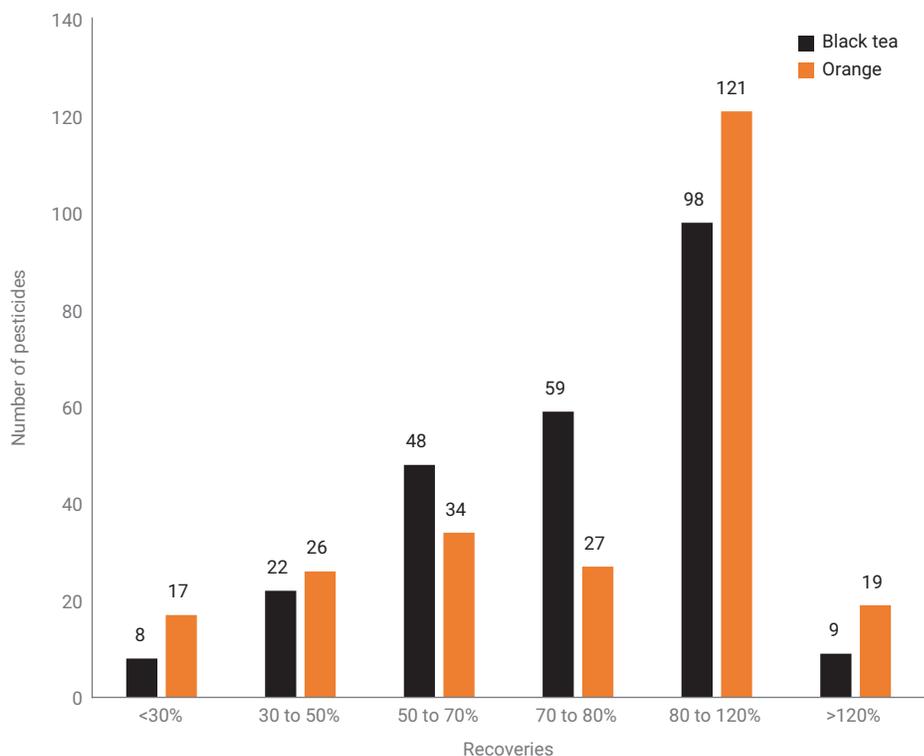


Figure 4. Histogram of recoveries for pesticides spiked into black tea and orange at the default MRL of 10 µg/kg.

Conclusion

The quantification performance of 244 pesticide residues in food matrices has been evaluated on an LC/MS platform, including the ultrahigh-performance 1290 Infinity II LC system coupled to the 6470B triple quadrupole LC/MS with the high-sensitivity Jet Stream technology ion source (AJS). Included with this 6470B triple quadrupole LC/MS system is the addition of VacShield and improved fine-tuned electronics, resulting in an easy-to-maintain and robust system for high-uptime operations, demanding the greatest robustness and sensitivity.

Two food matrices have been used in this application note, black tea and orange extract. Surprisingly, the organic products purchased from a local supermarket contained a number of synthetic pesticides. For both food matrices, matrix effects were apparent by differences in analyte response when compared to neat ACN. Even in heavy matrix, over 98% of all targeted 244 pesticides had an LLOQ at or lower than the default MRL of 10 µg/kg, as specified by the European Commission, demonstrating the high sensitivity of the LC/MS platform. Compound recoveries were calculated based on the response of pesticides in the matrix over their response in neat ACN. In the orange matrix, approximately 50% of the compounds achieved a recovery within SANTE guidelines of 80 to 120%, while approximately 40% of compounds were recovered within guidelines in the black tea matrix.

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