

Fast, Flexible and Reliable GC/MS/MS Analysis of Pesticides in High Chlorophyll Leafy Vegetables using Midcolumn Backflushing



Routine, high-throughput analysis of trace-level pesticide residues in food commodities comes with significant analytical challenges. Pesticide residue methods require reliable, high-sensitivity data under tight regulatory and time constraints. Hundreds of pesticide residues, of differing chemical classes, are routinely screened with cycle times ranging from 25 minutes to under 10 minutes.

Sample throughput can be significantly impacted when matrix accumulates and reduces data quality. Analysis is halted to perform routine maintenance—vent the MS, trim columns and change inlet supplies. This unplanned downtime is extremely costly to the laboratory. In addition, today, intermittent helium supply disruptions can cause additional, unplanned downtime. Methods that can be easily converted to hydrogen carrier offer an additional level of security against carrier gas supply disruptions.

The two GC/MS/MS residue screening methods shown offer the speed and sensitivity needed for routine, high sample throughput methods. Both utilize a midcolumn backflushing technique, which reverses flow after analysis and removes unwanted matrix interferences, extending time needed between maintenance intervals. This limits instrument downtime and improves data reliability.

Agilent high-throughput screening methods

Calibration performance was demonstrated over a broad range of concentrations. This satisfies SANTE/11312/2021 guidelines and meets the needs of government agencies and private companies that produce, package, and sell food to the public as well as laboratories that control food safety.

Midcolumn backflushing, in combination with a high matrix removal sample preparation procedure, were used to reduce run times, limit column trimming, and lower ion source contamination. Agilent MRM database P&EP 4.0 was used with retention time locking (RTL) to allow for fast method development, translation and to support efficient daily operations. This methodology can also be converted to hydrogen carrier gas with the addition of a HydroInert source and adjusting column dimensions, as shown.

The workflow was demonstrated in spinach, a significantly challenging leafy vegetable with a high abundance of chlorophyll. This workflow is also extendable to pesticides analysis in other high-chlorophyll leafy vegetables.

Agilent software and hardware have also been engineered for ease of use for pesticide workflows. The MRM database P&EP 4.0 delivers best in class time-to-value and retention time locking (RTL), and HydroInert sources allow for transition to hydrogen carrier gas.

Incorporating midcolumn backflushing, sample preparation, method translation, and retention time locking (RTL) techniques can provide cost savings for method development, maintenance, and translation, supporting daily operations. This methodology can also be converted to hydrogen carrier gas with the addition of a HydroInert source.

Recommendations for optimizing the system configuration for pesticide analysis

Sample preparation: A simplified two-step process was employed to improve matrix removal (Figure 1): sample extraction by traditional QuEChERS extraction, either AOAC or EN extraction, followed by Captiva enhanced matrix removal (EMR) with Carbon S pass-through clean up. Spinach is considered a high-chlorophyll leafy matrix, where the pigment removal is critical. Captiva EMR-HCF1 and HCF2 specifically target high-chlorophyll leafy vegetable matrix cleanup. Both cartridges provide highly efficient chlorophyll pigment removal, but without compromising recovery sensitive pesticide, including the planar compounds.

