Application Note

Food and Beverage Testing

Automated QuEChERS Extraction for Pesticides Analysis in Dry and Wet Matrix

Pesticide quantitation using the CTC PAL3 Series 2 RTC autosampler with the Agilent 7010D triple quadrupole GC/MS and the Agilent 6475 triple quadrupole LC/MS

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Abstract

For the quantitation of pesticides according to SANTE 11312/2021 guidelines, we developed an automated QuEChERS sample preparation using a CTC PAL3 Series 2 RTC autosampler LC/MS/MS and GC/MS/MS workflow with the Agilent 6475 triple quadrupole LC/MS and the Agilent 7010D triple quadrupole GC/MS. The workflow used a 20-minute LC/GC runtime and provides a fast and simple solution for routine laboratory food testing analysis.

After manual weighing and fortification of samples, the subsequent QuEChERS extraction and injection on LC or GC were performed by the CTC PAL3. A related script was developed using the CTC Method Composer and implemented into Agilent MassHunter software, thus requiring only one software platform for sample workup and data acquisition.

Applicability and method performance were assessed by fortifying five replicates at concentration levels of 10 or 20 µg/kg. Employing a relative standard deviation (RSD) of ≤ 20 %, about 350 analytes were analyzed from a single sample extraction using either LC- or GC-based detection. Matrix-matched calibration solutions were used to establish linear calibration with a weighing of 1/x. A coefficient of determination (R^2) ≥ 0.99 was obtained for 98% of the analytes, confirming excellent linearity across the working range.

Introduction

Pesticides are widely used in agriculture and the food industry to maximize crop yields and ensure food availability. However, residual pesticide traces on commodities such as fruits, vegetables, and other crops can pose significant health risks and environmental concerns. To address these issues, regulatory agencies have established maximum residue levels (MRLs), often in the low parts-per-billion (ppb) range, to strictly limit pesticide and metabolite concentrations.

Detecting trace levels of pesticides in food is challenging due to the complexity of food matrices. Pesticide analysis typically relies on QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction, which is widely used for its ability to accommodate a broad range of pesticide residues in complex food samples. However, the largely manual nature of QuEChERS sample preparation can be labor-intensive and prone to handling errors, potentially affecting data accuracy and reliability. These workflows require skilled analysts to perform the extractions and operate the analytical instruments. Variations in operator expertise can result in inconsistent outcomes, reducing the repeatability and robustness of pesticide analysis, particularly when high precision at trace concentration levels is required.

The PAL3 autosampler provides a broad range of modular components that can be individually configured to support complex sample-processing workflows. Although advanced customization through scripting is possible, routine method development is greatly simplified by the CTC Method Composer, which enables the creation of sequential operational steps via an intuitive drag-and-drop interface. Using this tool, a complete QuEChERS sample-preparation procedure—including extraction, salting-out, dilution, and injection—was reconstructed within the autosampler environment.

All module-specific parameters were subsequently optimized, and the finalized script was uploaded into the acquisition system. The PAL3 autosampler is fully integrated into MassHunter software, allowing the corresponding sample-workup procedures to be saved directly as part of the LC or GC acquisition method. Consequently, extraction, chromatographic acquisition, and even downstream data evaluation can be initiated with a single click, enabling seamless end-to-end automation. Additionally, the PAL3 autosampler also offers the option to be used in standalone mode without connection to an analytical system.

This application note introduces a comprehensive workflow combining LC/MS/MS and GC/MS/MS for the accurate and efficient analysis of about 350 pesticide residues in tomato and flour. The approach integrates automated sample preparation, chromatographic separation, mass spectrometric detection, targeted quantitation, and data interpretation—streamlining routine pesticide analysis and boosting laboratory productivity.

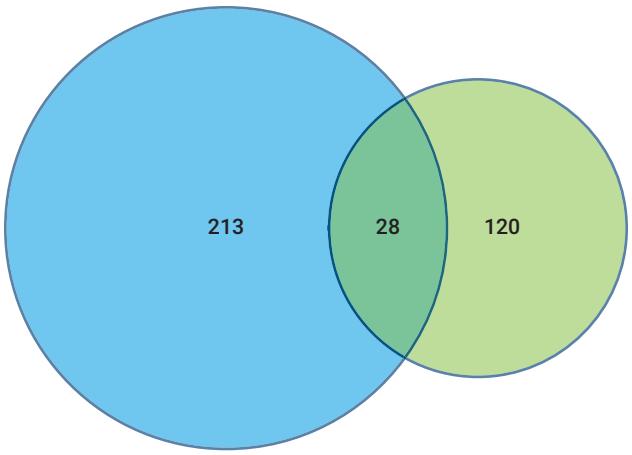

Figure 1. Venn diagram of compounds analyzed in tomato matrix using LC/MS/MS (blue) and GC/MS/MS (green).

