Application Note

Food Testing and Agriculture

Quantitation of 341 Pesticide Residues in Tomato According to SANTE 11312/2021 Guideline

Using the Agilent 7010C triple quadrupole GC/MS

Author

Teresa Klink
Agilent Technologies, Inc.

Abstract

A comprehensive, quantitative GC/MS/MS workflow using the Agilent 8890 GC and 7010C triple quadrupole GC/MS was developed for the quantitation of 341 pesticide residues in tomato samples following the SANTE 11312/2021 guideline. This workflow aimed to demonstrate an accelerated and simplified workflow for routine laboratory food testing. Over 99% of analytes demonstrated linearity with $R^2 \geq 0.99$. Method precision was assessed using recovery repeatability (RSD_r). At the 10 µg/kg level, RSD_r values of 99% of compounds were within the limit of 20%. The mean recoveries of the six technical replicates were within the limits of 70 to 120% for 96% of target analytes. This workflow, including sample preparation, chromatographic separation, MS detection targets quantitation, and results interpretation helps streamline routine pesticide analysis and, therefore, accelerates lab throughput and productivity.

Introduction

Pesticides play an import role in the agriculture and food industry to improve crop yield and food production. Residues of pesticides remaining in or on commodities such as fruits, vegetables, or cereals can cause adverse health effects as well as environmental concerns. Regulatory agencies have set maximum residue levels (MRLs) for hundreds of pesticides and their metabolites. Most MRLs are set at low parts per billion (ppb) levels, which poses significant challenges, especially if hundreds of analytes are screened and quantified simultaneously in complex food matrices. In Europe, pesticide testing laboratories adhere to the SANTE 11312/2021 guideline. This guideline ensures a consistent approach, controlling MRLs that are legally permitted in food or animal feed. Due to the vast number of pesticides, the analysis is very elaborate, often requiring multiple analytical approaches and laboratory-intensive workflows, resulting in high operating costs and slow turnaround times.

This application note describes a comprehensive, quantitative GC/MS/MS workflow for the quantitation of 341 pesticide residues within a 20-minute GC run time to accelerate and simplify routine laboratory food testing. The workflow applicability was demonstrated on tomato samples using an 8890 GC system coupled to a 7010C triple quadrupole GC/MS. For sample preparation, an Agilent Bond Elut QuEChERS EN extraction kit was used without the requirement for further cleanup. Compound transitions and optimized parameters were developed based on the Agilent pesticides and environmental pollutants (P&EP) MRM database, including curated parameters for fast and easy transfer into the analytical method. Workflow performance was evaluated and verified according to the SANTE 11312/2021 guideline based on instrument limit of detection (LOD), calibration curve linearity, recovery, and precision using matrix matched calibration standards from 0.5 to 100 µg/L.

Experimental

Chemicals and reagents

Agilent LC/MS-grade acetonitrile (ACN) was used for the study. All other solvents used were HPLC grade and from Merck (Darmstadt, Germany).

Standards and solutions

The following ready-to-use and custom premixed pesticide standards were acquired:

- Agilent GC pesticide standard 1 to 10, and 12 (part numbers PSM-100-A to -J, and -L)
- Agilent GC pesticide no. 1 and 2 (part numbers PSM-105-A and -B)

Other single standards, either as standard solution or powders, were purchased from AccuStandard (amchro GmbH, Hattersheim, Germany) and LGC (LGC Standards GmbH, Wesel, Germany).

When single standards were purchased as powders, single stock solutions with a concentration of 1,000 mg/L were prepared in acetone and stored at –20 °C.

Three intermediate standard mixes (1 to 3) were prepared from stock solutions and used for the rest of the experiments. Mixes 1 and 2 were prepared in ACN at concentrations of 1,000 µg/L each. Mix 3 was prepared in acetone at a concentration of 500 µg/L due to immiscibility with ACN. Working standards at 500 µg/L were diluted from mixes 1 and 2 and used with mix 3 for the preparation of prespiked quality control (QC) samples.

Solvent calibration standards were prepared for all standard mixes in ACN for preparation of matrix matched calibration and matrix effect (ME) assessment.[1] Serial dilutions were done from each mix to prepare eight calibration concentration levels of 5, 10, 20, 50, 100, 250, 500, and 1,000 µg/L (only mix 1 and 2). Calibration standards were prepared freshly and stored in a refrigerator at 4 °C if not used immediately.

Sample preparation

Pesticide-free and organic-labeled tomatoes were obtained from local grocery stores. The tomato was homogenized using a domestic blender and stored in the refrigerator at 4 °C before analysis.