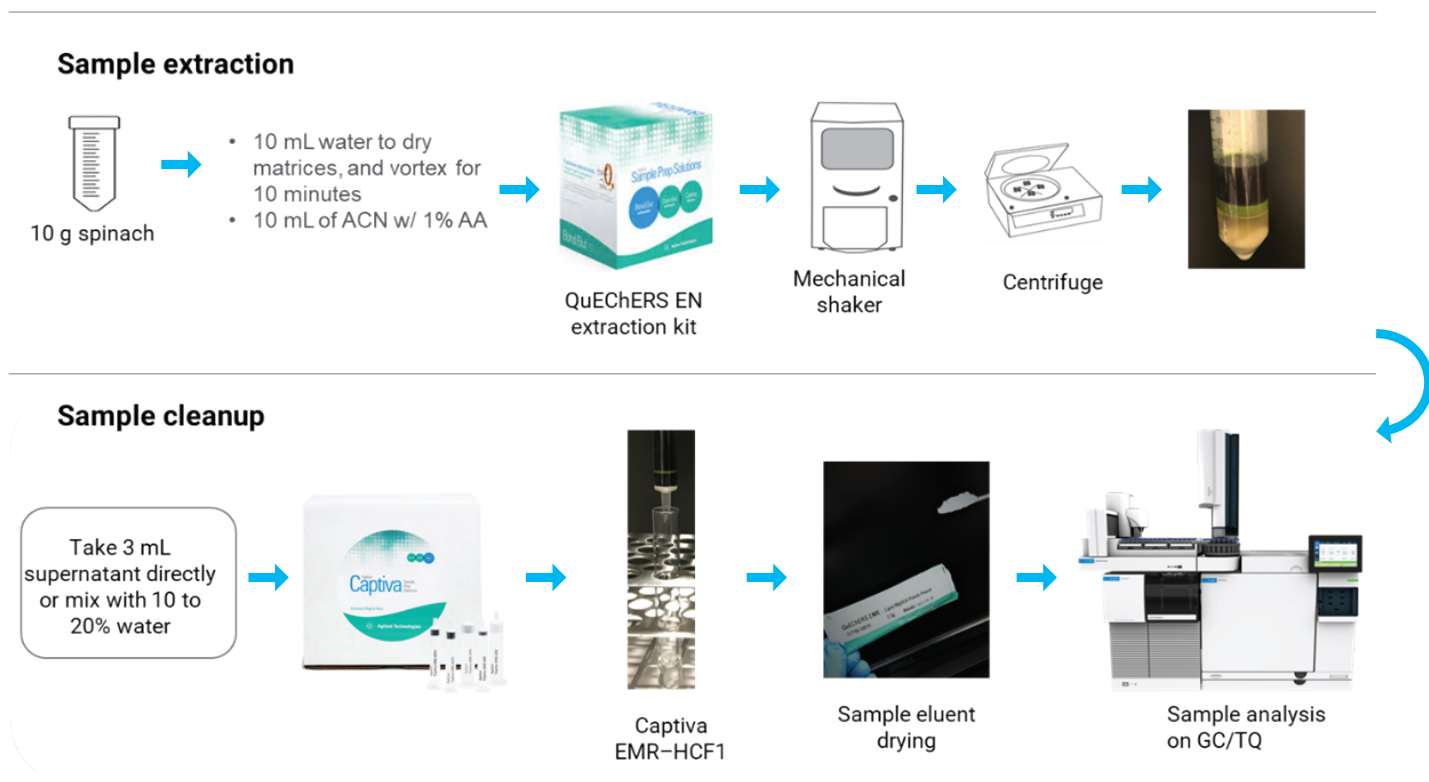


Figure 1. Sample preparation flowchart including traditional Agilent QuEChERS extraction, followed by Agilent Captiva EMR-HCF1 or HCF2 pass through cleanup.¹

System setup: Midcolumn backflush configurations (Figure 2 and 3) were employed to enable postrun column backflushing, lessening the frequency of inlet maintenance, GC column trimming, MS source cleaning, or MS retuning. Column configurations were shown with both helium and hydrogen carriers, and at conventional 20-minute and high-throughput 10-minute analysis times.

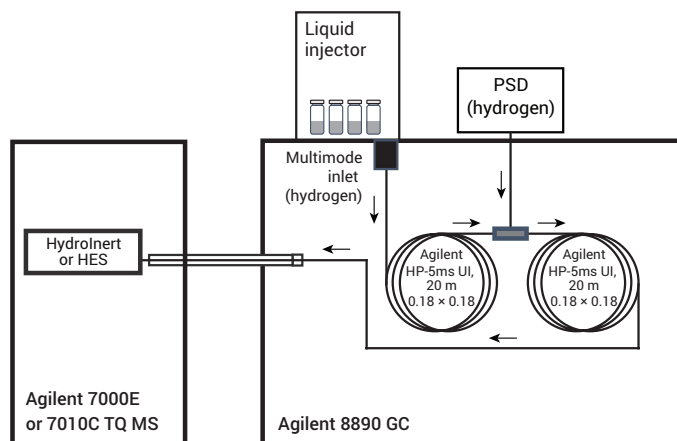
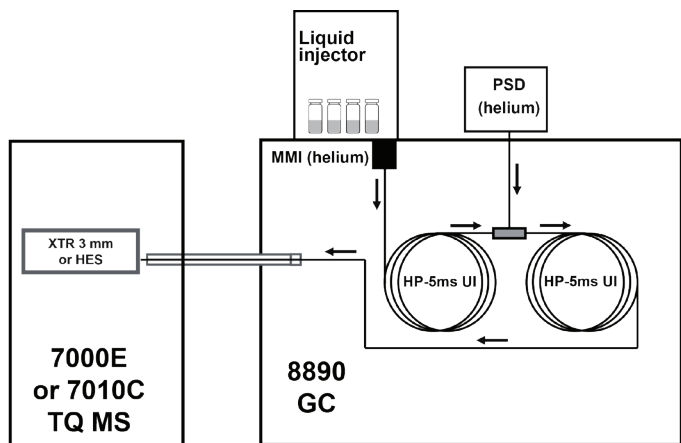
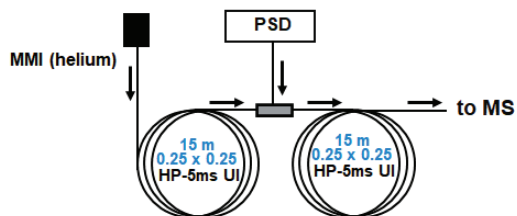


