Multiresidue Pesticide Analysis with the Agilent Intuvo 9000 GC and Agilent 7000 Series Mass Spectrometer

Application Note

Author

Rebecca Veeneman, PhD and
Joan Stevens, PhD
Agilent Technologies, Inc.

Abstract

This Application Note shows an evaluation of pesticides in seven different matrices for the Agilent Intuvo 9000 GC and an Agilent 7000 Series Mass Spectrometer. Calibration curves for 21 pesticides showed excellent linearity for concentrations ranging from 1 ng/mL to 1,000 ng/mL. Excellent response and peak shape consistency was obtained with the implementation of the Agilent Intuvo Guard Chip, which protects downstream components and eliminates the need to trim the column after matrix evaluation. Average recovery for a 50 ng/mL sample across 60 food extract injections was over 80 %, with an RSD of less than 10 %. With regular maintenance, including liner and Intuvo Guard Chip replacements, peak shape and recoveries were found to be unchanged for over 500 injections.

Introduction

As pesticide use has increased, so has the level of concern among environmentalists, regulators, and consumers. Regulations regarding the maximum residue limit (MRL) of pesticide that can be found in or on food have been established in North America (United States and Canada), Europe (European Union), Asia (Japan), and Australia. In the United States, MRLs can range from 0.02 ppm to 100 ppm depending on the matrix and pesticide in question[1], while the European Commission has a default value of 0.01 ppm[2].

To analyze pesticide residues in foods, some level of sample preparation must be done. At a minimum, the sample must be homogenized and extracted into a solvent suitable for chromatography. The QuEChERS extraction method is commonly used for pesticide extraction as it involves a single acetonitrile extraction, and simultaneous salting out with magnesium sulfate. In some cases, additional cleanup is performed with dispersive solid phase extraction (dSPE)[3]. The resulting sample is still relatively dirty, and can pose a problem for accurate identification and quantification due to high background signal.

The complexity of pesticide analysis lends itself well to gas chromatography/mass spectrometry (GC/MS). However, the quantitation limits and MRL ranges drive the need for a multiresidue method with a reasonable linear range and low limits of detection. For this reason, tandem mass spectrometry (MS/MS) can be used for screening, confirming, and quantifying low level pesticides. It not only provides low limits of quantitation, but also minimizes interferences from matrices[4].

While using multiple reaction monitoring (MRM), enabled with MS/MS, can reduce matrix interferences in the chromatogram, it does not remove the matrix from the sample. Injecting matrix can result in loss of signal and tailing. This can be mitigated, to some extent, by using backflush, but careful and frequent maintenance, including liner replacements and column trims, are needed to fully maintain the system. The Agilent Intuvo 9000 GC uses an Intuvo Guard Chip as part of the Intuvo inert flow path, eliminating the need to trim the column. By removing column trimming from the maintenance model, retention times are left unchanged while the column is maintained for longer lengths of time.

For this Application Note, an Agilent 7000 Series Triple Quadrupole GC/MS was coupled with an Intuvo 9000 GC with an Agilent Intuvo HP5-MS UI Column. Calibration curve linearity and analyte recovery over time was evaluated for seven different food matrices using sandwiched injections.

Experimental

Two custom pesticide standard mixes at 100 µg/mL were obtained from Ultra Scientific (North Kingston, RI). Stock solutions at 10 µg/mL were made for the two mixes in their respective solvents. The stock solutions were combined to make a working standard at 1 µg/mL in acetone. Calibration standards at 1, 5, 10, 50, 100, 200, and 500 ng/mL were diluted from the working standard in acetone. Individual deuterated polyaromatic hydrocarbons (PAHs) were obtained from AccuStandard (New Haven, CT), and a working stock solution of 8 µg/mL was made in acetone. This was added to the pesticide calibration standards at 40 ng/mL, and used as the internal standard. Standards were stored at 3 °C. Table 1 shows a list of pesticides and internal standards.

Table 1. Target pesticide and internal standard identification is given.

No.	Compound
1	1,4-Diclhlorobenzene-d4
2	Naphthalene-d8
3	Methacrifos
4	Acenaphthene-d10
5	Ethalfluralin
6	Sulfotep
7	Demeton-S
8	Simazine
9	Lindane
10	Phenthrene-d10
11	Chlorpyrifos methyl
12	Fenitrothion
13	Aldrin
14	Pendimethalin
15	Tolyfluanid
16	Dieldrin
17	Buprimate
18	Triazophos
19	Chrysene-d12
20	Iprodione
21	EPN
22	Phosalone
23	Mirex
24	Coumaphos
25	Perylene-d12
26	Pryaclostrobin
27	Deltamethrin

A stock solution of L-glulonolactone was made by weighing 500 mg into a 10-mL volumetric flask. Water (4 mL) was added before diluting to the mark with acetonitrile. Separately, 500 mg of D-sorbitol was added to a 10-mL volumetric flask, and diluted to the mark with acetonitrile after adding 5 mL of water. An analyte protectant solution (20 mg/mL L-glulonolactone and 10 mg/mL D-sorbitol) was made by combing the two stock solutions in a 10-mL volumetric flask, and diluting to the mark with acetonitrile.

Seven different matrices were prepared for this analysis. They were extracted using the QuEChERS method, in which various dSPEs were used for matrix cleanup.