The following products and equipment were used for sample preparation:

- Agilent Bond Elut QuEChERS EN extraction kit (part number 5982-5650CH)
- Vortex mixer (VWR International GmbH, Darmstadt, Germany)
- Centrifuge UNIVERSAL 320 R (Andreas Hettich GmbH, Tuttlingen, Germany)

Samples of 10 ± 0.1 g of homogenized tomato were weighed into a 50 mL tube. Prespiked QC samples were fortified by spiking 200 µL working standards (500 µg/L) to give a final concentration of 10 µg/kg. After spiking, the samples were capped tightly, vortexed, and equilibrated for 15 to 20 minutes. QuEChERS extraction was then performed and the samples were centrifuged. Before GC/MS/MS analysis, the extracts were diluted by a factor of 5 with acetonitrile. The preparation procedure is illustrated in Figure 1.

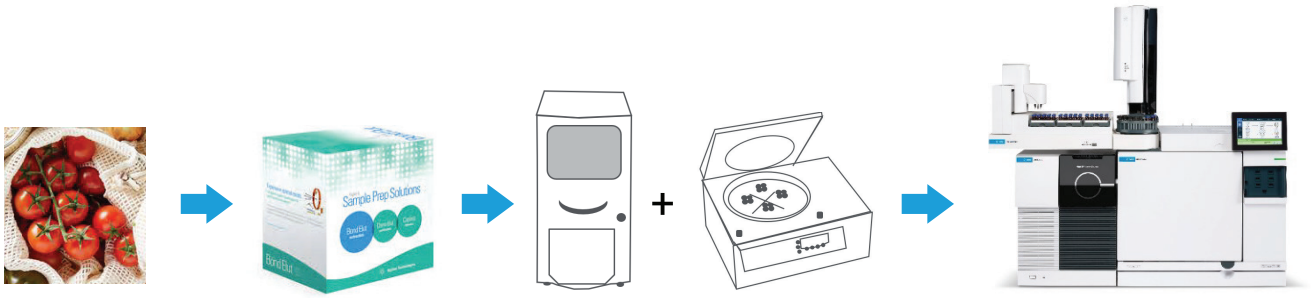

Figure 1. Sample preparation procedure using the Agilent Bond Elut QuEChERS EN extraction kit for sample cleanup before analysis with the Agilent 8890 GC and 7010C triple quadrupole GC/MS.

Preparation of matrix-matched calibration standards

Matrix matched calibration standards (postspiked standards) were used and prepared for the assessment of workflow performance. A matrix blank was prepared using an unfortified, blank sample of tomato. Preparation of matrix matched calibration levels was performed by mixing 100 µL solvent standard with 900 µL tomato blank matrix, resulting in a matrix matched calibration range from 0.5 to 100 µg/L. Before GC/MS/MS analysis, all solutions were diluted by a factor of 5 with ACN. The matrix-matched standard at 10 ppb was used to evaluate the ME by comparing responses with the corresponding solvent standard.[1]

Instrumentation

The 8890 GC was configured with the Agilent 7693A automatic liquid sampler and 150-position tray. The system used a multimode inlet (MMI) operated in temperature-programmed solvent vent injection mode. Chromatographic separation was performed using the conventional 15 m × 15 m midcolumn backflush configuration described in the P&EP database.

Therefore, two Agilent HP-5ms Ultra Inert (UI) GC columns (part number 19091S-431UI) were used, and midcolumn backflush capability was provided by the Agilent purged Ultimate union (PUU) installed between the two identical 15 m columns, and the pneumatic switching device (PSD) module on the 8890 GC. A schematic overview of this column setup is shown in Figure 2.

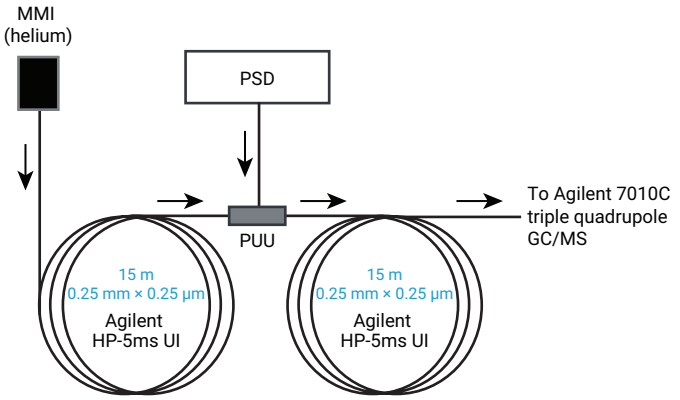

Figure 2. Midcolumn backflush configuration.

