Application Note

Food and Beverage Testing

Quantitation of Over 1,000 Pesticide Residues in Tomato According to SANTE 11312/2021 Guideline

Using LC/MS/MS and GC/MS/MS detection

Authors

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Abstract

A comprehensive multiresidue workflow was developed and validated for the simultaneous quantitation of over 1,000 pesticide residues in tomato to accelerate and simplify routine laboratory food testing. The workflow analyzes a wide range of pesticide residues simultaneously in 20 minutes and uses a single sample preparation method for both LC/MS/MS and GC/MS/MS analyses, leading to increased turnaround time, simplified analysis, and lower laboratory costs.

The workflow includes sample preparation, chromatographic separation, mass spectrometric (MS) detection, data analysis, and data interpretation using Agilent LC/MS/MS and GC/MS/MS systems. For sample preparation, the Agilent QuEChERS extraction kit was used without further cleanup. Compound transitions and associated optimized parameters were developed based on the Agilent pesticide MRM databases for both LC/MS and GC/MS workflows.

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. Over 98% 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 98% of compounds were within the limit of 20%. The mean recoveries of the six technical replicates were within the limits of 40 to 120% for 98% of target analytes.

Introduction

Pesticides play an important role in the agriculture and food industries 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.[1] This guideline ensures a consistent approach for 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.

In this study, an accurate and reliable analysis of over 1,000 pesticide residues in tomato was developed using a single QuEChERS extraction for sample preparation. As shown in the Venn diagram (Figure 1), 764 analytes were analyzed by LC/MS/MS and 341 analytes were analyzed by GC/MS/MS. The GC/MS/MS analysis included 84 analytes that can also be determined using LC/MS/MS; thus, this workflow covers a total of 1,021 unique substances.

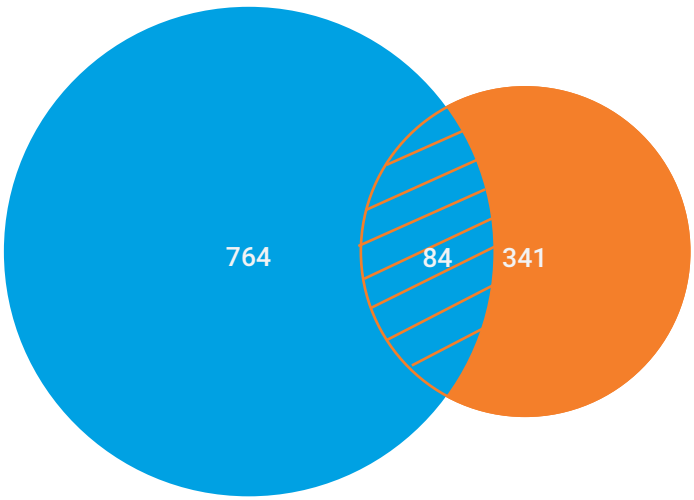

Figure 1. Venn diagram of compounds analyzed using LC/MS/MS (blue) and GC/MS/MS (orange).

This workflow, including sample preparation, chromatographic separation, MS detection, targeted quantitation, and results interpretation, helps streamline routine pesticide analysis and therefore accelerates lab throughput and productivity. Details of sample preparation procedures, instrumentation setup, and data analysis parameters are discussed, enabling the quantification and confirmation of pesticide residues.

Experimental

Chemicals and reagents

Agilent LC/MS-grade acetonitrile (ACN), methanol (MeOH), water, and ammonium formate were used in the study. LC/MS-grade formic acid was purchased from VWR. All other solvents used were HPLC grade and from VWR and Merck.

Standards and solutions

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

- Agilent LC/MS pesticide comprehensive test mix (part number 5190-0551)
- Agilent custom pesticide test mix (part numbers CUS-00000635 to CUS-00000643)
- Agilent custom organic standard (part number CUS-00004663)
- AccuStandard custom pesticide standard (part numbers S-96086-01 to S-96086-10), amchro GmbH, Hattersheim, Germany
- Agilent GC pesticide standard 1 to 10, and 12 (part numbers PSM-100-A to -J, and -L)
- Agilent GC pesticide standard 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.