Three grams of olive oil and 7 mL of water were vortexed for 2 minutes with two ceramic homogenizers. Ten milliliters of acetonitrile (ACN) were added, and the sample was vortexed for 2 minutes. The QuEChERS EN salts (p/n 5982-5650) were added, and the tubes were placed on a GenoGrinder vertical shaker for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. Five milliliters of water were added to an EMR—Lipid tube (p/n 5982-1010) containing 1 g of EMR—Lipid sorbent, and vortexed for 30 seconds. Five milliliters of the ACN extract were added to the activated EMR—Lipid, vortexed for 2 minutes, and centrifuged at 5,000 rpm for 5 minutes. The entire extract was decanted into a 50-mL centrifuge tube, and the entire contents of a Polish pouch (p/n 5982-0102) was added. The tube was capped, vortexed aggressively, and centrifuged at 5,000 rpm for 5 minutes. Four milliliters of the extract were transferred to a 15-mL centrifuge tube along with 300 mg/mL of extract from a Polish pouch. The tube was vortexed and centrifuged at 5,000 rpm for 5 minutes.

Ten grams of homogenized cucumber or 5 g of honey with 5 mL of water were vortexed with ceramic homogenizers for 2 minutes. Ten milliliters of ACN were added, and the mixture was vortexed for 2 minutes. The QuEChERS EN salts were added, and capped tubes were placed on a GenoGrinder vertical shaker for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. Six milliliters of the extract was transferred to the QuEChERS dSPE (p/n 5982-5056) for general fruit and vegetables, vortexed for 2 minutes, and centrifuged at 5,000 rpm for 5 minutes.

Ten grams of homogenized onion or homogenized orange or 3 g of homogenized rice with 7 mL of water were vortexed with two ceramic homogenizers. Ten milliliters of ACN were added, and the sample was vortexed for 2 minutes. The QuEChERS EN salts were added, and capped tubes were placed on a GenoGrinder vertical shaker for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. Six milliliters of the extract were transferred to QuEChERS dSPE (p/n 5982-5256) for fatty matrix, then vortexed for 2 minutes, and centrifuged at 5,000 rpm for 5 minutes.

Three grams of homogenized tea with 7 mL of water were vortexed with ceramic homogenizers. Ten milliliters of ACN were added, and the sample was vortexed for 2 minutes. The QuEChERS EN salts were added, and the tubes were placed on a GenoGrinder vertical shaker for 2 minutes, then centrifuged at 5,000 rpm for 5 minutes. Six milliliters of the extract were transferred to a QuEChERS dSPE (p/n 5982-5256) for pigmented matrix, vortexed for 2 minutes, and centrifuged at 5,000 rpm for 5 minutes.

After final centrifugation, the extracts were transferred to a 4-mL vial and stored at –20 °C until analysis.

Instrumentation

All testing was done on an Agilent Intuvo 9000 GC equipped with an Agilent 7693B Autosampler and an Agilent 7000 Series Triple Quadrupole MS. The Intuvo 9000 inert flow path was configured as a simple MS system with an Agilent Intuvo 15-m HP5-MS UI column. MRM was used by obtaining transitions from the P&EP MRM database (p/n G9250AA rev A.1.01). A 3-layer sandwich injection was made using the standard, matrix, and an analyte protectant solution to achieve matrix-matched calibration. Using sandwich injections allowed multiple matrices to be tested with only one set of calibration standards[5]. Table 2 gives detailed instrument conditions.

Results and Discussion

This study demonstrates the calibration linearity and chromatographic consistency achieved with the Intuvo 9000 GC and 7000 Series Triple Quadrupole MS system. A calibration curve consisting of triplicate 3-layer sandwiched injections of each standard was generated for each matrix. After calibrating, the 50 ng/mL standard was evaluated in triplicate as a calibration check before completing 60 matrix extract injections. The 60 matrix extract injections were sandwiched with the 50 ng/mL standard to monitor peak shape and recovery over time. Following the calibration, calibration check, and matrix injections, system maintenance was performed, which included replacing the septum, liner, and Guard Chip. No additional maintenance was performed before repeating the process for another matrix.

Table 2. Agilent 9000 Intuvo GC and Agilent 7000C MS/MS instrument conditions.

Parameter	Value
Agilent 9000 Intuvo GC	
Inert flow path configuration	Simple MS
Syringe	10 µL (p/n G4513-80204)
Solvent washes	Pre-injection 3x solvent A, acetone (3 µL) 3x solvent B, acetone (3 µL) Post-injection 3x solvent A, acetone (3 µL) 3x solvent B, acetone (3 µL)
Sample wash	1 × 1 µL
Sample pumps	6
Sandwich injection	3-layer sandwich L1 (matrix) 1 µL L2 (analyte protectant solution) 0.5 µL L3 (standard or sample) 1 µL
Carrier gas	Helium
Inlet	Split/splitless in pulsed splitless mode, 280 °C
Injection pulse pressure	30 psi until 0.5 minutes
Purge flow to split vent	15 mL/min at 0.5 minutes
Septum purge flow	3 mL/min
Gas saver	20 mL/min after 3 minutes
Intuvo Guard Chip	60 °C then 50 °C/min to 310 °C
Column	Agilent Intuvo HP5-MS UI (19091S-431UI-INT)
Column flow	1.4 mL/min
Column temperature program	60 °C (1.5 minutes), then 50 °C/min to 160 °C, then 8 °C/min to 240 °C, then 50 °C/min to 280 °C (2.5 minutes), then 100 °C/min to 290 °C (1.1 minutes)
Agilent 7000 Series triple quadrupole MS/MS	
Transfer line	280 °C
Source tempreature	280 °C
Quad temperature	150 °C
Solvent delay	3.1 minutes
Tune file	atunes.eiex.tune