Figure 3. The Agilent 8890/7010C GC/TQ system configuration with hydrogen carrier gas.²

Conventional 15 x 15 m midcolumn backflush configuration:



Narrow bore 10 x 10 m midcolumn backflush configuration:

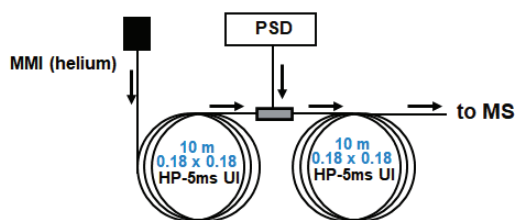


Figure 2. The Agilent 8890/7010C GC/TQ system (top) using two midcolumn backflush configurations (bottom).¹

Column dimensions:

Table 1. Recommended column dimensions for the rapid 10-minute analysis of over 200 pesticides.

	Conventional Analysis Times	High-throughput Analysis Times
Helium Carrier	(2) HP-5ms UI 15 m x 0.25 mm x 0.25 μm	(2) HP-5ms UI 10 m x 0.18 mm x 0.18 μm
Alternate Hydrogen Carrier	(2) HP-5ms UI 20 m x 0.18 mm x 0.18 μm	(2) HP-5ms UI 10 m x 0.18 mm x 0.18 μm

Flow rate: Optimal range 0.9–1.4 mL/min, dependent on column configuration and carrier gas.

Temperatures

Table 2. Sample oven program with helium carrier gas.¹

GC Oven Conditions		
	With 15 × 15 m	With 10 × 10 m
Initial Oven Temperature	60 °C	60 °C
Initial Oven Hold	1 min	0.5 min
Ramp Rate 1	80 °C/min	80 °C/min
Final Temp 1	170 °C	170 °C
Final Hold 1	0 min	0 min
Ramp Rate 2	35 °C /min	20 °C /min
Final Temp 2	310 °C	310 °C
Final Hold 2	3.625 min	1.125 min
Total Run Time	10 min	10 min
Post Run Time	1.5 min	1.5 min
Equilibration Time	0.25 min	0.25 min

Table 3. Sample oven program with hydrogen carrier gas.²

GC Oven Conditions	
Initial Oven Temperature	60 °C
Initial Oven Hold	1 min
Ramp Rate 1	40 °C/min
Final Temp 1	170 °C
Final Hold 1	0 min
Ramp Rate 2	10 °C /min
Final Temp 2	310 °C
Final Hold 2	2.25 min
Total Run Time	20 min
Postrun Time (Backflush Duration)	1.5 min
Equilibration Time	0.5 min

The workflows demonstrated can provide improved productivity and flexibility for multi-class pesticide analyses. Analysis times can range between conventional 20-minute analysis times or under 10 minutes when using high-efficiency mini-bore columns. These workflows can be transitioned to hydrogen carrier, while maintaining retention time locking, by adjusting column dimensions, as shown, and installing a HydroInert source. Midcolumn backflushing can be employed to extend routine maintenance due to matrix contamination.

References

List of application notes used in this workflow:

1. A Fast and Robust GC/MS/MS Analysis of 203 Pesticides in 10 Minutes in Spinach [5994-4967EN](#)
2. Hydrogen Carrier Gas for Analyzing Pesticides in Pigmented Foods with GC/MS/MS [5994-6505EN](#)

Easy selection and ordering information

To order items from the Agilent online store, click the part number hyperlinks in the table. Then enter the quantities for the products that you need, click Add to Cart, and proceed to checkout.

Alternatively, save the items in the table to your Favorite Products list by clicking the corresponding MyList header link. Enter the quantities for the products that you need, click Add to Cart, and proceed to checkout. The list will remain under your Favorite Products for future use.

If this is your first time ordering online, you'll be asked to enter your email address for account verification. If you have an existing Agilent account, you will be able to log in. If you don't have a registered Agilent account, you'll need to [register](#) for one.