Intermediate standard mixes were prepared from stock solutions and used for preparation of prespiked quality control (QC) samples, solvent calibration standards, and matrix-matched calibration. 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 tomatoes were 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 of 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. An aliquot of this extract was directly used for LC/MS/MS analysis. Before GC/MS/MS analysis, an aliquot of the extract was diluted by a factor of 5 with ACN. The preparation procedure is illustrated in Figure 2.

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 intermediate standard solutions with matrix blank extract. These solutions were used for LC/MS/MS analysis directly and diluted by a factor of 5 before GC/MS/MS analysis. The matrix-matched standard at 10 ppb was used to evaluate the matrix effect (ME) by comparing responses with the corresponding solvent standard.[1]

Instrumentation

The LC/MS/MS study was performed using an Agilent 1290 Infinity II LC system coupled to an Agilent 6470B triple quadrupole LC/MS. The modules of the LC/MS system included:

- Agilent 1290 Infinity II high-speed pump (G7120A)
- Agilent 1290 Infinity II autosampler (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 6470B triple quadrupole LC/MS (G6470B)
- Agilent pesticide dynamic MRM database (G1733CA)
- Agilent MassHunter software (version 10.1)

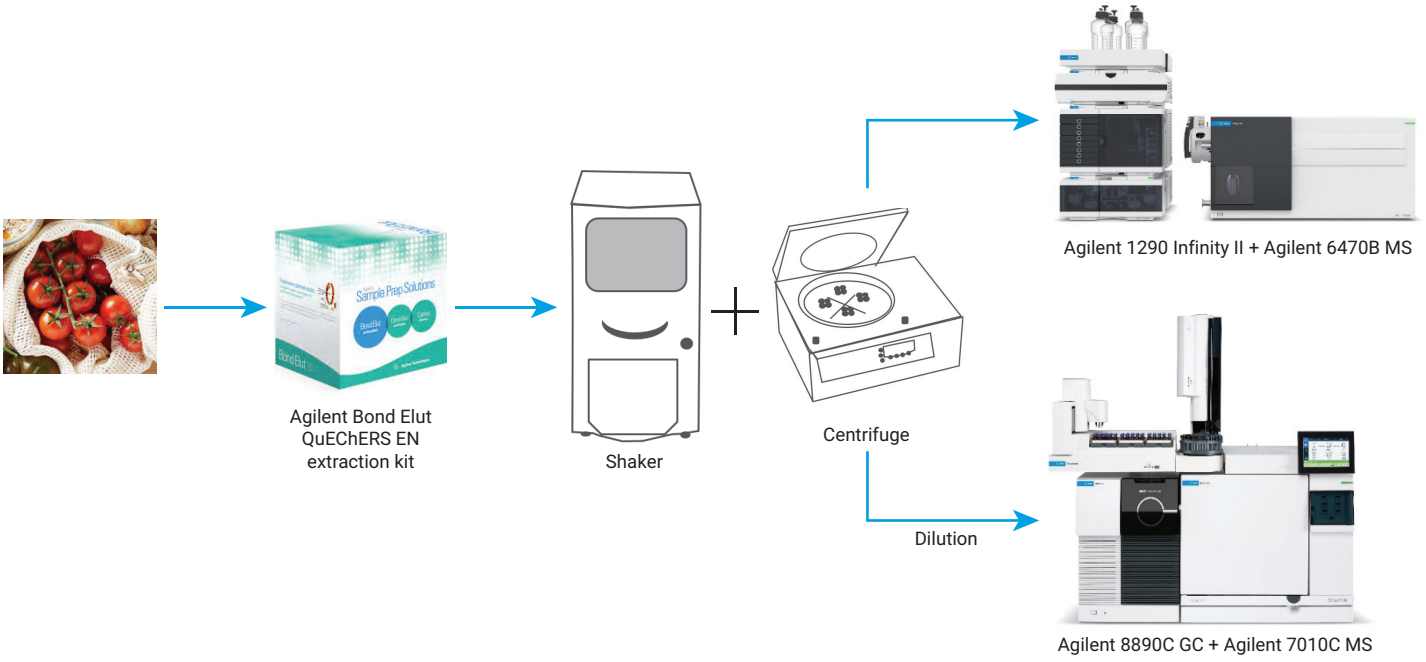

Figure 2. Sample preparation procedure using the Agilent Bond Elut QuEChERS EN extraction kit for sample cleanup before analysis.