Experimental

Chemicals and reagents

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

Standards and solutions

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

- Agilent LC/MS pesticide comprehensive test mix kit (p/n 5190-0551)
- Agilent comprehensive GC/LC pesticide RM kit (p/n PSM-100-A–J+L/PSM-105A+B)

Serial dilutions were created from the stock solution, to prepare nine calibration concentration levels of 0.5, 1, 2, 5, 10, 20, 50, and 100 µg/L for LC analysis in blank matrix. For GC, the calibration levels ranged from 0.5, 1, 2, 5, 10, 25, 50, and 100 µg/L for wet (tomato) matrix and 0.3, 0.5, 1, 2, 5, 10, and 20 µg/L for dry (flour) matrix. Calibration standards were freshly prepared and stored in a refrigerator at 4 °C if not used immediately.

Sample preparation

Pesticide-free and organically labeled strained tomatoes and wheat flour were obtained from local grocery stores.

The following products and equipment were used for sample preparation:

- Agilent QuEChERS extraction kit (p/n 5982-0550)
- 20 mL headspace vials (p/n 5182-0837)
- 10 mL headspace vials (p/n 5188-5392)

Instrumentation

Liquid chromatography

Chromatographic separation was performed using an Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 µm column (p/n 959759-902) installed on an Agilent 1290 Infinity III LC system.

The individual modules of the 1290 Infinity III LC system included:

- Agilent 1290 Infinity III High-Speed Pump (G7120A)
- Agilent 1290 Infinity III Multicolumn Thermostat Column Compartment (G7116B)

The LC system conditions are listed in Table 1.

Table 1. Parameters of LC method applied in this study.

Parameter	Value
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150; 1.8 µm (p/n 959759-902)
Column Temperature	40 °C
Injection Volume	2 µL
Autosampler Temperature	5 °C
Mobile Phase A	5 mM ammonium formate in water with 0.1% formic acid
Mobile Phase B	5 mM ammonium formate in MeOH 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	Step Time (sec) Solvent 1 7 ACN Seat backflush and needle wash 2 7 MeOH Seat backflush and needle wash 3 7 Water Seat backflush and needle wash

A 6475 triple quadrupole LC/MS (LC/TQ) with an Agilent Jet Stream (AJS) electrospray ion source was operated in dynamic MRM (dMRM) mode. All data acquisition and processing were performed using MassHunter software (version 12.3). The 6475 LC/TQ parameters are shown in Table 2.

Table 2. Parameters of MS method applied in this study.

Parameter	Value
Ionization Mode	Simultaneous positive/negative ESI with Agilent Jet Stream (AJS)
Scan Type	Dynamic MRM (dMRM)
Cycle Time	500 ms
Stop Time	20 min
MS1/MS2 Resolution	Unit / Unit
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

Gas chromatography

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

- Agilent 8890 GC (G3540A)
- Agilent 7693A automatic liquid sampler (G4513A and G4520A) or PAL autosampler
- Agilent 7010D triple quadrupole GC/MS (G7012D)
- Agilent MassHunter software (MassHunter Acquisition 13.1 and MassHunter Quantitative Analysis 12.1 Update 2)
- Agilent MassHunter Pesticide and Environmental Pollutants (P&EP) MRM database 4.0 (G9250AA)

The GC was either configured with the 7693A automatic liquid sampler (ALS) and 150-position tray or the PAL3 Series II RTC autosampler. 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 J&W HP-5ms Ultra Inert (UI) GC columns (p/n 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 main GC and MS parameters are listed in Table 3.

Table 3. Parameters of GC method applied in this study.