Table 3 shows the calibration curve coefficients (R^2) for a subset of the pesticide analytes in the seven matrices. The listed pesticides were chosen to represent a range of retention times, functionality, and difficulty. Depending on the matrix, average R^2 values for the 21 pesticides ranged from 0.972 to 0.997. Honey and rice yielded the best R^2 values, with an average value of 0.997, while black tea showed slightly lower R^2 values, especially for early-eluting compounds, due to light matrix interferences. The remaining matrices had calibration curve coefficients of approximately 0.994.

After calibrating the system with the given matrix, 60 extract injections, sandwiched with the 50 ng/mL standard and analyte protectant solution, were made. Figure 1 shows the average recovery of the 50 ng/mL standard over the course of the 60 extract injections for the seven matrices.

Table 3. Very good linearity was achieved for the seven matrices evaluated. Ten target pesticides were selected for tabulation based on their retention time and perceived difficulty.

	Honey	Rice	Orange	Black tea	Olive oil	Onion	Cucumber
Methacrifos	0.998	0.998	0.992	0.921	0.994	0.994	0.999
Sulfotep	0.998	0.996	0.991	0.917	0.994	0.993	0.996
Simazine	0.994	0.996	0.989	0.907	0.995	0.992	0.997
Aldrin	0.996	0.994	0.997	0.986	0.991	0.996	0.995
Fenitrothion	0.996	0.999	0.999	0.999	0.998	0.999	0.998
Dieldrin	0.997	0.997	0.997	0.998	0.998	0.997	0.998
EPN	0.998	0.998	0.999	0.999	0.999	0.999	0.999
Mirex	0.999	0.998	0.999	0.998	0.997	0.997	0.997
Pyraclostrobin	0.998	0.998	0.998	0.999	0.983	0.993	0.991
Deltamethrin	0.995	0.996	0.996	0.995	0.998	0.982	0.968

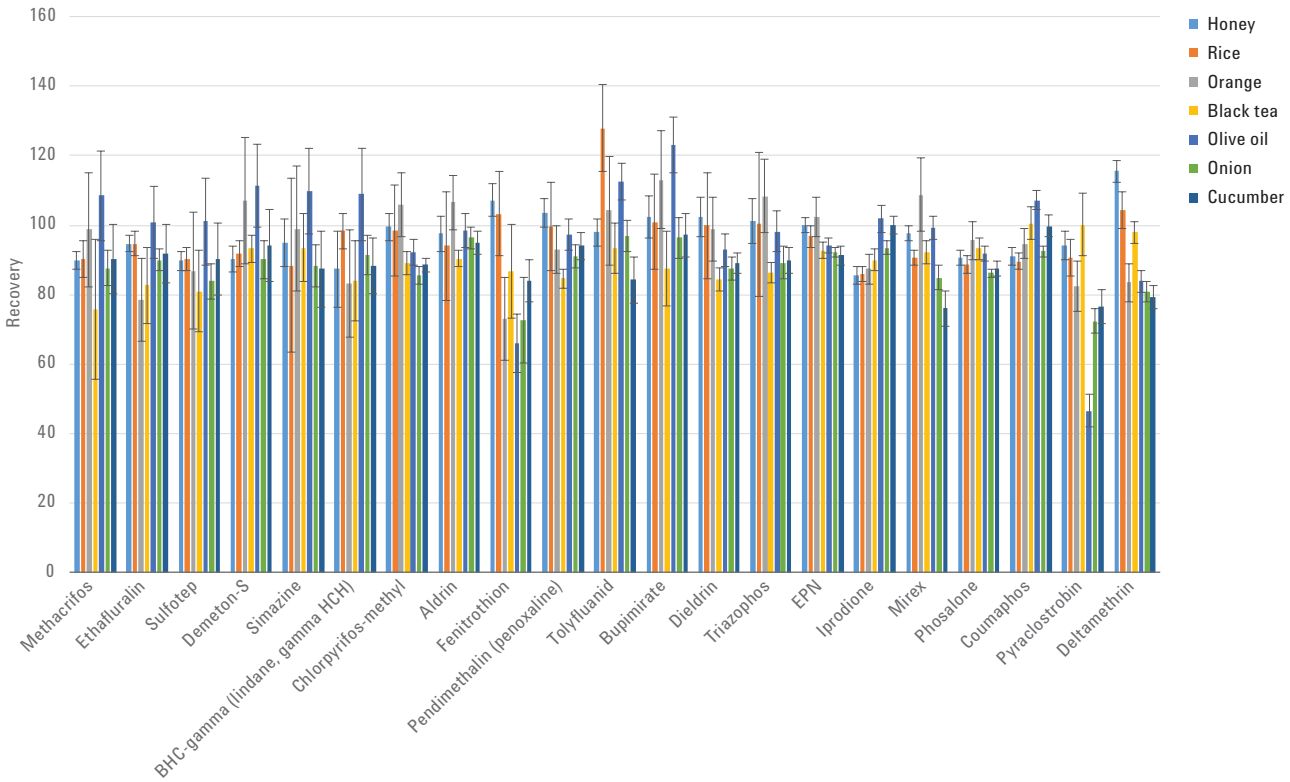

Figure 1. Average recoveries for 60 injections for seven different matrix types are nearly 100 % for a majority of the target analytes. Error bars denote the standard deviation of the measurement.