A 7010C triple quadrupole GC/MS with an Agilent high-efficiency source (HES) was operated in dynamic MRM (dMRM) mode. Data acquisition and processing was performed using Agilent MassHunter acquisition software (version 10.2) and MassHunter Quantitative Analysis software (version 12.0). All GC and MS conditions are listed in Table 1.

Table 1. GC and MS conditions.

Parameter	Value
GC	
Model	Agilent 8890 GC (220 V oven) with fast oven, auto injector, and tray
Inlet	Multimode inlet (MMI)
Mode	Solvent vent
Vent Flow and Pressure	20 mL/min at 5 psi until 0.06 min
Purge Flow to Split Vent	60 mL/min at 2 min
Septum Purge Flow	3 mL/min
Septum Purge Flow Mode	Switched
Injection Volume	1 µL
L1 Air Gap	0.1 µL
Gas Saver	15 mL after 4 min
Inlet Temperature Program	60 °C for 0.06 min, then to 280 °C with 720 °C/min
Postrun Inlet Temperature	310 °C
Carrier Gas	Helium
Inlet Liner	Agilent Ultra Inert dimpled liner (p/n 5190-2297)
Oven	
Initial Oven Temperature	60 °C
Initial Oven Hold	1 min
Ramp Rate 1	40 °C/min
Final Temperature 1	170 °C
Final Hold 1	0 min
Ramp Rate 2	10 °C/min
Final Temperature 2	310 °C
Final Hold 2	3 min
Total Run Time	20.75 min
Postrun Temperature	310 °C
Postrun Time	1.5 min
Equilibration Time	0.5 min
Column 1	
Type	Agilent J&W HP-5ms Ultra Inert
Length	15 m
Diameter	250 µm
Film Thickness	0.25 µm
Control Mode	Constant flow
Flow	0.94 mL/min
Inlet Connection	MMI
Outlet Connection	PSD 1
Postrun Flow (Backflushing)	–5.8 mL/min
Column 2	
Type	Agilent J&W HP-5ms Ultra Inert
Length	15 m
Diameter	250 µm
Film Thickness	0.25 µm
Control Mode	Constant flow
Flow	1.14 mL/min
Inlet Connection	PSD 1
Outlet Connection	MSD
Postrun Flow (Backflushing)	6.2 mL/min
Transfer Line Temperature	280 °C
MSD	
Model	Agilent 7010C triple quadrupole GC/MS
Source	Agilent HES
Vacuum Pump	Performance turbo
Tune File	Atunes.eihs.jtune.xml
Quadrupole Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM
Helium Quench Gas	2.25 mL/min
Nitrogen Collision Gas	1.5 mL/min
MRM Statistics	
Total MRMs (dMRM Mode)	2,093
Minimum Dwell Time	1.2 ms
Minimum Cycle Time	201.5
Maximum Concurrent MRMs	154
EM Voltage Gain Mode	10

Results and discussion

Development of triple quadrupole GC/MS method

A major part of this work was the development of dMRM transitions for 341 pesticide compounds based on the P&EP MRM database 4.0 (G9250AA).[2] Compounds whose MRM transitions were not listed in this database were developed using the Agilent MassHunter Optimizer for GC/TQ. Starting with a GC method that provides good chromatographic compound separation, the MassHunter Optimizer first identifies precursor and product ions, then optimizes collision energies for each promising precursor-product combination to identify the best MRM parameters. All other MRMs were taken from the database. Up to six MRMs for each compound were selected. Around 2,100 MRM transitions from 341 pesticides were stored in the dMRM method. Depending on the fragmentation behavior of the individual compound, two or three target-specific MRM transitions were selected per pesticide and later used for target quantification and qualification. This targeting was done to satisfy regulatory requirements for identification and confirmation by GC/MS/MS.[1]

Data were acquired in dMRM mode, which enables the capability for large multi-analyte assays and to accurately quantitate narrow peaks by an automated and most-efficient dwell time distribution. The dMRM capability enabled successful analysis of a large panel of 341 pesticides with 2,094 total MRM transitions and up to 154 concurrent MRMs (Figure 3). Furthermore, dMRM enables the analyst to add and remove additional analytes with ease.

The acquisition method was retention time locked to match the retention times in the P&EP database, which was used to seamlessly create the MS method. The use of the P&EP increased the ease and speed of setting up a targeted dMRM method. Retention time locking allows a new column or instrument to have retention times that match the MRM database or an existing method exactly, allowing methods to be easily ported from one instrument to another and across instruments globally. This simplifies method maintenance and system setup.

