Agilent CrossLab
From Insight to Outcome

Consumable Workflow Ordering Guide

Rapid Analysis of Pesticides in Tobacco Using LC/MS/MS and GC/MS/MS

The agricultural practices for cultivating, production, and storage of tobacco usually involve pesticide application for controlling pests, bacteria, and fungi. The wide concern about the environmental and health impacts of pesticides in tobacco means that analytical methods for safety evaluation must comply with guidance residue levels (GRLs) regulated by CORESTA.[1]

Tobacco contains a large variety of ingredients including carbohydrates, proteins, fatty acids, waxes, pigments, alkaloids, and nicotine, making it a challenging matrix for multiresidue pesticide extraction and matrix removal.

Common sample preparation methods usually involve the use of QuEChERS or modified QuEChERS extraction, followed by dispersive SPE cleanup. Agilent Captiva EMR with Carbon S cartridges applies passthrough cleanup methodology for fast and efficient sample matrix removal. Captiva EMR General Pigmented Dry (EMR–GPD) and EMR Low Pigmented Dry (EMR–LPD) cartridges are specifically targeted to complex dry matrices. Both cartridges contain the Agilent proprietary sorbents Carbon S and Captiva EMR–Lipid, blended with primary secondary amine (PSA) and C18 in an optimized formula.

Captiva EMR–Lipid sorbent provides highly selective and efficient lipid removal, while PSA sorbent efficiently removes fatty acids. Carbon S sorbent effectively removes pigment, and EC-C18 provides further hydrophobic matrix cleanup. The blended formula was carefully developed and optimized to deliver the best balance between matrix removal and target recovery for complex dry matrices with different levels of pigment components. For general pigmented dry matrix, Captiva EMR–GPD is usually recommended, while for low pigmented complex botanical dry matrix, Captiva EMR–LPD is favored.

Tobacco is considered a light pigmented dry matrix, therefore, Captiva EMR-LPD is more suitable for this matrix. After the traditional QuEChERS extraction, Captiva EMR–LPD cartridges were used for passthrough cleanup (Figure 1).[2]

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Weigh 1 g of ground tobacco into a 50 mL centrifuge tube.

↓

Spike appropriately with standard and IS spiking solution into tobacco QC samples. Vortex samples for 30 seconds to mix.

↓

Add 5 mL of water with 0.1% formic acid. Vortex the samples for 20 minutes.

↓

Add an aliquot of 10 mL of ACN with 1% acetic acid into the samples. Vortex the samples for 2 minutes to mix.

↓

Add salts from an EN salt packet and one to two ceramic homogenizers to the sample. Cap the tube tightly.

↓

Shake the samples vigorously using a Geno/Grinder at 1,500 rpm for 5 minutes. Then, centrifuge at 5,000 rpm for 5 minutes.

↓

Transfer 2.7 mL of crude extract and mix with 0.3 mL of water.

↓

Place Agilent Captiva EMR–LPD 6 mL cartridges onto PPM-48, with labeled collection tubes beneath. Transfer the entire 3 mL sample mixture into the Captiva EMR–LPD cartridge, and use gravity or apply 1 to 3 psi pressure for elution.

↓

Increase the pressure to 3 to 6 psi to dry the sorbent bed completely, until no more visible sample is left in the cartridge.

↓

Dry the sample eluent with anhydrous $MgSO_4$ (~200 mg), vortex for 2 minutes, and centrifuge at 5,000 rpm for 3 minutes.

↓

Transfer an aliquot of supernatant for GC/MS/MS analysis directly. Dilute supernatant 5x with water for LC/MS/MS analysis.

Figure 1. Sample preparation procedure for tobacco samples by Agilent Bond Elut QuEChERS AOAC extraction followed by Agilent Captiva EMR-LPD passthrough cleanup.

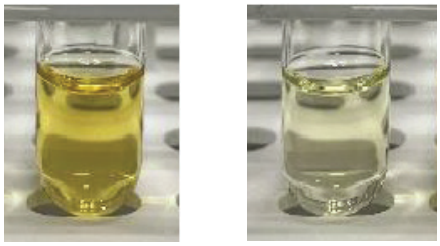

Figure 2. Tobacco extract: no cleanup (left), after Agilent Captiva EMR-LPD cleanup (right).

Table 1. LC/MS/MS method conditions.