While there are some analytes that show a higher degree of variability or lower recovery, the majority of the data trends to approximately 100 % recovery. Considering the standard deviation over the course of 60 matrix injections, most pesticides show recoveries between 80 % and 120 % for all seven matrices. The average recovery for the target pesticides across the seven extracts evaluated was 82 %. RSDs for the 60 injections were very low, highlighting the consistency. The average RSD for 60 injections and seven matrices was 6.3 %. The multimatrix plot is separated in Figure 2 to highlight specific matrices: honey (a popular adulteration commodity), black tea (a popular complaint and difficult commodity), olive oil (a popular commodity), and cucumber (a widely requested matrix).

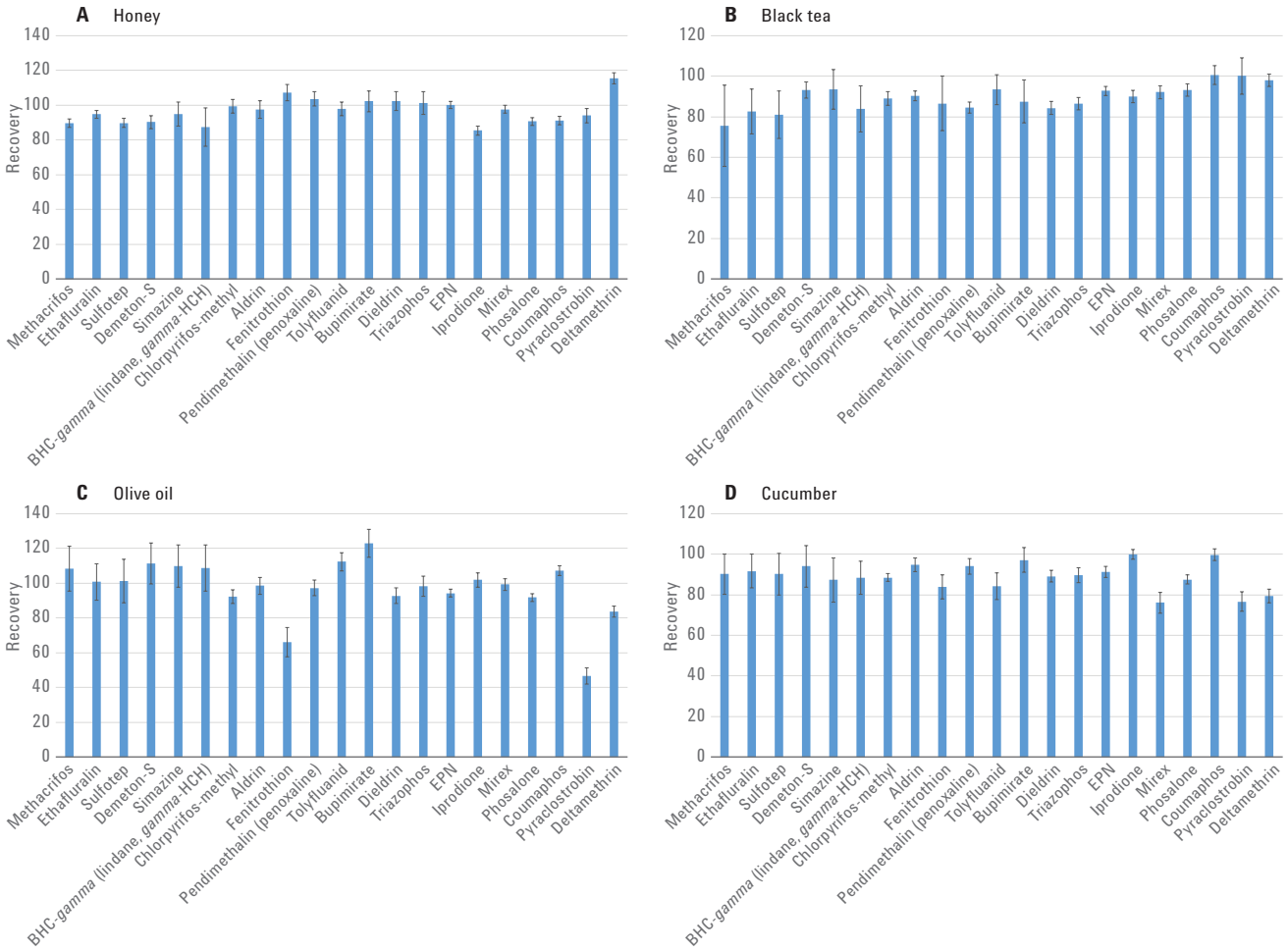

Figure 2. Honey, black tea, olive oil, and cucumber were plotted individually to demonstrate the performance consistency. Aside from a few difficult analytes, recoveries across 60 extract injections were found to be approximately 100 %.