Midcolumn backflushing improved method robustness by reducing the regular maintenance frequency, such as column-head trimming and source cleaning. Also, when used with a temperature-programmable MMI, the liner change and other inlet maintenance procedures can be conducted much more rapidly without cooling down and venting the MS source, compared to a conventional configuration with a column connecting the inlet directly to the mass spectrometer.[3]

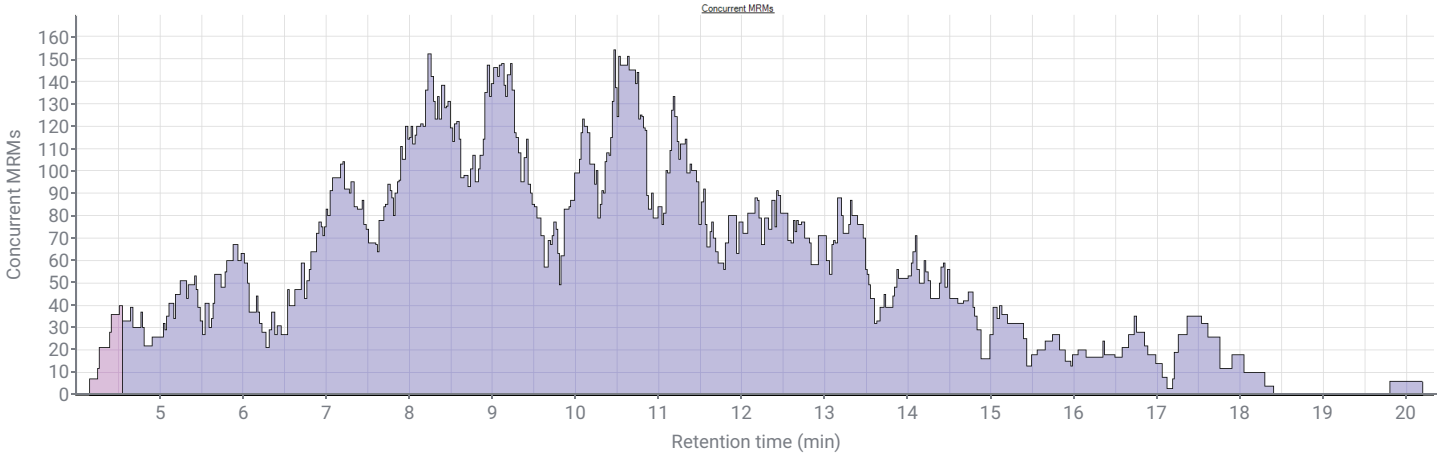

Figure 3. Overview of monitored MRMs over retention time.

A dMRM method with a cycle time of 300 milliseconds (ms) was used. Figure 4 shows a representative MRM chromatogram for all 341 pesticide targets postspiked at 10 µg/L in tomato extract.

Typical chromatographic peak widths were between 5 to 10 seconds. The selected cycle time of 300 ms ensured that sufficient data points were collected across the chromatographic peaks for reproducible quantitation and confirmation of results. Figure 5 shows the example of chlormephos, where 15 data points were collected across the peak for both quantifier and qualifier transition.