Parameter	Value
GC	
Columns	Agilent J&W HP-5ms, 15 m × 0.25 mm, 0.25 µm film thickness (two) (p/n 19091-431UI)
Carrier	Helium
Column 1 Flow	~1 mL/min*
Column 2 Flow	Column 1 + 20%
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	1–1.5 min postrun, 310 °C oven temperature
MSD	
Source	High-efficiency source 2.0 (HES 2.0)
Vacuum Pump	Performance turbo
Quad Temperature (MS1 and MS2)	150 °C
Source Temperature	280 °C
Mode	dMRM
EM Voltage Gain Mode	10

*Adjusted according to retention time locking of chlorpyrifos-methyl

PAL autosampler

A 120 cm CTC PAL3 Series II RTC autosampler was used for sample extraction and for performing injections onto the LC/TQ system. The PAL3 platform was equipped with various tools and modules, providing the necessary capabilities to achieve its designated functions. The following tools and modules were used in this study:

- Two PAL park stations with one liquid syringe tool (only for dilution for GC/TQ measurements)
- Dilutor tool, generic tool, and LC/MS tool
- Vortex mixer
- Centrifuge combi (for 2 mL/10 mL/20 mL vials)
- Dilutor multi
- Tray holders with rack R60 (for 10/20 mL vials) and rack VT54 (for 2 mL vials)
- Solvent module and fast wash module
- LC injection valve
- CTC Method Composer software

Results and discussion

Development of PAL sample preparation method

The primary objective of this study was to automate the conventional QuEChERS extraction workflow using the CTC PAL3 autosampler platform.

Initially, sample aliquots were weighed into 20 mL autosampler vials and fortified as required. In parallel, 0.5 g portions of QuEChERS extraction salts were manually transferred into 10 mL vials designated for the salting-out step. Although these preparatory steps were performed manually in this work, automated fortification can also be implemented within the autosampler's capabilities.

The automated workflow then commenced with the addition of a defined volume of acetonitrile, followed by vigorous vortex mixing to extract analytes into the organic phase. The vials were subsequently centrifuged to separate the solid matrix from the supernatant. A 3 mL aliquot of the resulting organic phase was transferred into the vial containing the QuEChERS salts and subjected to a second vortex step to induce phase partitioning. After a final centrifugation, an aliquot of the upper acetonitrile layer was either diluted for GC analysis or injected directly for LC analysis. Throughout all liquid-handling steps, the fast-wash functionality provided rigorous internal and external needle cleaning to minimize carryover and prevent cross-contamination.

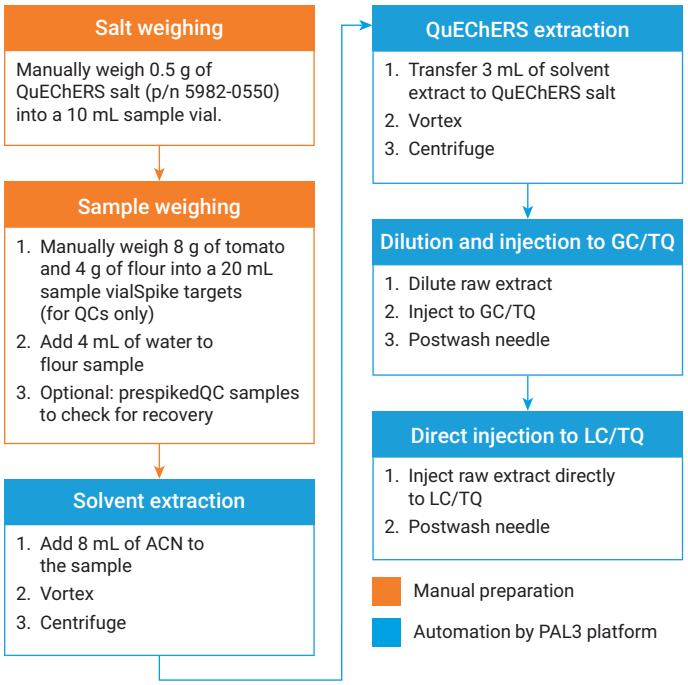

Figure 2. Automated sample preparation for wet and dry matrix by the Agilent CTC PAL3 Series 2 RTC autosampler.

With the exception of the initial weighing of sample material, all extraction, transfer, dilution, and injection steps are fully automated using the CTC PAL3 autosampler, enabling a streamlined, reproducible, and highly standardized QuEChERS workflow suitable for both LC- and GC-based pesticide analysis.

Verification of workflow performance

The workflow performance was evaluated with respect to linearity, method sensitivity, recovery, and precision. Each analytical batch consisted of matrix-matched calibration standards, matrix blanks, and prespiked quality control (QC) samples. For the prespiked QCs, five technical replicates were prepared.