Honey, black tea, and cucumber show relatively tight recoveries for the 21 pesticides, at 70 % or higher. Despite black tea being a difficult commodity and showing slightly lower R^2 values during calibration, the matrix evaluation showed excellent recovery across the range of pesticides. Olive oil proved to be the most challenging matrix in this evaluation, with recoveries for a few compounds (fenitrothion and pyraclostrobin) falling below 70 %, but otherwise showing very good recovery.

The consistency of area recoveries, seen in Figures 1 and 2, is complimented by chromatographic consistency. Figures 3 through 9 show overlaid chromatograms of the 50 ng/mL calibration check (performed after initial calibration), after 60 matrix injections were complete, and after maintenance (septum, inlet liner, and Intuvo Guard Chip replacement). Table 1 gives peak identification. The chromatogram after maintenance was not collected (Figure 4) due to an error. There was no difference in peak shape for any of the target analytes throughout the evaluation, and any response differences were accounted for by using relative response ratios. Peak shapes are generally sharp and symmetrical. Retention times were also consistent throughout the evaluation. Even after maintenance peaks were well overlaid, MRM transition times were not adjusted. The chromatograms show a high level of consistency among the different matrices for both retention times and peak shape. Because the Intuvo Guard Chip eliminates the need to trim the column by protecting downstream components from the matrix, retention time locking was not required. Furthermore, the Intuvo Guard Chip enabled consistent recovery and peak shape even without the use of backflushing techniques.

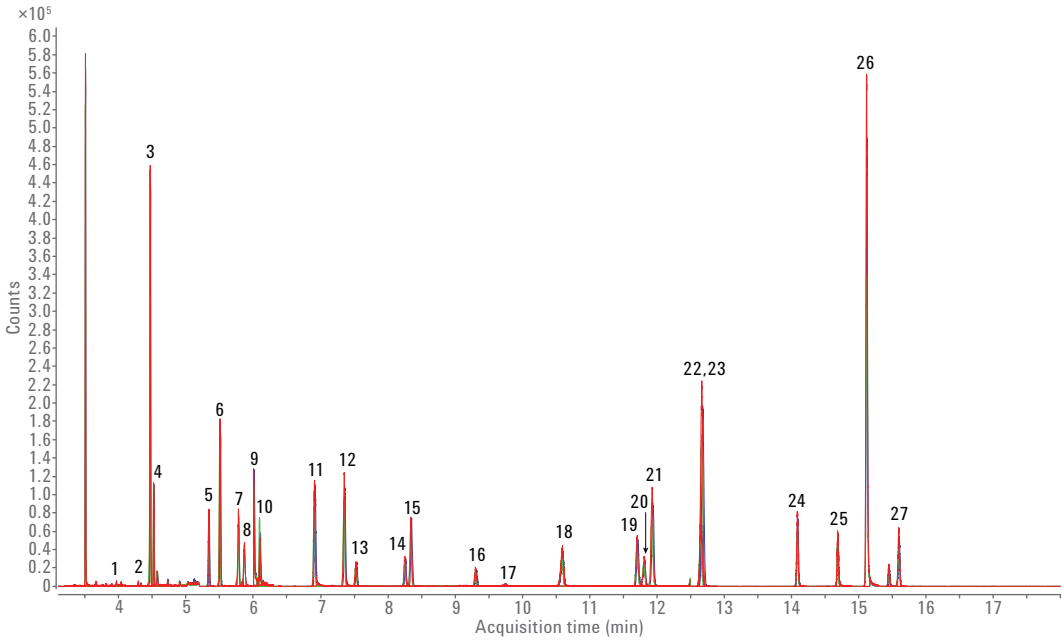

Figure 3. Overlaid chromatograms for the 50 ng/mL calibration check (blue) after 60 honey extract injections (red), and after liner and Agilent Intuvo Guard Chip replacement (green), show very good consistency.