The coupled 6470 triple quadrupole LC/MS was equipped with an Agilent Jet Stream (AJS) electrospray ion source and was operated in dynamic MRM (dMRM) mode.

The main LC and MS parameters are listed in Table 1. Please refer to the Agilent application note by Kornas for the detailed LC/TQ configuration.[2]

Table 1. LC and MS conditions.

Parameter	Value
LC	
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm (p/n 959759-902)
Column Temperature	40 °C
Injection Volume	2 µL
Autosampler Temperature	6 °C
Mobile Phase A	5 mM ammonium formate in water with 0.1% formic acid
Mobile Phase B	5 mM ammonium formate in methanol with 0.1% formic acid
Flow Rate	0.4 mL/min
Gradient	Time (min) A(%) B(%) 0 95 5 3 70 30 17 0 100 20 0 100
Postrun Time	3 min
Needle Wash	Multiwash
MSD	
Ionization Mode	Simultaneous positive/negative ESI with Agilent Jet Stream (AJS)
Scan Type	Dynamic MRM (dMRM)
Gas Temperature	200 °C
Gas Flow	9 L/min
Nebulizer	35 psi
Sheath Gas Temperature	400 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	2,500 V (+)/3,000 V (–)
Nozzle Voltage	0 V
Total MRMs	1,590
Min/Max Dwell Time	0.52 ms/242.30 ms

The GC/MS/MS study was performed using an Agilent 8890 GC and Agilent 7010C triple quadrupole GC/MS system. The modules of the GC/MS system included:

- Agilent 8890 GC (G3540A)
- Agilent 7693A automatic liquid sampler (G4513A and GG4520A)
- Agilent 7010C triple quadrupole GC/MS (G7012C)
- Agilent MassHunter pesticide & environmental pollutant (P&EP) MRM database 4.0 (G9250AA)[4]
- Agilent MassHunter software (MassHunter acquisition version 10.2 and MassHunter Quantitative Analysis version 12.0)

The GC was configured with the Agilent 7693A automatic liquid sampler (ALS) and 150-position tray. The system used a multimode inlet (MMI). 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. The acquisition method was retention time locked to match the retention times in the MassHunter P&EP 4.0.

The main GC and MS parameters are listed in Table 2. Please refer to the Agilent application note by Klink for the detailed GC/TQ configuration.[3] All data were acquired in dynamic MRM (dMRM) mode.

Table 2. GC and MS conditions.

Parameter	Value
GC	
Columns	Agilent HP-5ms, 15 m × 0.25 mm, 0.25 µm film thickness (two) (p/n 19091-431UI)
Carrier	Helium
Column 1 Flow	0.94 mL/min
Column 2 Flow	1.14 mL/min
Injection Volume	1 µL, solvent vent
Inlet Liner	Agilent Ultra Inert dimpled liner (p/n 5190-2297)
MMI Temperature Program	60 °C for 0.06 min, 720 °C/min to 280 °C and hold
Oven Temperature Program	60 °C for 1 min, 40 °C/min to 170 °C, 10 °C/min to 310 °C and hold for 3 minutes
Run Time	20.75 minutes
Transfer Line Temperature	280 °C
Backflush Conditions	1.5 min postrun, 310 °C oven temperature
MSD	
Source	High-efficiency source (HES)
Vacuum Pump	Performance turbo
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM
EM Voltage Gain Mode	10
Total MRMs (dMRM Mode)	2,093
Min/Max Dwell Time	1.2 ms/100.2 ms

Results and discussion

Development of multicompound methods

A major part of this study was the development of dMRM transitions for all pesticides from the Agilent databases. For LC/MS/MS, the Agilent pesticide dynamic MRM database was used. MRM transitions as well as fragmentor voltages, collision energies, and ionization polarity were optimized using the Agilent MassHunter Optimizer software by flow injection. Approximately 1,600 MRM transitions from 764 pesticides were stored in the final dMRM method. Typical chromatographic peak widths were between 8 to 12 seconds. The selected cycle time of 490 ms ensured that sufficient data points were collected across the chromatographic peaks for reproducible quantitation and confirmation of results.