Linearity

Matrix-matched calibration standards were used to generate calibration curves as described above. Calibration was performed using a linear regression model, with origin ignored and 1/x weighting applied. Overall, 98 % of the target compounds fulfilled the linearity acceptance criterion of $R^2 \geq 0.99$, demonstrating excellent linear response behavior across the investigated concentration range.

Method precision and reproducibility

Method precision was assessed based on recovery repeatability (RSD), determined from the variation in recovery values obtained from prespiked QC samples at 10 or 20 µg/kg. RSD was calculated as the percent relative standard deviation (%RSD) of recovery from five independent technical preparations. The commonly accepted criterion for RSD at this concentration level is ≤ 20 %.

According to SANTE 11312/2021, mean recoveries are considered acceptable when they fall within 40% to 120% and are consistent (RSD ≤ 20 %). Using LC/TQ, these criteria were met for approximately 96% of the target analytes in tomato and 93% in flour QuEChERS raw extracts at 10 µg/kg (Figure 3). Using GC/TQ, the criteria were fulfilled for approximately 97% of the target analytes in both tomato and flour QuEChERS raw extracts at the same concentration level (Figure 4).

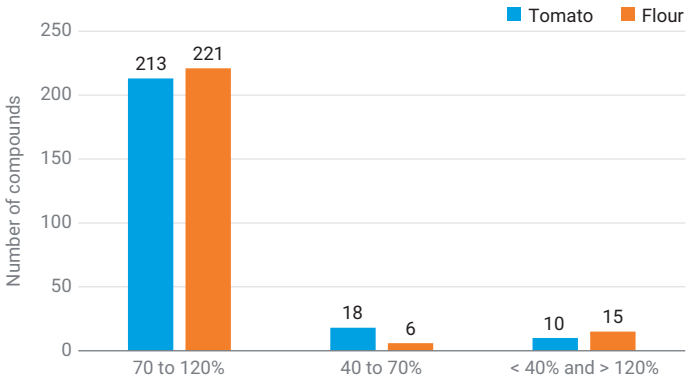

Figure 3. Recovery rates in tomato and flour QuEChERS raw extract measured with LC/TQ (RSD ≤ 20%).

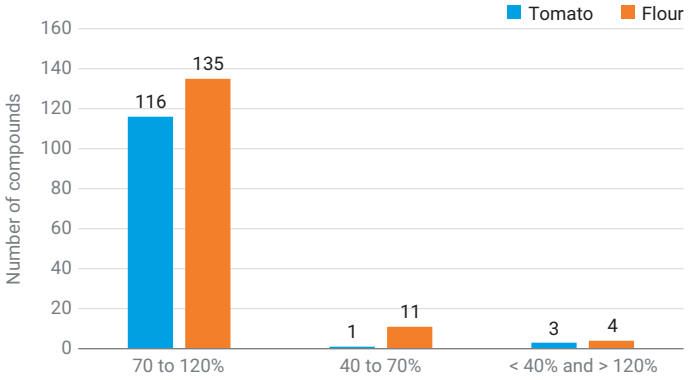

Figure 4. Recovery rates in tomato and flour QuEChERS raw extract measured with GC/TQ (RSD ≤ 20%).

Conclusion

This application note demonstrates a fully automated QuEChERS-based pesticide analysis workflow using the Agilent CTC PAL3 Series 2 RTC autosampler combined with the Agilent 7010D triple quadrupole GC/MS and the Agilent 6475 triple quadrupole LC/MS. By integrating sample extraction, cleanup, dilution, and injection into a single automated platform, the workflow significantly reduces manual handling while improving standardization and reproducibility for routine food testing laboratories.

The method showed excellent analytical performance across wet and dry food matrices, with 98% of target compounds achieving linear calibration ($R^2 \geq 0.99$) using matrix-matched standards. Recovery and reproducibility fulfilled the acceptance criteria defined in SANTE/11312/2021, with the majority of analytes exhibiting recoveries between 40% and 120% and RSD values ≤ 20 % at low µg/kg levels. These results confirm the robustness of the automated extraction and its suitability for reliable quantitative analysis of several hundred pesticide residues from a single sample preparation.

Reference

1. SANTE 11312/2021: Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. European Commission, **2026**.

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