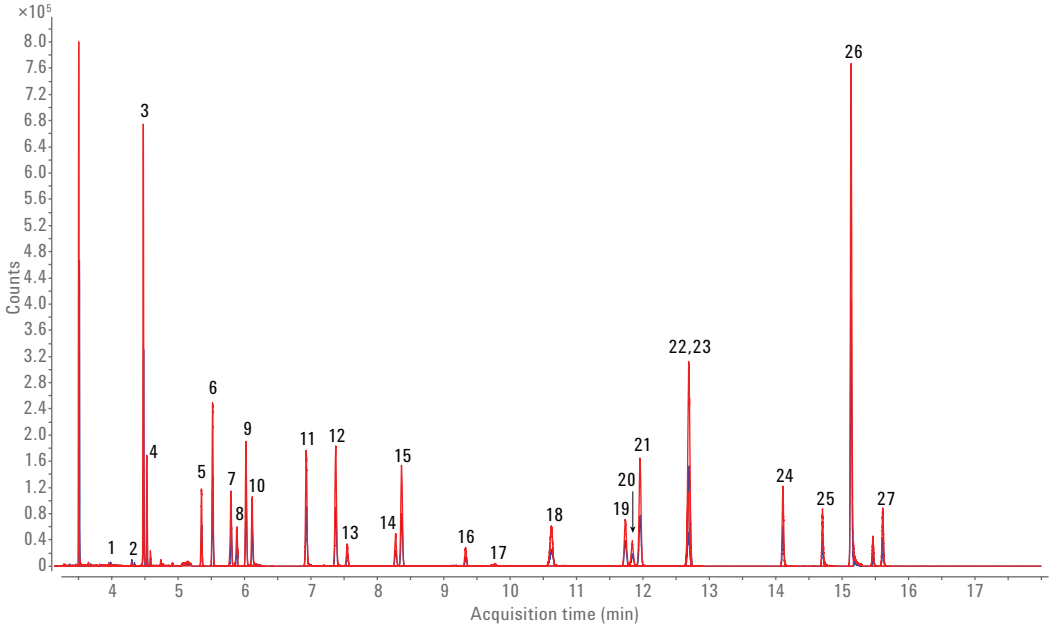

Figure 4. Overlaid chromatograms for the 50 ng/mL calibration check (blue), and after 60 rice extract injections (red) show very good consistency for peak shape. There is a slight increase in response after matrix injections due to evaporation from the vial.

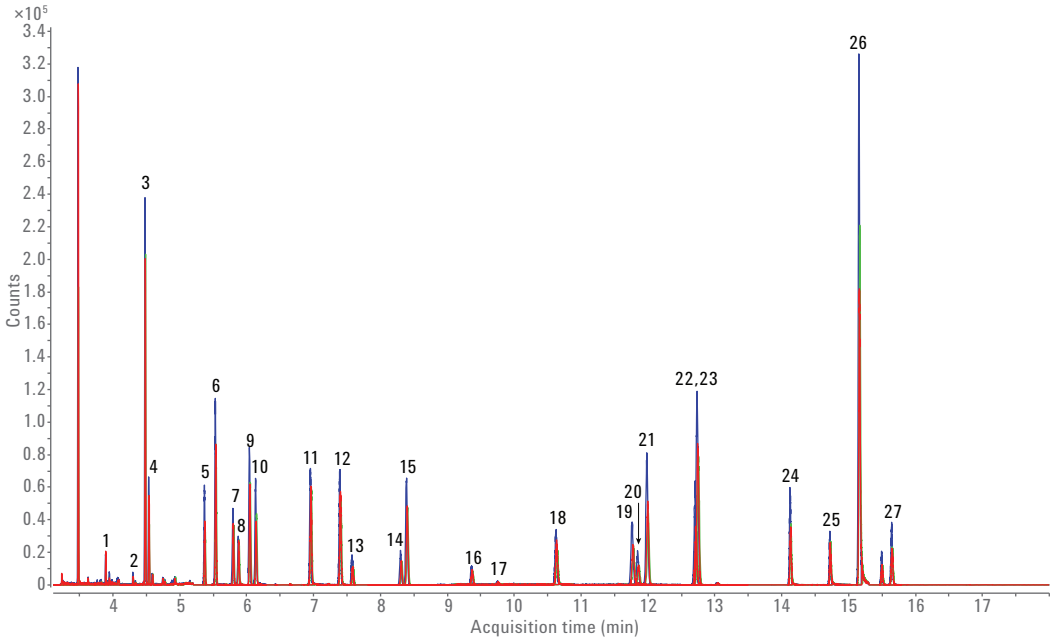

Figure 5. Overlaid chromatograms of the 50 ng/mL calibration check (blue), after 60 orange extract injections (red), and after liner and Intuvo Guard Chip replacement (green), show very good consistency. Slight differences in response is easily accounted for in response ratios.