LC Conditions			
Columns	Agilent ZORBAX Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm (p/n 959758-902) Agilent ZORBAX Eclipse Plus C18 column, UHPLC guard, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)		
Flow Rate	0.3 mL/min		
Column Temperature	40 °C		
Injection Volume	2 µL		
Mobile Phase	**A:** 10 mM ammonium formate, 0.5 mM ammonium fluoride in water, 0.125% formic acid (FA) **B:** 10 mM ammonium formate, 0.5 mM ammonium fluoride in 95:5 ACN:water, 0.125% FA		
Needle Wash	1:1:1:1 ACN:MeOH:IPA:water, 0.2% FA		
Gradient	**Time (min)** 0.0 6.0 8.01	**%B** 15 95 100	**Flow (mL/min)** 0.3 0.3 0.3
Stop Time	10 min		
Post Time	2.3 min		
MS Conditions			
Ionization Mode	Electrospray ionization (ESI)		
Gas Temperature	120 °C		
Gas Flow	20 L/min		
Nebulizer	40 psi		
Sheath Gas Heater	225 °C		
Sheath Gas Flow	11 L/min		
Capillary Voltage	4,500 V (positive and negative)		
Nozzle Voltage	0 V (both positive and negative)		
iFunnel Parameters	**High-pressure RF:** 150 V (positive), 90 V (negative) **Low-pressure RF:** 60 V (positive), 60 V (negative)		
Polarity	Positive and negative, refer to Table 4 from reference 1.		

Table 2. GC/MS/MS method conditions.

GC Conditions	
Columns	Agilent J&W HP-5ms Ultra Inert GC column, 15 m x 0.25 mm, 0.25 µm film thickness (two) (p/n 19091S-431UI-KEY)
Carrier Gas	Helium
Column 1 Flow	1.016 mL/min
Column 2 Flow	1.216 mL/min
Injection Volume	1 µL cold splitless
Inlet Liner	Agilent Ultra Inert 2 mm dimpled liner (p/n 5190-2297)
MMI Temperature Program	60 °C for 0.1 min, 600 °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 Hold for 2.25 min
Run Time	20 min
Backflush Conditions	1.5 min postrun 310 °C oven temperature Postrun total flow 25 mL/min
Transfer Line Temperature	280 °C
Source	Agilent Inert Extractor Source with a 3 mm lens, 280 °C
Vacuum Pump	Performance turbo
Quadrupole Temperature	150 °C
Source Temperature	280 °C
Data Monitoring	Dynamic MRM mode (dMRM)
EM Voltage Gain Factor	10
Solvent Delay	3 min

Note: For GC/MS/MS, the sample eluent needs to be dried using the anhydrous $MgSO_4$ powder. You can use a small spatula of anhydrous $MgSO_4$ powder (200 to 300 mg) from the Agilent Bond Elut QuEChERS EMR—Lipid polish pouch.

Table 1 lists the LC/MS/MS conditions. For target dynamic multiple reaction monitoring (dMRM) parameters, please see the application note by Zhao.[3]

Table 2 lists the GC/MS/MS conditions. For target dMRM parameters, please see the Agilent MassHunter Pesticides & Environmental Pollutant MRM Database (P&EP 4, part number G9250AA).

References

1. CORESTA GUIDE No. 1, Agrochemical Guidance Residue Levels, 2021
2. Determination of Over 300 Pesticides in Tobacco Using Agilent Captiva EMR–LPD Passthrough Cleanup and LC/MS/MS and GC/MS/MS Detection 5994-5777EN
3. Determination of Multiclass, Multiresidue Pesticides in Spring Leaf Mix Using Captiva EMR-HCF Passthrough Cleanup and LC/MS/MS 5994-4765EN

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To order listed items from the Agilent online store, simply click the MyList header links. Then, enter the quantities for the products you need, click Add to Cart, and proceed to checkout. Your list will remain under Favorite Products for your use with future orders. This feature is valid only in regions that are e-commerce enabled.

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MyList 1: Sample preparation supplies for the analysis of pesticides in tobacco

Description	Part No.
Extraction	
Agilent Bond Elut QuEChERS extraction kit, AOAC 2007.01, without ceramic homogenizers	5982-5755
Ceramic homogenizers, 50 mL tubes, 100/pk*	5982-9313
Agilent Bond Elut QuEChERS extraction kit, AOAC 2007.01, with ceramic homogenizers	5982-5755CH
Cleanup	
Agilent Captiva EMR–LPD cartridge, 6 mL, 30/pk	5610-2092
Agilent Bond Elut QuEChERS EMR–Lipid polish pouch, 3.5 g anhydrous MgSO4	5982-0102
Other Essential Supplies for Sample Preparation	
Agilent positive pressure manifold 48 processor**	5191-4101
Centrifuge tube and cap, polypropylene, 50 mL, 25/pk	5610-2049
6 mL SPE cartridge rack for PPM-48**	5191-4104
3 mL SPE cartridge rack for PPM-48**	5191-4103
Collection rack for 16 x 100 mm tubes**	5191-4108