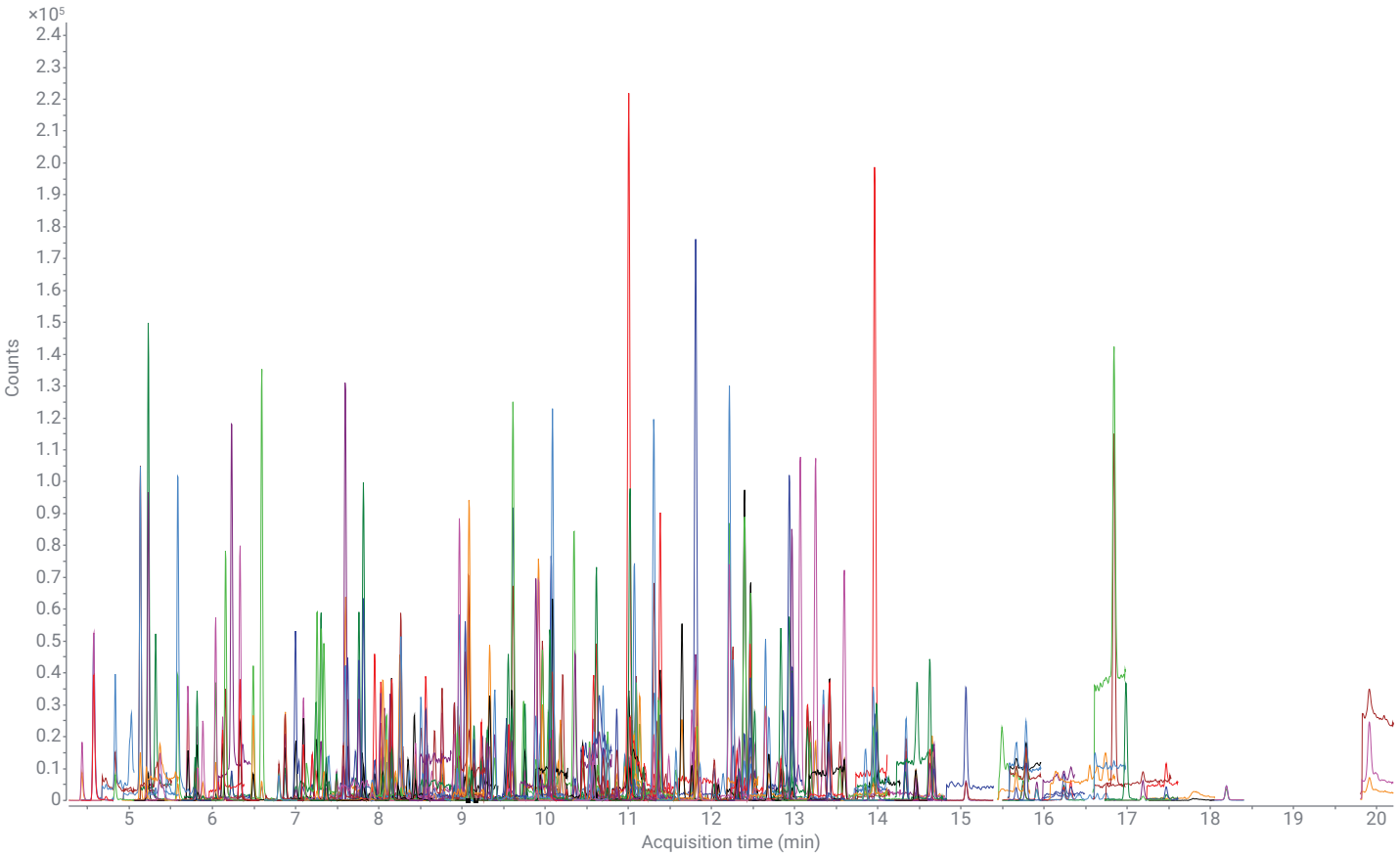

Figure 4. Overlay of MRM chromatograms of 341 pesticides postspiked at 10 µg/L in tomato extract.