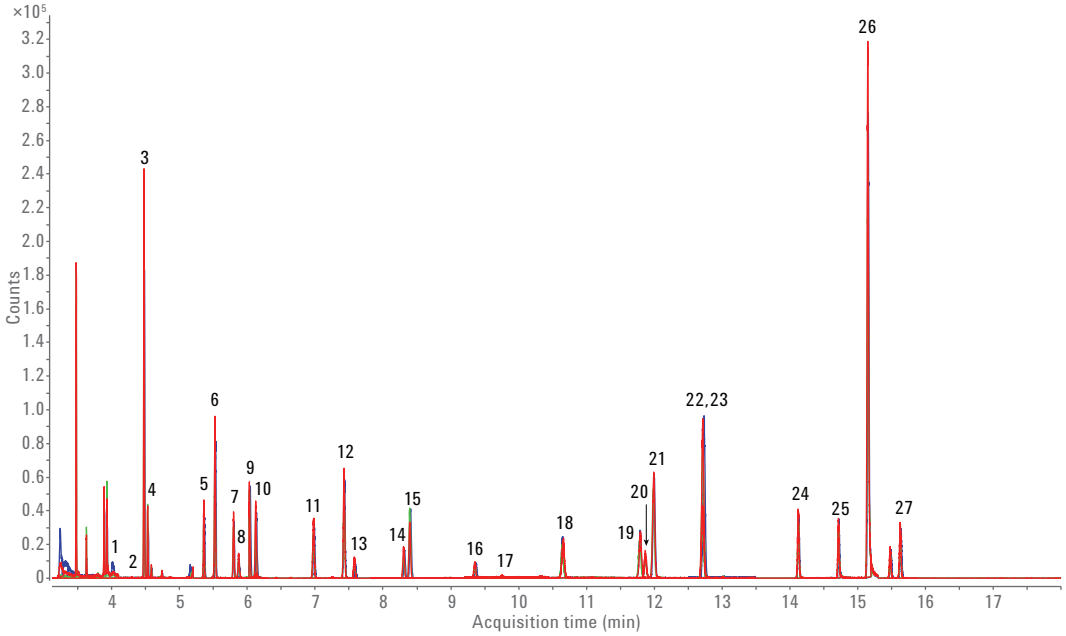

Figure 6. Overlaid chromatograms for the 50 ng/mL calibration check (blue), after 60 black tea extract injections (red), and after liner and Intuvo Guard Chip replacement (green), shows very good consistency for peak shape and response.

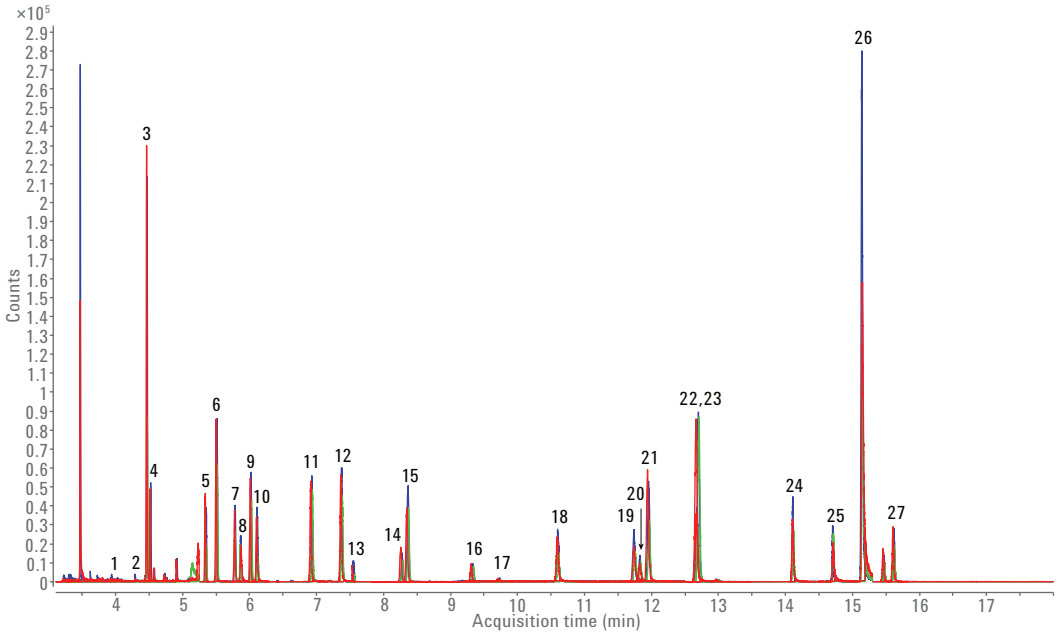

Figure 7. Overlaid chromatograms for the 50 ng/mL calibration check (blue), after 60 olive oil extract injections (red), and after inlet and Intuvo Guard Chip replacement (green), shows only slight differences in retention time or response.

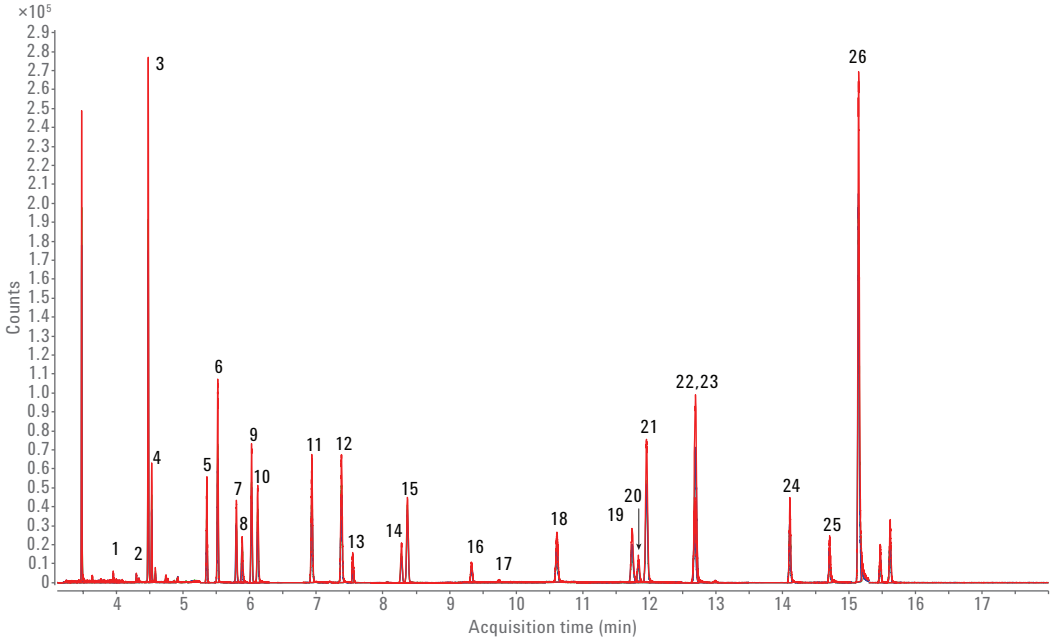

Figure 7. Overlaid chromatograms for the 50 ng/mL calibration check (blue), after 60 onion extract injections (red), and after liner and Intuvo Guard Chip replacement (green), shows excellent consistency for peak shape and response.