**Only needed when extraction kits without ceramic homogenizers are used.*

***One-time purchase.*

MyList 2: LC/MS/MS columns and supplies for the analysis of pesticides in dry spices and tobacco

Description	Part No.
Standards*	
Ready-to-use 254-compound standards mix, 8 x 1 mL, 100 µg/mL each	5190-0551
HPLC Columns	
Agilent ZORBAX RRHD Eclipse Plus C18 column, 95Å, 2.1 x 100 mm, 1.8 µm, 1200 bar pressure limit	959758-902
Agilent ZORBAX Eclipse Plus C18 column, UHPLC guard, 2.1 × 5 mm, 1.8 µm	821725-901
HPLC Supplies	
Agilent 1290 Infinity inline filter 0.3 µm	5067-6189
InfinityLab Quick Connect assembly, 0.12 x 105 mm, for column inlet connection on UHPLC	5067-5957
InfinityLab Quick Connect assembly, 0.17 x 105 mm, for column inlet connection on HPLC	5067-6166
InfinityLab Quick Turn fitting, for column outlet	5067-5966
Quick Turn capillary 0.12 x 280 mm, for connection from column to detector	5500-1191
Kit of Stay Safe waste cap GL45 with 4 ports and waste can 6 L	5043-1221
Charcoal filter with time strip for waste container	5043-1193
Stainless steel solvent inlet filter, 10 µm pore size	01018-60025
Solvents & Reagents	
InfinityLab Ultrapure LC/MS acetonitrile	5191-4496
InfinityLab Ultrapure LC/MS methanol	5191-4497
InfinityLab Ultrapure LC/MS water	5191-4498
MS solution, formic acid, 99.5% purity, 10 mL**	US-700002341
5M Ammonium formate solution	G1946-85021
Solvent Filtration Assembly***	
InfinityLab solvent filtration assembly, includes glass funnel, 250 mL, membrane holder glass base, glass flask, 1 L, and aluminum clamp	5191-6776
Regenerated Cellulose Filter membrane 47 mm, 0.20 µm 100/pk	5191-4340
Vials & Caps	
Agilent A-Line certified amber (screw top) vials, 100/pk	5190-9590
Agilent deactivated vial inserts, 400 µL, 500/pk	5183-2086
Agilent screw caps, PTFE/silicone/PTFE septa, cap size: 12 mm, 100/pk	5190-7024

**Please contact Agilent for custom premixed pesticides standards.*

***Not available for online sale. Please contact your local Agilent representative for ordering information.*

****Recommended if using solvents other than those listed in this table.*

MyList 3: GC/MS/MS columns and supplies for the analysis of pesticides in dry spices and tobacco

Description	Part No.
GC Columns	
Agilent HP-5ms UI, 15 m × 0.25 mm, 0.25 µm film thickness (two)	19091S-431 UI-KEY
GC Supplies	
Agilent Ultra Inert 2 mm dimpled liner	5190-2297
Agilent Blue Line 10 µL PTFE-tip plunger tapered syringe	G4513-80203
Agilent Advanced Green, nonstick 11 mm septum	5183-4759
Column nut, collared, self-tightening, inlet/detector	G3440-81011
Column nut, collared, self-tightening, MSD	G3440-81013
Flexible Metal ferrule, gold-plated, 0.4 mm id, for 0.1 to 0.25 mm id fused silica tubing, 10/pk	G2855-28501
MS Supplies	
HES Filament for 7010 Triple Quadrupole GC/MS	G7002-60001
Extractor lens, 3 mm	G7000-20444
Vials & Caps	
Agilent A-Line certified amber (screw top) vials, 100/pk	5190-9590
Agilent deactivated vial inserts, 250 µL, 100/pk	5181-8872
Agilent screw caps, PTFE/silicone/PTFE septa, cap size: 12 mm, 100/pk	5190-7024

Note: *The Pesticide and Environmental Pollutant MRM Database includes over 1,100 pesticides and environmental pollutants representing multiple compound classes for easy and fast setup of GC/MS/MS analysis. Ask your local representative for part number G9250AA.*

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