For GC/MS/MS, most of the compounds were already listed in the MassHunter P&EP database.[4] Compounds whose MRM transitions were not listed in this database were developed using the MassHunter Optimizer for GC/TQ. Starting with a GC method that provides good chromatographic compound separation, the MassHunter Optimizer first identifies precursor ions and product ions, then optimizes collision energies for each promising precursor-product combination to identify the best MRM parameters. Around 2,100 MRM transitions from 341 pesticides were stored in the final dMRM method. 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. The GC acquisition method was retention time locked to match the retention times in the Agilent P&EP database, which was used to seamlessly create the MS method. The use of 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.

Two or three target specific MRM transitions were selected per pesticide in each method to satisfy the regulatory requirements for identification and confirmation by LC/MS/MS and GC/MS/MS, respectively.[1]

Data were acquired in dynamic MRM (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. Furthermore, dMRM enables the analyst to add and remove additional analytes with ease.

Matrix effect assessment

Effects caused by the sample matrix are frequent and cause suppression or enhancement of the MS detection system response.[1] ME was assessed by the ratio of target response in matrix-matched standards to that in corresponding solvent standards. Typically, there is no strict requirement on acceptance 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 their metabolites.

More than 45% of the 1,021 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 all compounds using matrix-matched standards ranging from 0.5 to 100 µg/L, and eight calibration points. Linear or quadratic regression with 1/x weight and unspecified origin were used for calibration curve generation. The calibration range was determined based on LOQ sensitivity and selectivity requirements. Results in Figure 3 show that more than 98% of the targets met the calibration curve linearity requirement of $R^2 \geq 0.99$.[1] Only some compounds showed a modified calibration range due to either lack of sensitivity at low calibration levels or detector saturation at high concentration levels.

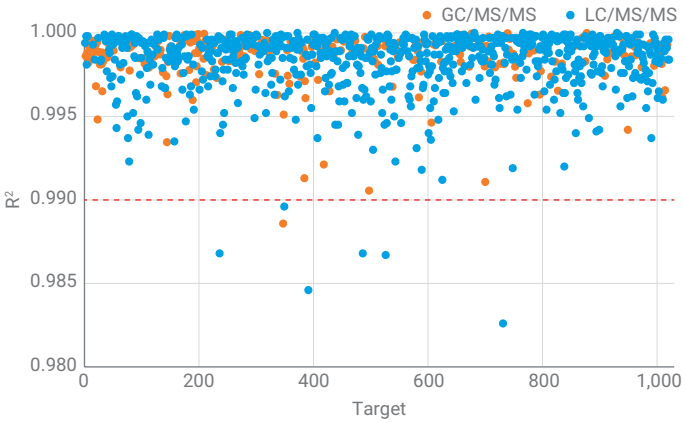

Figure 3. R^2 distribution of linearity curves for 1,021 pesticides, compounds below $R^2 = 0.98$ are not shown (9 in total).

Instrument limit of detection (LOD)

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 minutes.

More than 97% of target compounds showed an instrument LOD of ≤ 10 µg/L, and, even at a concentration level of 1 µg/L, more than 88% of compounds had an S/N of 10 and up (Figure 4). These results demonstrate the high sensitivity of both systems, the 6470 triple quadrupole LC/MS and the 7010 triple quadrupole GC/MS, against a complex matrix such as a tomato QuEChERS raw extract.