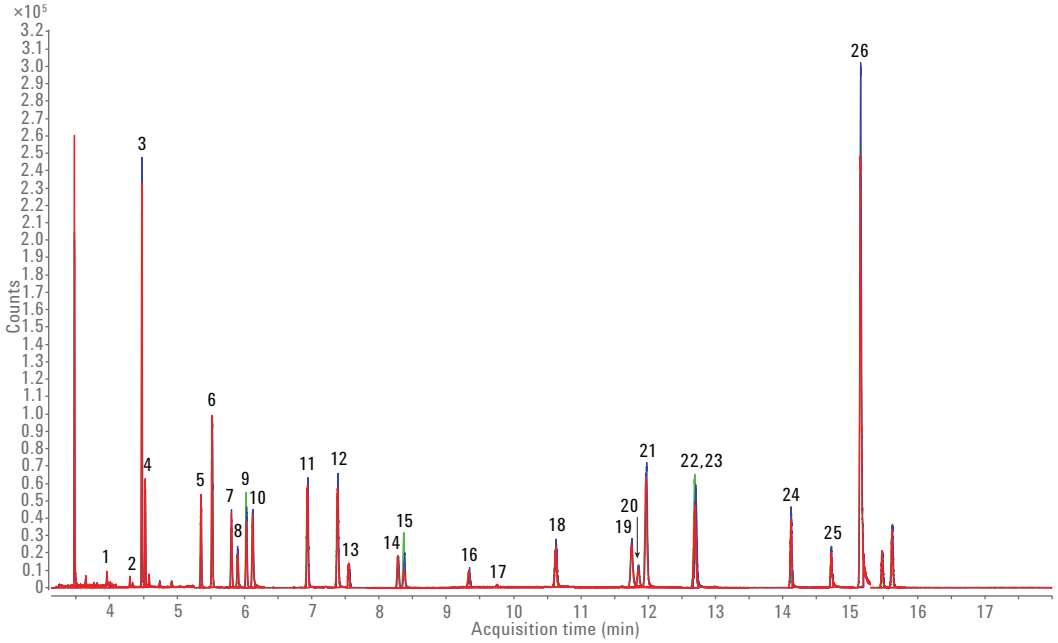

Figure 9. Overlaid chromatograms for the 50 ng/mL calibration check (blue), after 60 cucumber extract injections (red), and after liner and Intuvo Guard Chip replacement (green), shows very good peak shape and response consistency.

Conclusions

A calibration and matrix evaluation was performed on an Agilent Intuvo 9000 GC equipped with an Intuvo HP5-MS UI column and an Agilent 7000 Series Triple Quadrupole GC/MS. Twenty-one pesticides were evaluated with seven matrices to represent a range of commodities, with varying levels of difficulty. The instrument showed excellent calibration linearity and recovery. With the implementation of the Agilent Intuvo Guard Chip, the following was observed:

- The need to trim the column to maintain peak shape and recovery was eliminated.
- Retention time locking was not required.
- The source did not require cleaning throughout the entire evaluation.
- Excellent peak shape and recovery was maintained, even without backflushing.
- Replacement of the Intuvo Guard Chip did not affect retention times.

In this evaluation, calibration curve coefficients were usually 0.995 or better, regardless of matrix. Average recovery for a 50 ng/mL sample across 60 food extract injections was approximately 100 % for all seven matrices. This demonstrates consistent responses over the course of a batch analysis. Peak shapes and retention times were also exceptionally consistent both before matrix, after matrix exposure, and after maintenance. By preemptively replacing the Intuvo Guard Chip after approximately 100 injections, the system was well maintained for over 500 injections with minimal intervention.

References

1. Maximum Residue Limits Database. (2016, July 7). Retrieved from United States Department of Agriculture Foreign Agriculture Service: http://www.fas.usda.gov/maximum-residue-limits-mrl-database
2. EU legislation on MRLs. (2016, July 7). Retrieved from http://ec.europa.eu/food/plant/pesticides/max_residue_levels/docs/maximum_residue_levels_factsheet_en.pdf
3. M. Churley, J. Stevens, Reduce Cost of Pesticide Residue Analysis, *Agilent Technologies Application Note*, publication number 5991-6069EN, **2015**.
4. M. Churley, Lowering Detection Limits for Routine Analysis of Pesticies Residues in Foods Using the Agilent 7000C Triple Quadrupole GC/MS, *Agilent Technologies Application Note*, publication number 5991-4131EN, **2015**.
5. L. Zhao, MS Analysis of Fruit and Vegetable Pesticies by GC/MS/MS Using Agilent Inert Flow Path, *Agilent Technologies Application Note*, publication number 5991-3234EN, **2013**.

www.agilent.com/chem/intuvo

Published in the USA, September 1, 2016
5991-7216EN