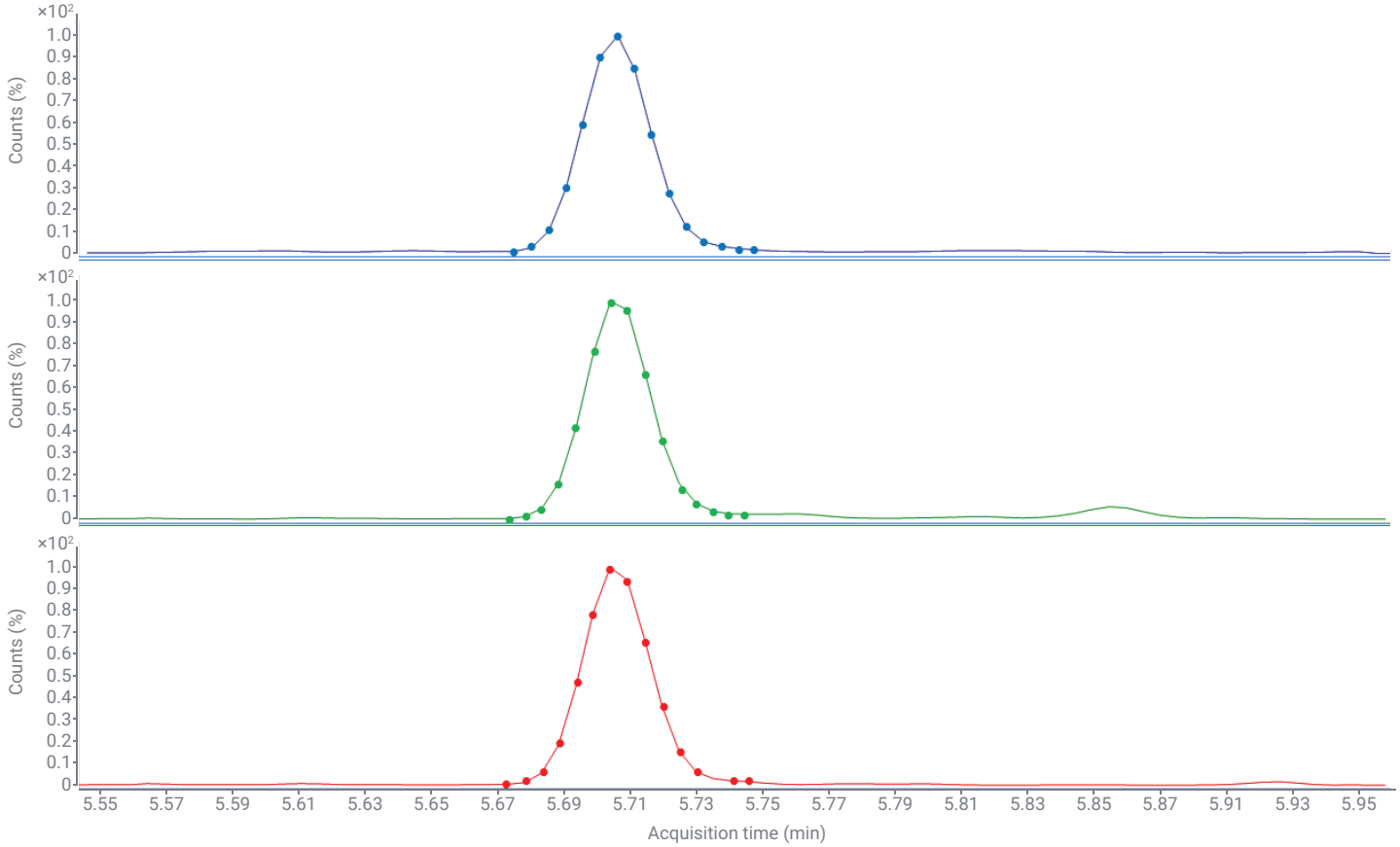

Figure 5. Data points per peak for quantifier and qualifier transitions for chlormephos.

Matrix effect assessment

Effects caused by sample matrix are frequent and cause suppression or enhancement of the MS detection system response.[1] This effect can be observed especially when analyzing pesticides using GC/MS in pure solvent. It can lead to poor peak shape and loss of analytes due to interaction with active sites in the inlet, column, and surface of ion source. Matrix components can mask these active sites in the flow path and can significantly increase peak area and improve peak shape.[4]

ME was assessed by the ratio of target response in matrix matched calibration standard at 10 ppb to that in the corresponding solvent standard. Typically, there is no strict requirement on acceptance of ME criteria, because ME can be corrected by the matrix-matched calibration curve. However, ME is an important parameter for method sensitivity and reliability assessment, and less than 20% signal suppression or enhancement is usually considered as insignificant ME.[1] In this study, ME was investigated using a 10 µg/L standard in tomato extract (postspiked standard) and the response was compared to the corresponding solvent standard. The 10 µg/L standard was chosen as this is the lowest MRL for pesticides and its metabolites. More than 95% of the 341 targets in tomato showed significant ME at 10 µg/L. Based on the results of the ME assessment, matrix matched calibration standards were used to compensate MEs in this study.

Verification of workflow performance

The workflow performance criteria were verified based on linearity, method sensitivity, recovery, and precision. The batch included solvent blank, matrix matched calibration standards, matrix blank, and prespiked QCs. Six technical replicates were prepared for the prespiked QCs.

Linearity

Calibration curves were generated for mixes 1 to 3 using matrix matched standards ranging from 0.5 to 100 µg/L, and eight calibration points. For some compounds, the low calibration point(s) were removed from calibration due to low sensitivity. To determine the best linearity response function, various regression models were evaluated and the best calibration model was with type: linear or quadratic; origin: ignore; weight: 1/x. More than 99% of the targets met the calibration curve linearity requirement of $R^2 \geq 0.99$.

Instrument limit of detection

A sensitive workflow for pesticide residue analysis is beneficial for users to perform routine operations following various regulatory guidelines. Instrument LODs were used to evaluate method sensitivity. Instrument LOD was established based on matrix matched calibration standards for signal-to-noise ratio (S/N) of 10 and up. The S/N was defined using the peak height and peak-to-peak algorithm embedded in MassHunter Quantitative Analysis software. The noise region was manually chosen and had a minimum length of 0.1 minute.