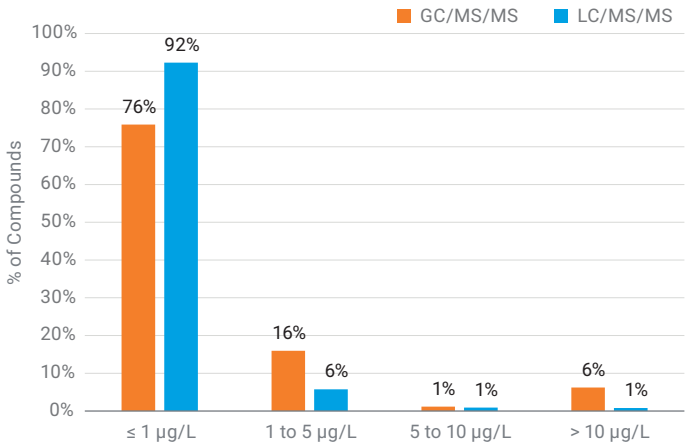

Figure 4. Instrument LOD in tomato QuEChERS raw extract.

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 of 98% of all targets were within 20%, demonstrating consistent behavior with each technical preparation. These results confirmed the high repeatability of this workflow. Figure 5 shows that the vast majority of compounds had RSD of recovery rates below 20%.

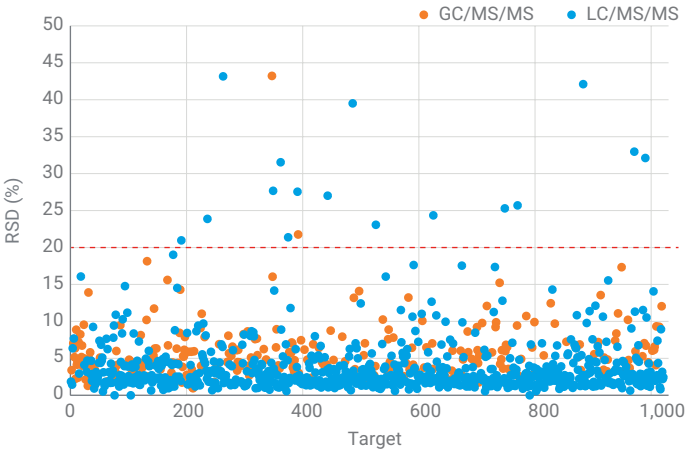

Figure 5. RSD_r of recovery rates at 10 µg/kg in QuEChERS tomato raw extract.

Recovery was used in this experiment to evaluate the capability of a quantitative analytical workflow for over 1,000 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 are acceptable 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 97% of targets in tomato QuEChERS raw extract at 10 µg/kg met the acceptance criteria. The vast majority of compounds (975) were within the recovery range of 70% to 120% and only 26 compounds (3%) were below 70% or above 120%, respectively (Figure 6).

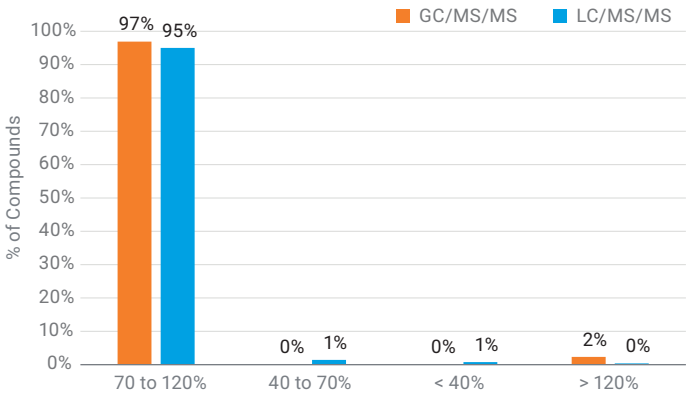

Figure 6. Recovery rates in tomato QuEChERS raw extract ($RSD_r \leq 20\%$).

Combination of methods

The combination of LC/MS/MS and GC/MS/MS allows users to cover the widest range of pesticides and metabolites occurring in food. Due to the molecular structure of this huge class of compounds, it is impossible to analyze various pesticides solely by GC or LC techniques. Exploiting both techniques makes it possible to get a wide coverage of these residues that can potentially endanger human health.

The presented workflow used both techniques and covered in total 764 pesticides analyzed by LC/MS/MS and 341 compounds analyzed by GC/MS/MS. All detailed results can be found in references 2 and 3. Furthermore, the analyses covered pesticide residues (84) that can be analyzed by either technique. This gives a clear benefit when, for example, positive results must be confirmed or higher sensitivity is needed.