All items can also be ordered through your regular sales and distributor channels.

Description	Part Number
MyList Sample Preparation	
Bond Elut QuEChERS EN Extraction Kit	5982-5650CH
Bond Elut QuEChERS AOAC Extraction Kit	5982-5755CH
Captiva EMR-HCF1, 3 mL Cartridge	5610-2088
Captiva EMR-HCF2, 3 mL Cartridge	5610-2089
Anhydrous MgSO ₄	5982-0102
Positive Pressure Manifold 48 Processor	5191-4101
MyList Analytical Columns	
J&W HP-5ms Ultra Inert GC column, 15 m, 0.25 mm, 0.25 µm, with smart key, 1/pk (need qty 2) (recommended for conventional and fast helium carrier gas method with conventional column configuration)	19091S-431UI-KEY
J&W HP-5ms Ultra Inert GC Column, 10 m, 0.18 mm, 0.18 µm, 7 inch cage, 1/pk (need qty 2) (recommended for fast helium or fast hydrogen carrier gas method)	19091S-571UI
J&W HP-5ms Ultra Inert GC Column, 20 m, 0.18 mm, 0.18 µm, 7 inch cage, 1/pk (need qty 2) (recommended for conventional hydrogen carrier gas method)	19091S-577UI
MyList GC Supplies	
Agilent Ultra Inert 2 mm Dimpled Liner	5190-2297
Gold-plated flexible metal ferrules	G2855-28501
Self-tightening collared column nuts for GC inlet	G3440-81011

Description	Part Number
Self-tightening collared column nuts for MS transfer line	G3440-81013
85:15 Vespel/Graphite Ferrules, 0.4 mm ID, 10/pk	5181-3323
Inlet septa, Advanced Green, non-stick, 11 mm, 50/pk	5183-4759
ALS syringe, Blue Line, 10 µL, fixed needle, 23/42/cone, PTFE-tip plunger	G4513-80220
Purge Ultimate Union (PUU) kit, deactivated	G3186-80580
Purged ultimate union (PUU) assembly, inert	G3186-60581
8890 with PSD (pneumatic switching device)	Option #310
MyList Sample Containment	
Vials, screw top, amber, write-on spot, deactivated (silanized), certified, 2 mL	5183-2072
Caps, screw, blue, certified, PTFE/silicone/PTFE septa	5182-0723
Vial insert, 250 µL, deactivated glass with polymer feet	5181-8872
MyList MSD Source Parts	
Filament, high temperature, EI ion source	G7005-60061
9 mm HydroInert extraction lens* (recommended for H ₂ carrier gas)	G7078-20909
Repeller - HydroInert	G7078-20902
MyList Gas Filters	
Gas Clean carrier gas kit, 1-position, for 7890, 1/8 in. Includes one 1-position 1/8 in connecting unit; Purifiers: one carrier gas (p/n CP17973); one 7890 mounting bracket	CP17988
Gas Clean kit, for 8890 and 8860 GC. Includes mounting bracket, connecting unit, and carrier gas filter	CP179880
Gas Clean carrier gas purifier replacement cartridge	CP17973
Agilent big universal trap (recommended for H ₂ carrier gas)	RMSH-2-SS
Agilent Gas Clean purifier kit for carrier gas	CP17976
HydroInert Source for Transition to H₂ Carrier Gas	
HydroInert complete source assembly for 7000 TQ	G7006-67930
HydroInert GC/TQ upgrade	5505-0084
Stainless steel installation kit	19199S
Software	
Standalone Pesticides and Environmental Pollutants MRM Database	G9250AA
MassHunter GC/MS Software Upgrade (including MassHunter Acquisition and MassHunter Qualitative and Quantitative Analysis)	G6845AA
MassHunter GC/MS Data Analysis Software	G6849AA

Agilent CrossLab: Real insight, real outcomes

CrossLab goes beyond instrumentation to bring you services, consumables, and lab-wide resource management. So your lab can improve efficiency, optimize operations, increase instrument uptime, develop user skill, and more.

Learn more about Agilent CrossLab, and see examples of insight that leads to great outcomes, at www.agilent.com/crosslab

Learn more or for additional ordering guides, visit:

www.agilent.com/chem/ordering-guides

U.S. and Canada

1-800-227-9770

agilent_inquiries@agilent.com

Europe

info_agilent@agilent.com

Asia Pacific

inquiry_lsca@agilent.com

DE17956593

This information is subject to change without notice.