More than 94% of target compounds showed an instrument LOD of ≤ 10 µg/L, and, even at a concentration level of 1 µg/L, more than 71% of compounds had an S/N of 10 and up (Figure 6). These results demonstrate the high sensitivity of the 7010C triple quadrupole GC/MS against a complex matrix such as a tomato QuEChERS raw extract.

Method precision and recovery

Method precision was estimated using recovery repeatability (RSD_r) based on the variation of recovery values from technical replicates of prespiked QC samples that were spiked at 10 µg/kg. The RSD_r was determined by calculating percent relative standard deviation (%RSD) of recovery using these six technical preparations. Typically, the acceptable RSD_r is 20% or less. The RSD_r values for approximately 99% of all targets were within 20%, demonstrating consistent behavior with each technical preparation. These results confirmed the high repeatability of this workflow. Example chromatograms of the six technical replicates for flurenol-butyl, deltamethrin, and nitralin are given in Figure 7.

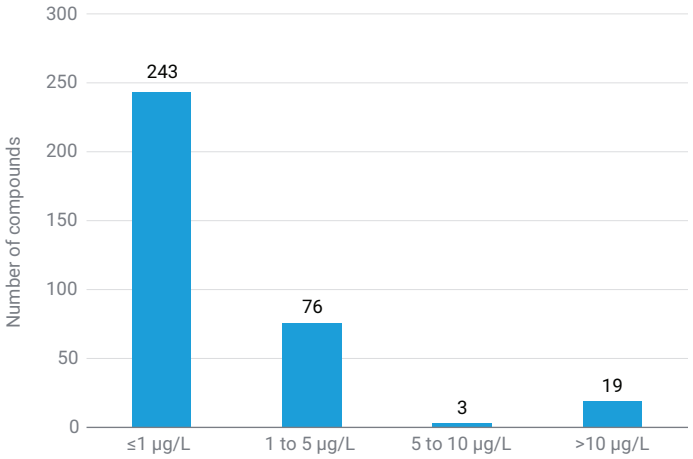

Figure 6. Instrument LOD in tomato QuEChERS raw extract.

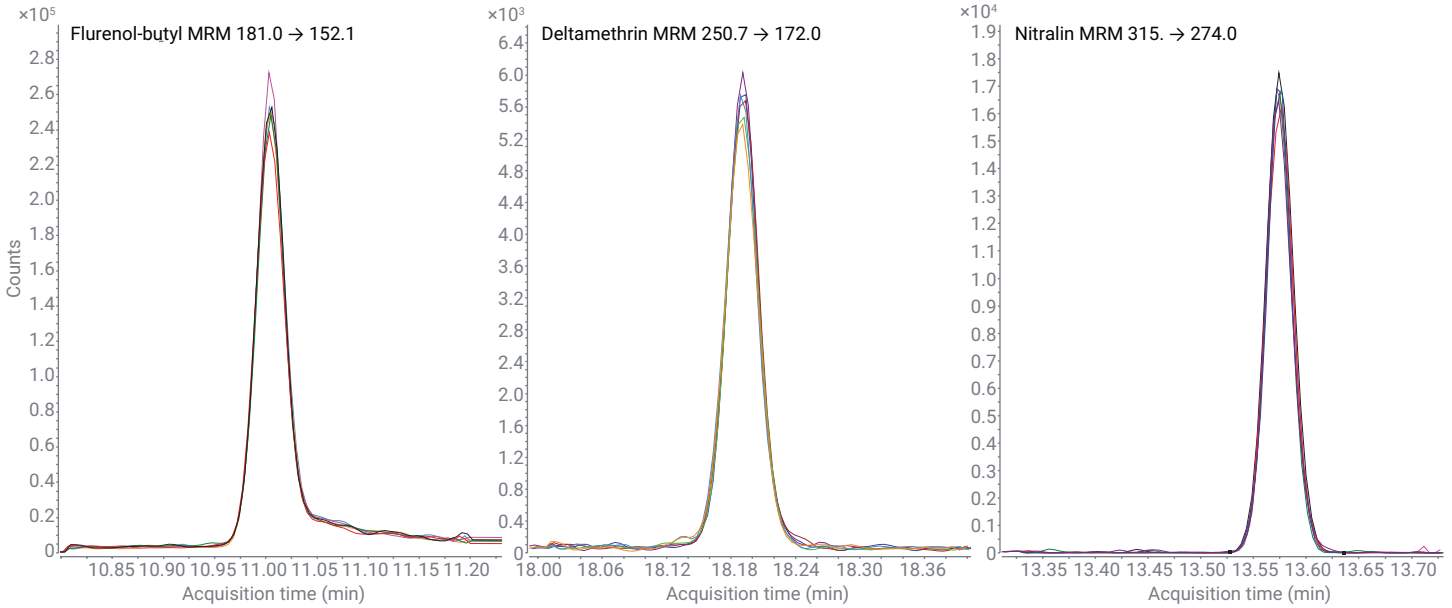

Figure 7. MRM chromatogram overlay for six technical replicates at 10 µg/kg in tomato extract.