In Figure 7, the chromatograms of silafluofen in a spiked matrix sample at 10 µg/kg are shown. The left chromatogram shows that sensitivity using LC/MS/MS was not good enough to get reliable results at MRL of 10 µg/kg. The full Agilent solution allows analysis of this compound using GC/MS/MS, resulting in much better sensitivity (right chromatogram).

The use of the other technique for confirmatory analysis can be demonstrated for bifenthrin. This compound can be reliably quantified using both techniques. The chromatograms in Figure 8 clearly demonstrate that sensitivity is high enough to determine and confirm positive results by either LC or GC technique.

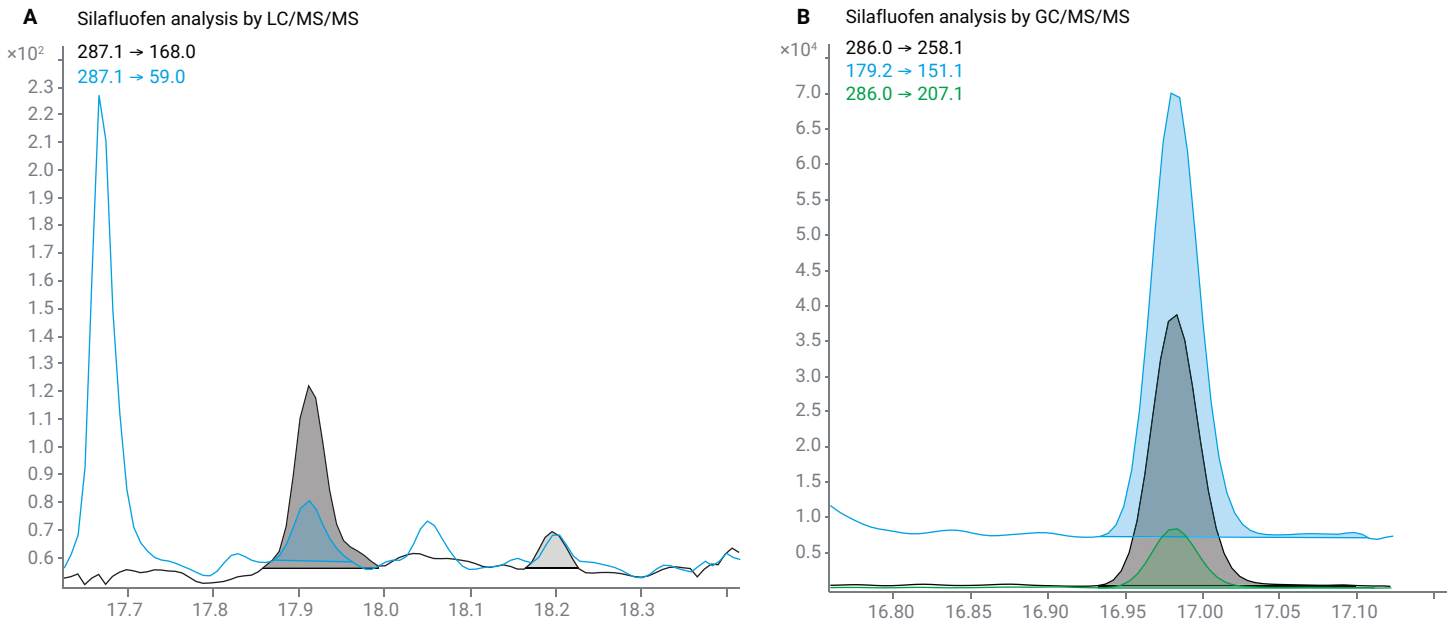

Figure 7. Analysis of silafluofen by LC/MS/MS (A) and GC/MS/MS (B).