Recovery was used in this experiment to evaluate the capability of a quantitative analytical workflow for 341 pesticides. Recovery was calculated based on analyte response ratios between prespiked QCs and corresponding matrix matched calibration levels. Mean recovery at 10 µg/kg level was obtained for six technical replicates. According to SANTE 11312/2021, mean recoveries can be accepted within the range of 40 to 120% if they are consistent ($RSD_r \leq 20\%$). Based on these criteria, the mean recovery results for more than 96% of targets in tomato QuEChERS raw extract at 10 µg/kg met the acceptance criteria. Most compounds (328) were within the recovery range of 70 to 120% with $RSD_r \leq 20\%$, and only 9 compounds (3%) were below 70% or above 120% respectively (Figure 8).

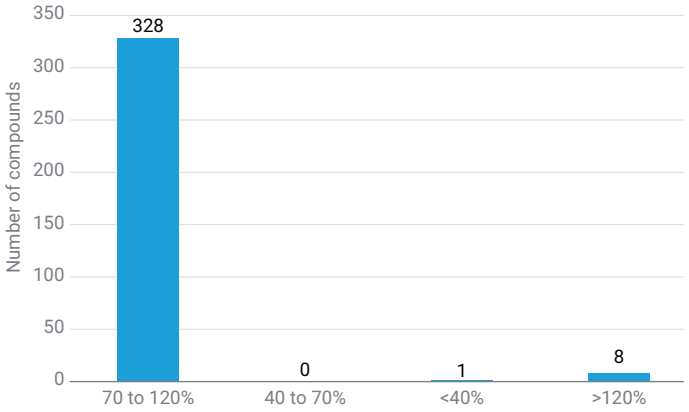

Figure 8. Recovery rates in tomato QuEChERS raw extract ($RSD_r \leq 20\%$).

Conclusion

This application note demonstrates the applicability of a sensitive and reproducible workflow for fast and reliable quantitation of 341 pesticide residues in tomato QuEChERS raw extract while conforming to the SANTE 11312/2021 guideline. The simple sample preparation protocol uses the Agilent Bond Elut QuEChERS EN extraction kit for facile extraction without requiring further sample cleanup.

The Agilent 8890 GC coupled to the Agilent 7010C triple quadrupole GC/MS were used to quantify 341 pesticide residues with matrix matched calibration. The 20-minute GC method using the 15 m × 15 m Agilent J&W HP-5ms UI column setup offered good chromatographic separation and even retention time distribution of all targets.

To achieve the most efficient use of instrument cycle time, the triple quadrupole MS data acquisition was acquired in dMRM mode. The dMRM method was created and developed based on the Agilent pesticides and environmental pollutants MRM database, which covers more than 1,100 compounds. This database is readily customizable, allowing the addition or removal of compounds or transitions as required (i.e., by adding new compounds or adding transitions to existing compounds).

The overall workflow performance was assessed for linearity, instrument LOD, recovery, and precision, demonstrating its suitability for the quantitation of 341 pesticide residues in a QuEChERS raw extract.

References

1. SANTE 11312/2021: Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.
2. The Agilent MassHunter Pesticide and Environmental Pollutants MRM Database (P&EP 4.0). G9250AA. https://www.agilent.com/en/product/gas-chromatography-mass-spectrometry-gc-ms/gc-ms-application-solutions/gc-ms-ms-pesticides-analyzer
3. Andrianova, A. *et al.* A Fast and Robust GC/MS/MS Analysis of 203 Pesticides in 10 Minutes in Spinach, *Agilent Technologies application note*, publication number 5994-4967EN, **2023**.
4. Westland, J. Advantages of Reversed Sandwich Injection for Pesticide Residue Analysis, *Agilent Technologies application note*, publication number 5994-7973EN, **2017**.

www.agilent.com

DE98064942

Printed in the USA, September 28, 2023
5994-6761EN

Agilent
Trusted Answers