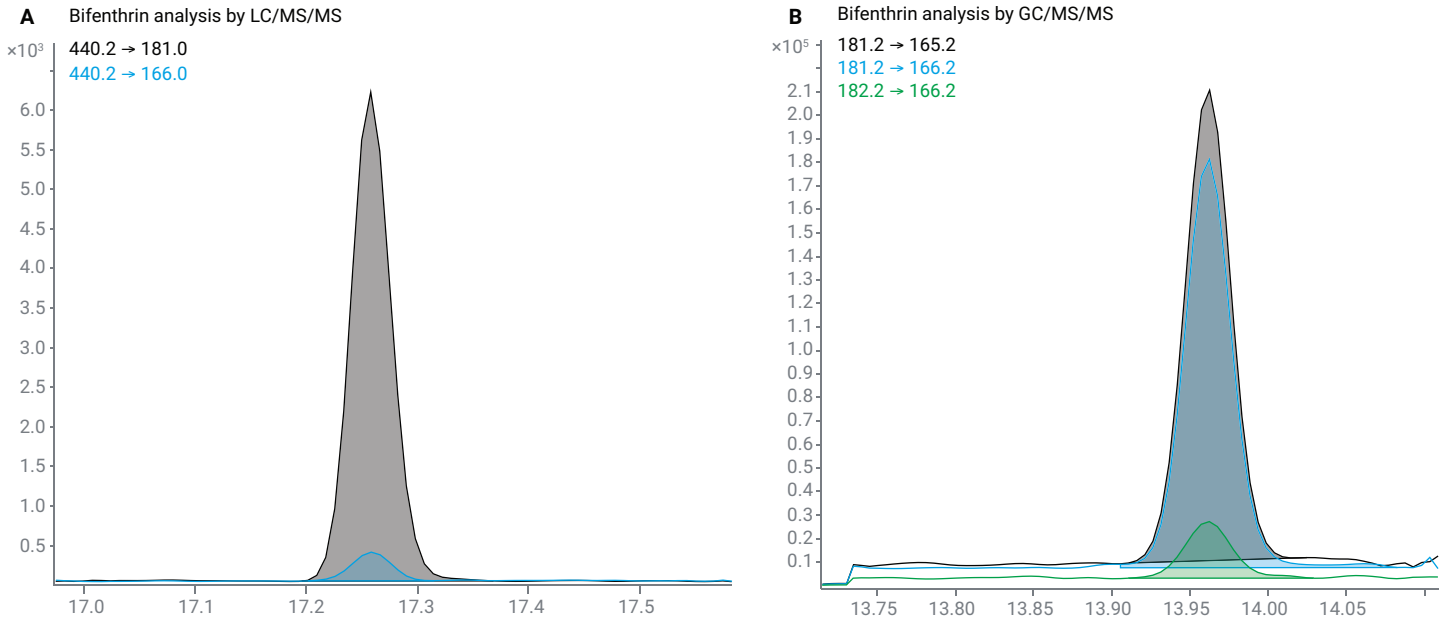

Figure 8. Analysis of bifenthrin by LC/MS/MS (A) and GC/MS/MS (B).

Conclusion

This application note demonstrates the applicability of a sensitive and reproducible workflow for fast and reliable quantitation of more than 1,000 pesticide residues in tomato QuEChERS raw extract 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. A single sample preparation procedure can be used and then split into two aliquots for subsequent analysis by LC/MS/MS and GC/MS/MS.

An Agilent 1290 Infinity II LC system coupled to an Agilent 6470 triple quadrupole LC/MS was used to quantify 764 pesticides, and an Agilent 8890 GC coupled to an Agilent 7010C triple quadrupole GC/MS was used to quantify 341 pesticide residues with matrix-matched calibration. Both methods had 20-minute run times, and column setups offered good chromatographic separation and even retention time distribution of all targets.

To achieve the most efficient use of instrument cycle time, all data were acquired in dMRM mode. The dMRM methods were created and developed based on the Agilent pesticide MRM databases.

The overall workflow performance was assessed for linearity, instrument LOD, recovery, and precision, demonstrating its suitability for the quantitation of over 1,000 pesticide residues in the same QuEChERS raw extract.

References

1. SANTE 11312/2021: Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.
2. Kornas, P. Quantitation of 764 Pesticide Residues in Tomato by LC/MS According to SANTE 11312/2021 Guidelines. *Agilent Technologies application note*, publication number 5994-5847EN, **2023**.
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4. 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.

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